Limited emergence of resistance to Integrase strand transfer inhibitors (INSTIs) in HIV-experienced patients failing dolutegravir-based antiretroviral therapy: Cross-sectional analysis from a Northeast Nigerian cohort

Adam Abdullahi[1,2,3$], Ibrahim Musa Kida[4*], Umar Abdullahi Maina[5], Amina Husaini Ibrahim[6], James Mshelia[4], Haruna Wisso[3], Abdullahi Adamu[5], James Ezenwa Onyemata[3], Haruna Yusuph[4], Sani H. Aliyu[7], Man Charurat[8], Alash'le Abimiku[3], Lucie Abeler-Dorner[9], Christophe Fraser[9], David Bonsall[9], on behalf of the PANGEA consortium, Steven A. Kemp[1,2*] and Ravindra K. Gupta[1,2,10*$*]

[1]Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), Cambridge, UK
[2]Department of Medicine, University of Cambridge, Cambridge, UK
[3]Institute of Human Virology, Abuja, Nigeria
[4]Department of Infectious Disease and Clinical Immunology University of Maiduguri, Borno, Nigeria
[5]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Borno, Nigeria
[6]Oman Medical College, Oman
[7]Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
[8]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, USA
[9]Big Data Institute, University of Oxford, UK
[10]Africa Health Research Institute, Durban, South Africa

*Equal senior author contribution
$Correspondence:
Ravindra K. Gupta
rkg20@cam.ac.uk
or
Adam Abdullahi
aa2291@cam.ac.uk

Abstract

Background:

Owing to high levels of resistance to previous first-line non-nucleoside reverse transcriptase inhibitors (NNRTI)-based antiretroviral therapy (ART), consolidated recommendations since 2019, from the WHO and others, have indicated that dolutegravir (DTG) is the preferred drug of choice for HIV treatment, globally. There is a paucity of resistance outcome data from non-B HIV subtypes circulating across West Africa.

Aim:

We aimed to characterise the mutational profiles of HIV-positive patients from a small North-East Nigeria cohort, failing a DTG-based ART regimen.

Methods:

Plasma samples were collected and stored from 61 HIV-1 infected participants. Following failure of DTG-based ART, all samples were sequenced by Illumina whole-genome, ultra-deep sequencing. Sequencing was successful in (n=33) participants with median age of 40 years and median time on ART of 9 years. HIV-1 subtyping was performed using SNAPPy. Haplotype reconstruction and transmission were inferred using standard phylogenetic methods.

Result:

Most patients had mutational profiles that were reflective of prior exposure to first- and second-line ART including exposure to thymidine analogues, efavirenz and nevirapine. One patient had evidence of major INSTI DRMs (T66A, G118R, E138K and R263K), reducing efficacy of DTG. The participant was aged 18, infected with a subtype G virus and likely vertically infected.

Conclusion:

This study found low level resistance to DTG in the cohort, with one patient having high-level resistance to DTG and other INSTIs. Critical population level and long-term data on DTG outcomes are required to guide implementation and policy action across the region.

Introduction

In the context of rising pre-treatment NNRTI drug resistance[1,2], the World Health Organisation (WHO) recommended dolutegravir (DTG) as the preferred antiretroviral therapy (ART) drug of choice for both newly diagnosed and individuals transitioning from previous regimens[3]. Safety, potency, tolerability and cost-effective characteristics of dolutegravir (DTG) supported this change[4] subsequently, countries across sub-Saharan Africa (SSA) have consequently rolled-out dolutegravir as part of standard treatment. Roll out across the region started in 2019 and is expected to continue, aided by the availability of a low-cost, generic fixed-dose co-formulation of tenofovir, lamivudine and dolutegravir (TLD)[5].

Dolutegravir-based antiretroviral therapy (ART) have been commercialised and sold in Nigeria since 2019 with the national treatment guideline recommending transitioning to DTG-based ART in both virally suppressed and unsuppressed patients since 2020[6]. There is no indication of virological or resistance testing prior to transitioning to DTG-based ART and therefore, majority of patients transitioned without prior viral load or resistance testing. Data from the ADVANCE and NAMSAL clinical trials[7,8], which recruited ART naïve participants exclusively in SSA showed no evidence of emergence of drug resistance mutations (DRMS) on DTG-based ART. Data from treatment experienced patients transitioning to TLD is limited although data is starting to emerge.

Given the high proportion of treatment experienced HIV patients with resistance following failure of previous first and second-line ART[9], data on resistance outcome following failure of DTG in non-B subtypes is highly valuable. Here, we present data on drug resistance using Next-Generation Sequencing (NGS) in a small Nigerian cohort failing DTG-based ART following roll-out.

Methods

Study population and design

This study was a cross-sectional study performed at the University of Maiduguri Teaching Hospital, Borno State, Nigeria between January, and June 2021. Study criteria included participants who were failing a DTG-based ART, ≥18 years of age and attending routine clinic visits. We defined virological failure as two consecutive HIV-1 RNA > 1000 copies/ml following exposure to a DTG-based ART for ≥ 6 months. Patients who met inclusion criteria voluntarily signed informed consent. Available demographic data including age, gender, ART regimen, duration on ART and current CD4 count were collected from clinical files and recorded in Microsoft excel.

Laboratory methods:

Plasma was separated from whole venous blood in EDTA within 2 hours of collection and stored immediately at -80°C. Plasma viral load testing and CD4 count were performed at the Defence Reference Laboratory, Asokoro Abuja using the COBAS AmpliPrep/COBAS TaqMan HIV type 1 (HIV1) v2.0 test (Roche Diagnostics, Basel, Switzerland). Whole genome sequencing of 61 blood samples was performed according to the Bonsall et al protocol[10]. Briefly, total RNA was extracted from HIV-infected plasma samples, washed in ethanol, and eluted using the NUCLISENS easyMAG system (bioMérieux). Libraries were prepared using the SMARTer Stranded Total RNA-Seq Kits v2 (Clontech, Takara Bio) according to the manufacturer protocol. Total RNA was denatured, and reverse transcribed to cDNA and a total of 500ng of pooled libraries were hybridised to custom HIV-specific biotinylated 120-mer oligonucleotides (xGen Lockdown Probes, Integrated DNA Technologies). Captured libraries were then PCR amplified to produce a final pool for sequencing with an Illumina MiSeq (San Diego, CA, USA) to produce up to 300-nucletoide paired-end reads. FastQ files were trimmed of adapters and mapped iteratively to the best available reference from a curated alignment of 3000 HIV-1 genomes with SHIVER[11]. Resistance genotyping was performed using an in-house script that determines the prevalence of DRMS in each sequencing reads and calculates an overall score (1-4) for each ART, according to the Stanford HIV drug resistance algorithm (v9.1).

Bioinformatics analysis:

Haplotypes were reconstructed using CliqueSNV v2.0.3[12]. Phylogenies were inferred with IQTREE2 v2.2.2[13] using a GTR+F+I+R4 model with 1000 rapid bootstraps. Inference of transmission was made Phyloscanner v1.8.1[14] using overlapping windows of 150 bp across the whole genome. Phylogenies were rooted on a HIV-1 subtype G consensus sequence downloaded from the Los Alamos National HIV Database. HIV-1 subtyping was performed using SNAPPy v1.0. Prediction of co-receptor usage was made using TROPHIX (prediction of HIV-1 tropism). Available at: http://sourceforge.net/projects/trophix/).

Statistical analyses:

The characteristics of the study population were summarized as either categorical or continuous variables and reported as either proportions or medians with interquartile ranges (IQRs), respectively. Analyses were performed with STATA v17 (StataCorp, College Station, TX).

Ethics:

The study was approved by the University of Maiduguri Teaching Hospital Ethics Committee (UMTH/REC/21/714). All participants provided written informed consent.

Results

61 had samples available for resistance testing. Following sequencing and quality control, 33 samples (61.7%) were of sufficient quality (i.e., fully in-tact *pol* gene and depth of ≥500x) to determine DRMS and minority variants (Median viral load in these samples was 4.1 $\log_{10}$ copies/ml (range 3.0-5.2). Using a minimum threshold of 20%, NRTI, NNRTI, PI and INSTI DRMS occurred in 17 (50%), 24 (70.6%), 4 (11.8%) and 1 (2.9%) of samples respectively (Figure 1a). Dual-class resistance occurred in 17 (50%) patients and tri-class mutations occurred in 5 (14.7%) patients. Consistent with likely long-term exposure to lamivudine, the most prevalent NRTI mutation DRMS was M184V. The most prevalent NNRTI DRMS was K103N, reflecting previous exposure to nevirapine and efavirenz. Mutational profiles were similar across 2, 10 and 20% interpretative thresholds (Figure 1b).

One patient of interest was found to have high-level resistance to NRTIs, NNRTIs and INSTIs, including almost complete resistance to the novel long-acting injectable, cabotegravir. Mutations included inE138K, inG118R, inT66A, inR263K, rtH221Y, rtV108I, rtK103N, rtM184V, rtM41L, rtA98G and rtT215Y, all at frequencies of >40%, with a mean read depth of 770x. This patient was established on DTG for a median of 1.2 years and on ART for a median of 12 years. Clinical data on other clinical data including nadir CD4 counts were unavailable. Using the SNAPPy HIV-1 subtyping tool, almost 40% of viruses were assigned to Subtype G, and 15% were G_A1 subtypes. The remaining viruses were recombinants (Table 1).

To identify within-host diversity and potential transmission of DRMs between patients, we reconstructed viral haplotypes (Figure 2) for each patient. These were homogeneous and the same resistance mutations were identified on all reconstructed haplotypes for each patient. Following this, we investigated whether there was evidence of direct transmission between any patients in this cohort (Supplementary Figure 1). However, no significant transmission pairs were identified, indicating that there were several intermediaries between patients which have not yet been sampled. Of note, two patients' virus was predicted to use the CXCR-4 co-receptor.

Discussion

After rollout of previous first-line NNRTI based ART, DRMS were previously observed within first year of failure in around 15 and 35% of patients with resistance emerging against both lamivudine, tenofovir and NNRTIs[16,17]. Drug resistance has been associated with mortality in hospitalised individuals in LMIC settings[18]. The second generation INSTI DTG has been systematically adopted and rolled out across the SSA region since 2019, with concerns of the emergence of resistance associated mutations following failure under a limited monitoring infrastructure. In a recent analysis of pooled evidence on virological and resistance outcomes following DTG failure in SSA region, there was an overall high rate of virological response to DTG; 88.5% (95% CI: 73.8-97.8) with the overall proportion of patients failing showing limited evidence of DRMS[19] over short periods of time. It is likely that prolonged virologic failure will select for DRM to components of ART regimens within the viral quasispecies as a result of intrahost evolution[20,21].

It is important to note that pre-existing DRMS prior to switch to DTG may be critical to both virological and resistance outcomes with study evidence suggesting pre-existing NNRTI mutations reducing the short term efficacy of DTG[22], although other studies across the region have shown similar rates of both virological and resistance outcomes in ART naïve[23,24] and experienced patients[25,26] (with no evidence of DRMs) and ART experienced patients with historical evidence of NRTI mutation (especially M184V/I)[27–29].

Here, in this cross-sectional analysis, we assessed drug resistance using next-generation sequencing in a small cohort of HIV-1 infected subjects failing DTG-based ART using a failure threshold of 1000 copies/ml. Most patients were treatment experienced and amongst 33 participants with sequence data, mutational patterns observed were reflective of exposure to previous first-line NNRTI with only 1/34 (3%) showing evidence of DRMS against DTG or protease inhibitors. The individual with DTG resistance was vertically infected with evidence of selection of mutations conferring high level resistance to dolutegravir and other INSTIs i.e T66A, G118R, E138K (accessory) and R263K. The T66A mutation is non-polymorphic and primarily selected by elvitegravir (EVG) and raltegravir (RAL) with ~9-fold reduction in susceptibility to EVG but minimal impact on other INSTIs whilst the E138K mutation has negligible effect on susceptibility to INSTIs although a combination of E138K and other DRMs may lead to further decreased susceptibility to DTG[30]. Further, the G118R and R263K mutations observed in this patient, which causes between 2 to 15 fold reduction to DTG susceptibility[31,32] have also been observed in patients experiencing virological failure to INSTI drug agents in non-B HIV subtypes[33–36]. It is likely that the G118R mutation emerged first and led to the

accumulation of other mutations including the E138K compensatory mutation as the G118R mutation has been described as the DTG-resistance pathway in non-B subtype[31,37].

In context of the continuously expanding use of DTG, the most convenient approach to managing patients on DTG with persistent viraemia remains uncertain especially in resource limited setting such as this, where drug resistance testing capacity remains limited[38]. Several factors may increase the likelihood of the emergence of DTG resistance across the region including prolonged virological failure due to lack of routine virological monitoring[39,40]and poor treatment adherence which is an independent determinant of virological outcome[41] in these settings. Further analyses of resistance across SSA are warranted over extended periods, as well as surveillance for INSTI resistance in newly diagnosed individuals. This is even more critical given INSTI based long acting injectables are being considered as PreP[42].

Acknowledgements:

We are thankful to the volunteers who participated in the study. A.A. is supported by Africa Research Excellence Fund Research Development Fellowship (AREF-318-ABDUL-F-C0882) and Cambridge-Africa award. A.A. and S.A.K. are supported by Bill and Melinda Gates Foundation via the Phylogenetics and Networks for Generalised Epidemics in Africa (PANGEA) (grant number OPP1175094). R.K.G. is supported by a Wellcome Trust Senior Fellowship in Clinical Science (WT108082AIA).

Author contributions

Study conception, design and administration: A.A, R.K.G and I.M.K; data collection: A.A, I.M.K, A.H.I, J.M, A.Ad, J.E.O, H.Y, S.H.A, A.Ab, S.K and R.K.G; data analysis: A.A., I.M.K, S.K, A.Ab and R.K.G; data interpretation: A.A, S.K, A.Ab and R.K.G; manuscript preparation; A.A wrote the first draft of the manuscript with the critical input of all co-authors. All authors reviewed the results and approved the final version of the manuscript.

Table 1: Characteristics of study participants with successful genotyping

Characteristic		
Total number (%)		33 (100)
Female, n (%)		20 (61)
Age, median years (IQR)		40 (35, 48)
CD4 count, median cells/mm^3 (IQR)		200 (300, 467)
ART regimen, (%)		
	TDF+3TC+DTG	33 (100)
ART regimen prior to DTG		
	TDF+3TC+LPV/r	17 (52)
	TDF+3TC+ATV/r	4 (12)
	AZT+3TC+EFV	8 (24)
	ABC+3TC+EFV	2 (6)
	No prior ART	2 (6)
Time on DTG, median years (IQR)		1.8 (1.4, 1.9)
Time on ART, median years (IQR)		9.3 (5.8, 15.0)
ART status		
	Switching to DTG	31 (94)
	Starting DTG	2 (6)
HIV-1 subtype, n (%)[b]		
	G	13 (39)
	G/A1	5 (15)
	CRF02_AG	3 (9)
	A (A1)	2 (6)
	CRF02_AG/G	2 (6)
	02AG/A1	2 (6)
	Others	6 (18)

[a]Data available from 25 participants; [b]Other subtypes comprising CRF06_CPX (n=1); CRF13_CPX-like (n=1); CRF13_CPX/G (n=1); CRF 11_CPX (n=1); G/C (n=1); G/J/A (n=1).

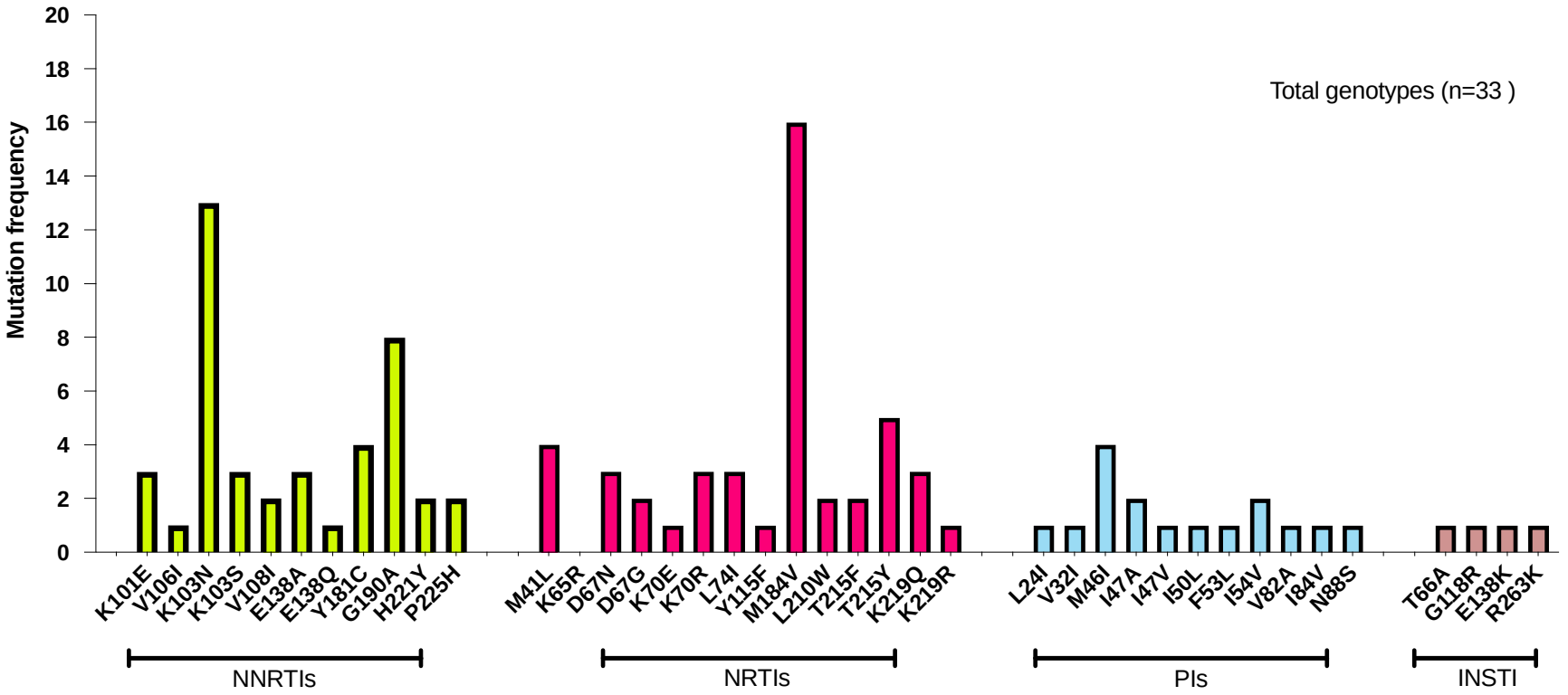


Figure 1a: Proportion of patients with resistance associated mutations using the Stanford algorithm.

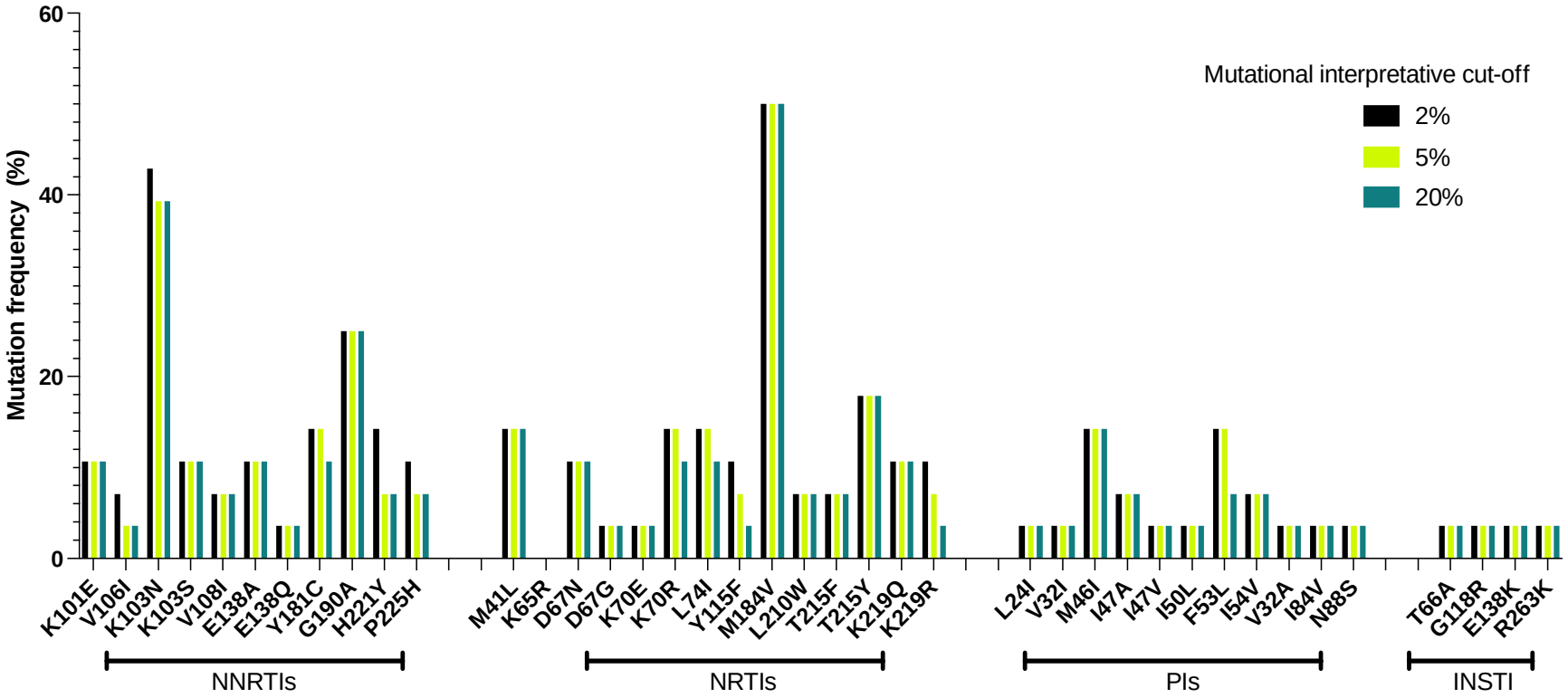


Figure 1b: Proportion of patients with resistance associated mutations using the Stanford algorithm, sub-divided into interpretational cut-offs of 2, 5 and 20%. Evidence suggest that minority variants may play a role in drug resistance (https://doi.org/10.1128/mbio.00269-22).

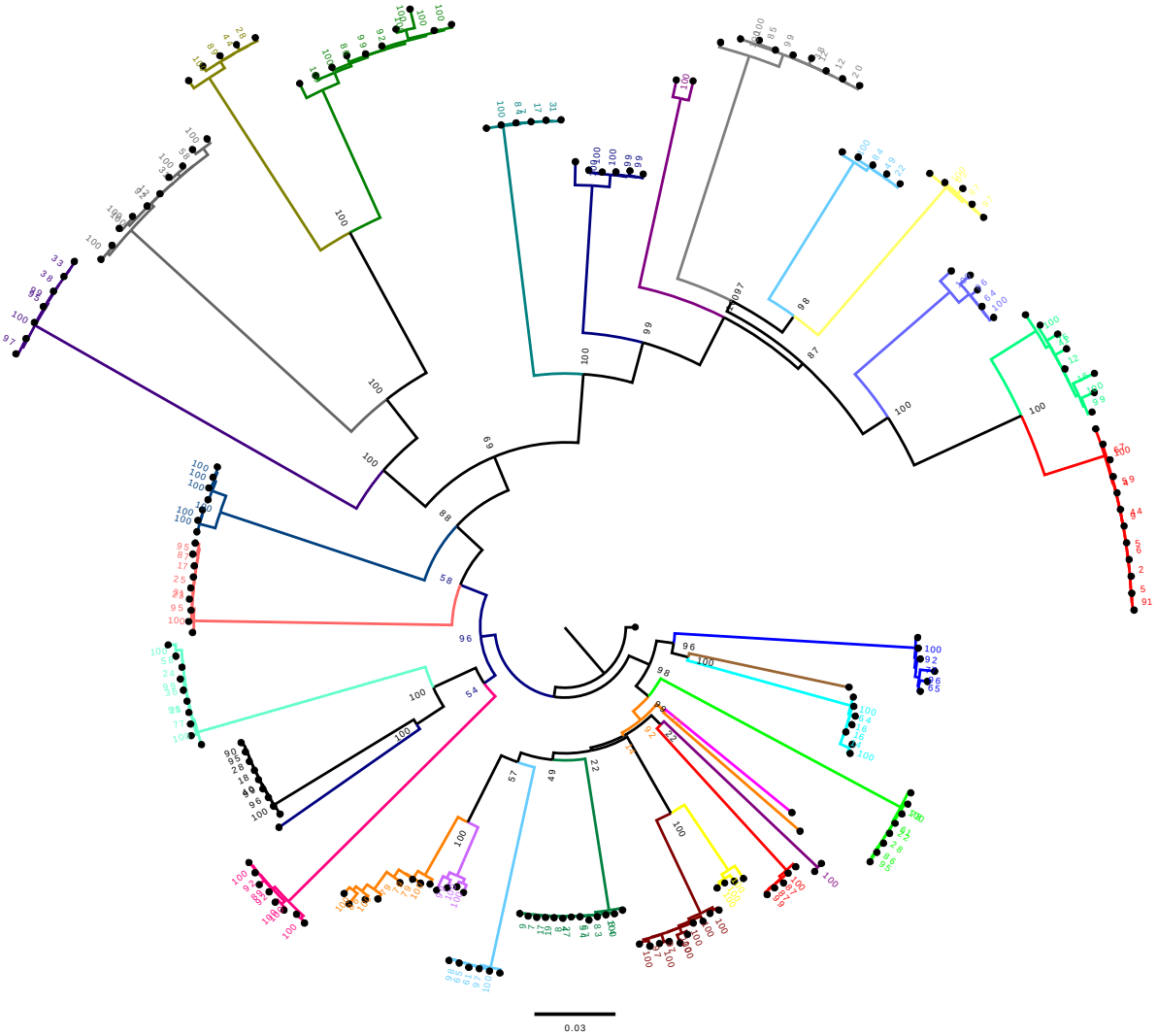
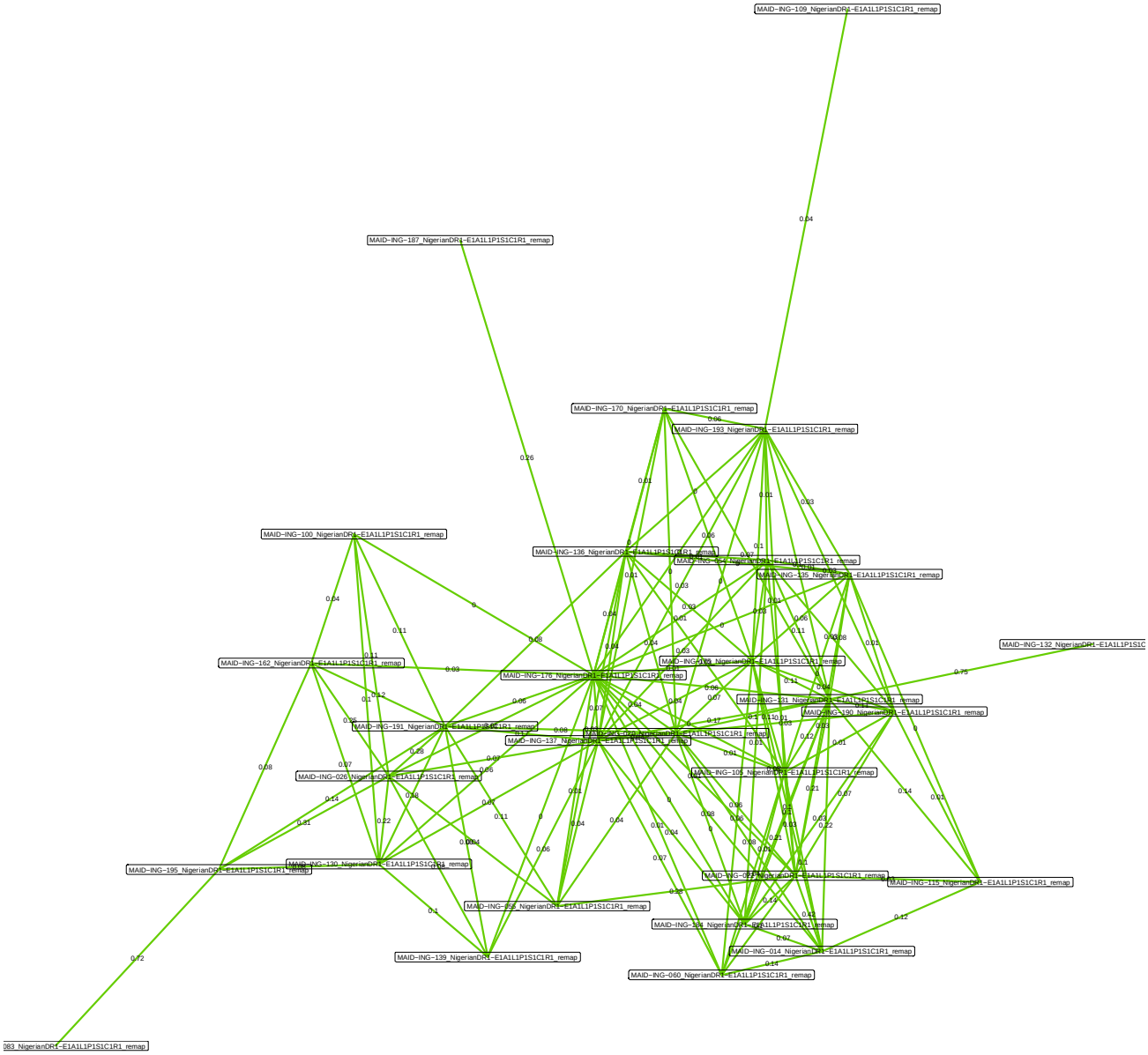


Figure 2. Maximum-likelihood phylogeny of reconstructed haplotypes from each patient. Haplotypes were homogeneous and had the majority of the same mutations on each haplotype. Bootstraps are indicated at each node.



294 **Supplementary Figure 1:** Inferred transmission network of all patients in the cohort. Green lines
295 indicate a degree of linkage between two sequences, but without sufficient statistical support to
296 indicate a direct transmission event has occurred.
297
298
299

References:

1. Gupta RK, Jordan MR, Sultan BJ, *et al.* Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet (London, England)* 2012; **380**: 1250–8.

2. Gupta RK, Gregson J, Parkin N, *et al.* HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. *Lancet Infect Dis* 2018; **18**: 346–55.

3. World Health Organization. *Update of recommendations on first- and second-line antiretroviral regimens*. 2019. Available at: https://apps.who.int/iris/bitstream/handle/10665/325892/WHO-CDS-HIV-19.15-eng.pdf?ua=1.

4. Jamieson L, Serenata C, Makhubele L, *et al.* Cost and cost-effectiveness of dolutegravir-based antiretroviral regimens: an economic evaluation of a clinical trial. *AIDS* 2021; **35**. Available at: https://journals.lww.com/aidsonline/Fulltext/2021/12152/Cost_and_cost_effectiveness_of_dolutegravir_based.9.aspx.

5. Dorward J, Hamers RL. Dolutegravir in sub-Saharan Africa: context is crucial. *Lancet HIV* 2019; **6**: e72–3. Available at: https://doi.org/10.1016/S2352-3018(18)30331-X.

6. Federal Ministry of Health Abuja Nigeria. *National guideline for HIV prevention, treatment and care*. 2020.

7. Calmy A, Tovar Sanchez T, Kouanfack C, *et al.* Dolutegravir-based and low-dose efavirenz-based regimen for the initial treatment of HIV-1 infection (NAMSAL): week 96 results from a two-group, multicentre, randomised, open label, phase 3 non-inferiority trial in Cameroon. *lancet HIV* 2020; **7**: e677–87.

8. Venter WDF, Sokhela S, Simmons B, *et al.* Dolutegravir with emtricitabine and tenofovir alafenamide or tenofovir disoproxil fumarate versus efavirenz, emtricitabine, and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection (ADVANCE): week 96 results from a randomised, phase 3, . *lancet HIV* 2020; **7**: e666–76.

9. Stockdale AJ, Saunders MJ, Boyd MA, *et al.* Effectiveness of Protease Inhibitor/Nucleos(t)ide Reverse Transcriptase Inhibitor–Based Second-line Antiretroviral Therapy for the Treatment of Human Immunodeficiency Virus Type 1 Infection in Sub-Saharan Africa: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2017; **66**. Available at: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/cix1108/4767830.

10. Bonsall D, Golubchik T, de Cesare M, *et al.* A Comprehensive Genomics Solution for HIV Surveillance and Clinical Monitoring in Low-Income Settings. *J Clin Microbiol* 2020; **58**.

11. Wymant C, Blanquart F, Golubchik T, *et al.* Easy and accurate reconstruction of whole HIV

334 genomes from short-read sequence data with shiver. *Virus Evol* 2018; **4**: vey007.
335 12. Knyazev S, Tsyvina V, Shankar A, *et al.* Accurate assembly of minority viral haplotypes from next-
336 generation sequencing through efficient noise reduction. *Nucleic Acids Res* 2021; **49**: e102–e102.
337 Available at: https://doi.org/10.1093/nar/gkab576.
338 13. Minh BQ, Schmidt HA, Chernomor O, *et al.* IQ-TREE 2: New Models and Efficient Methods for
339 Phylogenetic Inference in the Genomic Era. *Mol Biol Evol* 2020; **37**: 1530–4. Available at:
340 https://doi.org/10.1093/molbev/msaa015.
341 14. Wymant C, Hall M, Ratmann O, *et al.* PHYLOSCANNER: Inferring Transmission from Within- and
342 Between-Host Pathogen Genetic Diversity. *Mol Biol Evol* 2018; **35**: 719–33. Available at:
343 https://doi.org/10.1093/molbev/msx304.
344 15. Araújo PMM, Martins JS, Osório NS. SNAPPy: A snakemake pipeline for scalable HIV-1 subtyping
345 by phylogenetic pairing. *Virus Evol* 2019; **5**: vez050. Available at: https://doi.org/10.1093/ve/vez050.
346 16. Gregson J, Kaleebu P, Marconi VC, *et al.* Occult HIV-1 drug resistance to thymidine analogues
347 following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or
348 efavirenz in sub Saharan Africa: a retrospective multi-centre cohort study. *Lancet Infect Dis* 2017; **17**:
349 296–304.
350 17. Tenores Study Group. Global epidemiology of drug resistance after failure of WHO
351 recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study.
352 *Lancet Infect Dis* 2016; **16**: 565–75.
353 18. Gupta-Wright A, Fielding K, van Oosterhout JJ, *et al.* Virological failure, HIV-1 drug resistance, and
354 early mortality in adults admitted to hospital in Malawi: an observational cohort study. *lancet HIV*
355 2020; **7**: e620–8.
356 19. Ngoufack Jagni Semengue E, Santoro MM, Ndze VN, *et al.* HIV-1 integrase resistance associated
357 mutations and the use of dolutegravir in Sub-Saharan Africa: A systematic review and meta-analysis.
358 *PLOS Glob Public Heal* 2022; **2**: e0000826. Available at:
359 https://doi.org/10.1371/journal.pgph.0000826.
360 20. Datir R, Kemp S, El Bouzidi K, *et al.* In Vivo Emergence of a Novel Protease Inhibitor Resistance
361 Signature in HIV-1 Matrix. *MBio* 2020; **11**.
362 21. A. KS, J. CO, Anne D, *et al.* HIV-1 Evolutionary Dynamics under Nonsuppressive Antiretroviral
363 Therapy. *MBio* 2022; **13**: e00269-22. Available at: https://doi.org/10.1128/mbio.00269-22.
364 22. Siedner MJ, Moorhouse MA, Simmons B, *et al.* Reduced efficacy of HIV-1 integrase inhibitors in
365 patients with drug resistance mutations in reverse transcriptase. *Nat Commun* 2020; **11**: 5922.
366 23. Maggiolo F, Gulminetti R, Pagnucco L, *et al.* Lamivudine/dolutegravir dual therapy in HIV-
367 infected, virologically suppressed patients. *BMC Infect Dis* 2017; **17**: 215.

24. Joly V, Burdet C, Landman R, *et al.* Dolutegravir and lamivudine maintenance therapy in HIV-1 virologically suppressed patients: results of the ANRS 167 trial (LAMIDOL). *J Antimicrob Chemother* 2019; **74**: 739–45.

25. Taiwo BO, Marconi VC, Berzins B, *et al.* Dolutegravir Plus Lamivudine Maintains Human Immunodeficiency Virus-1 Suppression Through Week 48 in a Pilot Randomized Trial. *Clin Infect Dis an Off Publ Infect Dis Soc Am* 2018; **66**: 1794–7.

26. Cahn P, Madero JS, Arribas JR, *et al.* Dolutegravir plus lamivudine versus dolutegravir plus tenofovir disoproxil fumarate and emtricitabine in antiretroviral-naive adults with HIV-1 infection (GEMINI-1 and GEMINI-2): week 48 results from two multicentre, double-blind, randomised, non-inferio. *Lancet (London, England)* 2019; **393**: 143–55.

27. Hidalgo-Tenorio C, Cortés LL, Gutiérrez A, *et al.* DOLAMA study: Effectiveness, safety and pharmacoeconomic analysis of dual therapy with dolutegravir and lamivudine in virologically suppressed HIV-1 patients. *Medicine (Baltimore)* 2019; **98**: e16813.

28. Jary A, Marcelin A-G, Charpentier C, *et al.* M184V/I does not impact the efficacy of abacavir/lamivudine/dolutegravir use as switch therapy in virologically suppressed patients. *J Antimicrob Chemother* 2020; **75**: 1290–3. Available at: https://doi.org/10.1093/jac/dkaa019.

29. De Miguel R, Rial-Crestelo D, Dominguez-Dominguez L, *et al.* Dolutegravir plus lamivudine for maintenance of HIV viral suppression in adults with and without historical resistance to lamivudine: 48-week results of a non-randomized, pilot clinical trial (ART-PRO). *eBioMedicine* 2020; **55**. Available at: https://doi.org/10.1016/j.ebiom.2020.102779.

30. Mesplède T, Osman N, Wares M, *et al.* Addition of E138K to R263K in HIV integrase increases resistance to dolutegravir, but fails to restore activity of the HIV integrase enzyme and viral replication capacity. *J Antimicrob Chemother* 2014; **69**: 2733–40.

31. Rhee S-Y, Grant PM, Tzou PL, *et al.* A systematic review of the genetic mechanisms of dolutegravir resistance. *J Antimicrob Chemother* 2019; **74**: 3135–49.

32. Brenner BG, Thomas R, Blanco JL, *et al.* Development of a G118R mutation in HIV-1 integrase following a switch to dolutegravir monotherapy leading to cross-resistance to integrase inhibitors. *J Antimicrob Chemother* 2016; **71**: 1948–53.

33. Ahmed N, Flavell S, Ferns B, *et al.* Development of the R263K Mutation to Dolutegravir in an HIV-1 Subtype D Virus Harboring 3 Class-Drug Resistance. *Open forum Infect Dis* 2019; **6**: ofy329.

34. Chimbetete C, Chirimuta L, Pascoe M, Keiser O. A case report of untreatable HIV infection in Harare, Zimbabwe. *South Afr J HIV Med* 2019; **20**: 885.

35. Ndashimye E, Avino M, Kyeyune F, *et al.* Absence of HIV-1 Drug Resistance Mutations Supports the Use of Dolutegravir in Uganda. *AIDS Res Hum Retroviruses* 2018; **34**: 404–14.

402 36. Mahomed K, Wallis CL, Dunn L, Maharaj S, Maartens G, Meintjes G. Case report: Emergence of
403 dolutegravir resistance in a patient on second-line antiretroviral therapy. *South African J HIV Med*
404 *Vol 21, No 1 (2020)DO - 104102/sajhivmed.v21i11062* 2020. Available at:
405 https://sajhivmed.org.za/index.php/hivmed/article/view/1062.
406 37. Semengue ENJ, Armenia D, Inzaule S, *et al.* Baseline integrase drug resistance mutations and
407 conserved regions across HIV-1 clades in Cameroon: implications for transition to dolutegravir in
408 resource-limited settings. *J Antimicrob Chemother* 2021.
409 38. da Silva J, Pals S, Chang J, Hackett S, Godfrey C, Raizes E. Monitoring Emerging Human
410 Immunodeficiency Virus Drug Resistance in Sub-Saharan Africa in the Era of Dolutegravir. *J Infect Dis*
411 2022; **225**: 364–6. Available at: https://doi.org/10.1093/infdis/jiab382.
412 39. Villa G, Abdullahi A, Owusu D, *et al.* Determining virological suppression and resuppression by
413 point-of-care viral load testing in a HIV care setting in sub-Saharan Africa. *EClinicalMedicine* 2020;
414 **18**. Available at: https://doi.org/10.1016/j.eclinm.2019.12.001.
415 40. Gupta RK, Hill A, Sawyer AW, *et al.* Virological monitoring and resistance to first-line highly active
416 antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic
417 review and meta-analysis. *Lancet Infect Dis* 2009; **9**: 409–17.
418 41. Geretti AM, Abdullahi A, Mafotsing Fopoussi O, *et al.* An apparent paradox: resistance mutations
419 in HIV-1 DNA predict improved virological responses to antiretroviral therapy. *J Antimicrob*
420 *Chemother* 2019; **74**: 3011–5.
421 42. World Health Organization. *Guidelines on long-acting injectable cabotegravir for HIV prevention*.
422 2022. Available at: https://www.who.int/publications/i/item/9789240054097.
423