Primary resistance against integrase strand transfer inhibitors in integrase strand transfer inhibitor-naive patients failing first- and second-line ART in Tanzania

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Introduction: Sub-Saharan African countries are introducing integrase strand transfer inhibitors (INSTIs) in their ART programmes as the preferred first-line regimen, and dolutegravir is the INSTI of choice due to its potency, tolerability and high genetic barrier to resistance. Dolutegravir was introduced into the first-line ART regimen in Tanzania in 2019. However, there is a paucity of data on the occurrence of mutations in HIV lineages circulating in Tanzania. This study aimed to determine the prevalence of INSTI primary resistance mutations in Tanzanian patients exposed to ART but not INSTIs.

Methods: Plasma samples from 50 INSTI-naive patients failing first- or second-line ART [median (IQR) age: 40 (21.93–46.41) years; 68% women] were subjected to Sanger sequencing of the HIV integrase gene. Participants had been on ART for a median (IQR) duration of 7.32 (4.73–9.29) years, with 80% and 20% failing first- and second-line ART, respectively.

Results: No major INSTI mutations were found, but 2 (4%) participants had the accessory mutation T97A. Using the REGA HIV-1 subtyping tool, HIV subtype A1 (53.1%) was found to be dominant, followed by subtypes C (30.6%) and D (16.3%).

Conclusions: This study found no current evidence for transmitted resistance against INSTIs among unexposed patients failing ART and supports the scale-up of INSTI-based regimens. However, the presence of accessory mutations calls for the surveillance of INSTI resistance mutations to ensure that the anticipated long-term desired outcomes are achieved.
a lower prevalence, also PI-based first- and second-line regimens,1–7 which until recently were the mainstream of ART regimens of most SSA countries.1–7 Due to increased transmitted NNRTI resistance, higher effectiveness, low cost and good tolerability of dolutegravir-based regimens the WHO recommended the switch to integrate strand transfer inhibitor (INSTI)-based first-line regimens.8 Dolutegravir, a second-generation INSTI, was introduced in the Tanzanian ART programme in 2019 as a fixed-dose combination with tenofovir disoproxil fumarate and lamivudine (tenofovir/lamivudine/dolutegravir).8,9 Dolutegravir has been proven to have a better safety profile and higher effectiveness in suppressing viral load,10 a higher genetic barrier to resistance and to be better tolerated and more cost-effective than efavirenz-based regimens, which until recently were used as first-line regimens, and to be superior to other first-generation INSTIs.8,11–15

Despite the welcome advantages possessed by dolutegravir over other antiretrovirals, including first-generation INSTIs, the question of dolutegravir primary resistance is yet to be answered in the context of ART adherence challenges, insufficient HIV viral monitoring and limited access to HIV drug resistance testing.16–18 There is still a lack of data on dolutegravir resistance to inform a public health approach to ART prescription.17 As the prevalence of polymorphisms in integrase play a role in primary resistance against dolutegravir,18,19 variation by subtype might impact INSTI response.20 This was shown by a study, conducted in Cameroon, showing 5% of 255 ART-naive HIV-positive individuals harboured major mutations against INSTIs (E92G, E138K, G140S and Y143C) even though largely as minority variants as deep sequencing was used. A high prevalence of polymorphic accessory mutations was reported in this study (54.9% of the 255 individuals), with L74I, E157Q, T97A and L74M mutations being prevalent.21 To help inform policy, there is a need to further investigate the presence of resistance-associated mutations (RAMs) in the integrase gene with the potential to confer resistance to dolutegravir and other INSTIs in dolutegravir-naive patients virologically failing their first- or second-line ART.

Materials and methods

Study participants

This was a cross-sectional study, conducted between 2018 and 2020, of patients with virological failure. Virological failure was defined as a plasma viral load above 1000 copies/mL based on two consecutive viral load measurements separated by 3 months, with adherence support. The treatment history of the study participants included first-line treatment failure with NRTI backbones with tenofovir disoproxil fumarate/zidovudine or efavirenz/nevirapine-based regimens and second-line treatment failure with ritonavir-boosted PIs (atazanavir/r and/or lopinavir/r). Those who met the inclusion criteria voluntarily signed an informed consent form. Two study sites participated in the recruitment process: the Pastoral Activities and Services for people with AIDS Dar es Salaam Archdiocese (PASADA) clinic and a tertiary hospital for the north-western zone of Tanzania, Bugando Medical Centre (BMC). Participants with an INSTI exposure history were excluded from taking part in the study to minimize the confounding effect of INSTI exposure. EpiData software version 3.1 (http://www.epidata.dk/documentation.php) was used to record patient demographics, medical history and other information derived from structured case report forms, patient files and the national care and treatment (CTC II) database. These data were then exported to Microsoft Excel software (2010 version), cleaned, and made ready for analysis.

Viral load testing

A whole blood sample (4.5 mL) was collected in EDTA collection tubes (BD, Franklin Lakes, NJ, USA). Plasma was obtained by centrifuging the collected whole blood sample at 956 x g for 15 min. Viral load testing was done at the respective study sites following the national guidelines for the management of HIV and AIDS,9 using the HIV-1 assay on the COBAS 6800/8800 platforms from Roche (Roche Diagnostics, Branchburg, NJ, USA), which employ the use of automatic RNA extraction using magnetic glass particle technology and purification followed by real-time PCR using TaqMan hydrolysis probes.22

HIV genotyping and sequencing

To analyse the integrase gene, RNA was extracted from plasma using the QIAamp (QIAGEN, Valencia, CA, USA) Virus RNA Mini extraction kit following the manufacturer’s instructions.23 The extracted RNA was reverse transcribed and amplified using the QIAGEN OneStep RT-PCR kit (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions.24 All the PCR processes and PCR product purification were performed following the protocol described by Brada et al.25 Sequencing of PCR product was performed using a capillary ABI Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions and the abovementioned protocol.25 All the primers and thermocycling conditions were as described in the protocol.25

Sequences were checked for quality using the HIV-1 Sequence Quality Analysis tool (http://www.hiv.lanl.gov/content/sequence/QC/index.html) before being submitted to Stanford’s HIV drug resistance database for analysis and subtyping.26 The HIV-1 subtypes were identified using the REGA HIV-1 online subtyping tool version 3.4;27 these sequences were also submitted to GenBank and assigned accession numbers from OL962487 to OL962535.

Subtyping

REGA HIV-1 subtyping tool version 3.4.6 was used to assign subtypes to our sequences as well as obtaining the reference sequences, for which Clustal W alignment was then performed in Bio-Edit sequence alignment editor version 7.2.5.27 A neighbour-joining phylogenetic tree was inferred in MEGA version 10.2.5 and evolutionary distances computed using the Kimura 2-parameter substitution model.28–30

Statistical analysis

Median and IQR were used to present numerical variables, while categorical variables were presented in proportions with their 95% CIs. Proportions and 95% CIs were used to present prevalence values. All the statistical analyses were conducted using Stata 13 (Stata Corp, College Station, TX, USA).

Ethics approval

This study was approved by the Tanzania National Institute of Medical Research ethics review board (reference number: NIMR/HQ/R.8a/Vol.1X/2462). The study was part of a clinical trial entitled ‘The impact of HIV drug resistance testing, and subsequent change to an individualized therapy based on the resistance profile, in children, adolescents and adults with virological failure of their HIV therapy, in three HIV clinics in Tanzania’, which is registered at ClinicalTrials.gov with registration number NCT03557021. All patients gave written informed consent, and the study was conducted following principles described in the Declaration of Helsinki.
Results

Patient demographics

We successfully sequenced all 50 samples for analysis of the integrase gene. Median (IQR) patient age was 40 (21.93–46.41) years and 40 (80%) were on a first-line regimen, while 10 (20%) were on a second-line regimen. Sixty-eight percent of the participants were female. The median (IQR) of 260 (110–416) cells/mm³ and 77,700 (22,000–303,000) copies/mL were obtained for CD4+ cells and viral load, respectively. The duration on ART for the participants was observed to be a median (IQR) of 7.32 (4.73–9.29) years. This information is detailed in Table 1.

DRMs

No sequence included major DRMs with the potential to confer resistance to any of the INSTIs. Accessory mutation T97A was found in 2 (4%) of samples, all of subtype A1.

Subtyping

A neighbour-joining phylogenetic tree for HIV-1 integrase sequences was inferred in MEGA version 10.2.5 with evolutionary distances computed using the Kimura 2-parameter substitution model.28–30 The tree shows clusters of the following subtypes: A1, 26 (53.1%); C, 15 (30.6%); and D, 8 (16.3%). During this analysis, one patient’s sequence was excluded because it was short; this information is detailed in Figure 1.

Discussion

This study observed no major RAMs against any INSTI drug in INSTI-naive patients experiencing treatment failure in their first- and second-line ART regimens across the subtypes that were found (A1, C and D). This finding is consistent with a study conducted in Tanzania that observed no major mutations in treatment-experienced (INSTI-naive) patients, but accessory mutations T97A, E157Q, G163E/K and A128T were observed in 8 (16.3%) of the study participants.29 The subtypes identified in this study are similar to those reported in other studies also conducted in Tanzania7,29,30 and this provides very good contextual information to policymakers, as the regimen can be used for HIV management in our patients. As documented recently in Tanzania,29 accessory resistance mutation T97A was observed in 2/50 participants (4%). T97A is a polymorphic INSTI-selected mutation that, depending on the subtype, occurs in about 1%–5% of viruses from untreated patients with the exception of subtype A (5%–10%), subtype J (33%) and group P (50%), and in our study it occurred in individuals infected with HIV-1 subtype A.31 This mutation alone has minimal effects on INSTI susceptibility. In synergy with other major mutations it reduces susceptibility to each of the INSTIs.31 The occurrence of T97A in individuals with subtype A is similar to that in a study that reported the mutation as most prevalent in individuals with subtype A.31

A study conducted in Uganda on treatment-naive and treatment-experienced individuals observed no major mutations in INSTI-naive individuals, but were observed in treatment experienced individuals failing a raltegravir-based third-line regimen.12 This study also reported the presence of accessory mutations T97A, M50I and L74M whereby T97A was found in 8 (5%) and 9 (7.4%) of patients failing their first-and-second-line ART, respectively, and 7 (8%) of ART-naive individuals.32

Another study that has reported the occurrence of major DRMs against dolutegravir in treatment-naive patients was conducted in Cameroon, wherein major INSTI mutations were observed in about 5% of the study participants, with E92G, E138K, G140S and Y143C mutations being prevalent.23 However, the majority of these mutations were detected in minority variants as a low variant threshold of 1% was used.21 Accessory mutations have also been reported in various studies conducted in low- and middle-income settings; a study conducted by Mikasi et al.19 reported that other accessory mutations (E157Q, G163E/K and A128T) were found in 5% of the study population. Further accessory mutations have been reported by a modelling study, which indicated that E157Q and D232N have the potential to affect dolutegravir binding and lead to resistance development.33 In addition, it has also been documented that the T97A accessory mutation is associated with increased dolutegravir resistance in conjunction with other DRMs in INSTI-experienced patients.34 Our study has shown that DRMs against dolutegravir in INSTI-naive patients are rare. However, it is possible to occur, as shown in a study conducted in Uganda, which detected major INSTI-associated mutations (E138T, E138K and T66I) in the sequences of 6/511 (1.2%) ART-naive individuals, and accessory mutations were observed in 145 sequences, which accounted for 24% of the 511 sequences.35

Apart from the fact that the majority of the countries in the sub-Saharan region introduced dolutegravir in their first-line regimen, major INSTI-associated mutations have started to occur in patients on dolutegravir-based regimens. A study conducted by Joep et al.16 in Malawi reported the occurrence of R263K, E138K and S147G in 8/27 (29.6%) of the samples from virologically failing patients on tenofovir/lamivudine/dolutegravir.

These possibilities have also been described in some case reports in South Africa, Botswana and Uganda.36–38 The ineffectiveness of some emergent drug resistance against dolutegravir has also been explained by mutations outside the integrase gene, arising in patients receiving dolutegravir-containing

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**Table 1.** Demographic characteristics

| Characteristics                  | All patients, N=50 |
|----------------------------------|--------------------|
| Age (years), median (IQR)        | 40 (21.93–46.41)   |
| Sex, n (%)                       |                    |
| Female                           | 34 (68)            |
| Male                             | 16 (32)            |
| Viral load (copies/mL), median (IQR) | 77700 (22000–303000) |
| ART regimen, n (%)               |                    |
| First line, 40 (80)              |                    |
| ZDV + 3TC + EFV/NVP              | 30 (75)            |
| TDF + 3TC/FTC + EFV              | 10 (25)            |
| Second line, 10 (20)             |                    |
| TDF + FTC + LPV/ATV/r            | 6 (40)             |
| ABC + 3TC + ATV/r                | 4 (40)             |
| CD4+ cells (cells/mm³), median (IQR), N=44 | 260 (110–416)     |
| Duration on ART (years), median (IQR) | 7.32 (4.73–9.29)  |
regimens with no mutations in the integrase gene,\textsuperscript{39} as well as by the role of natural polymorphisms.\textsuperscript{33,40}

All these phenomena stress the need for surveillance of INSTI-associated resistance mutations. Though our findings showed that resistance against INSTIs is rare in INSTI-naive patients, the widespread use of dolutegravir-containing regimens may be associated with the emergence and spread of INSTI-associated mutations, which further calls for continued surveillance of INSTI-associated mutations.

**Study limitations**

This study did not sequence the protease (PR) and reverse transcriptase (RT) genes and therefore does not determine whether DRMs in these locations influence the emergence of primary resistance against dolutegravir. Our study was also limited by the smaller sample size that was used considering the rarity of dolutegravir resistance.

**Conclusions**

This study observed no major mutations against INSTIs in INSTI-naive treatment-experienced patients and only a single accessory mutation, T97A, supporting the use of dolutegravir-containing regimens in Tanzania. However, the widespread use of INSTIs calls for continued surveillance of INSTI resistance mutations to ensure that the desired long-term treatment outcomes are achieved.

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**Transparency declarations**

All authors declared no conflict of interest. The findings, conclusions and opinions in this report are those of the authors, funding agencies had no role in the conceptualization, data collection, analysis and reporting of this study.

**Author contributions**

C.K., D.M. and S.H. conceptualized and designed the study. S.H. and E.L. did the laboratory analyses, captured demographic information and...
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