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RESEARCH ARTICLE

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Pharmaco-virological algorithm to target risk of drug resistance among a population of HIV-infected key populations in Togo

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Abstract

No data about antiretroviral (ARV) treatment coverage and virological response are available among key populations (female sex workers [FSW] and Men having Sex with Men [MSM]) in Togo. This study aimed to both describe Human Immunodeficiency Virus (HIV) immunovirological status and evaluate the pertinence of an original algorithm combining pharmacology (PK) and viral load (VL) to identify subjects at risk of ARV drug resistance. A cross-sectional multicentric study was conducted in 2017 in Togo. Our PK-virological algorithm (PK-VA) defines subjects at risk of resistance when exhibiting both detectable plasma drug concentrations and VL > 200 c/mL. Among the 123 FSW and 136 MSM included, 50% and 66% were receiving ARV, with 69% and 80% of them successfully-treated, respectively. Genotypes showed drug-resistance mutation in 58% and 63% of nonvirologically controlled (VL > 200 c/mL) ARV-treated FSW and MSM, respectively. PK-VA would have enabled to save 75% and 72% of genotypic tests, for FSW and MSM, respectively. We reported first data about HIV care cascade among key populations

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in Togo, highlighting they are tested for HIV but linkage to care remains a concern. Furthermore, 70%–80% of ARV-treated participants experienced virological success. In limited resources settings, where genotyping tests are beyond reach, PK-VA might be an easiest solution to sort out patients needing ARV adaptation due to resistance.

KEYWORDS
Africa, ARV resistance, FSW, HIV, MSM, Togo

1 | INTRODUCTION

In Togo, in 2015, a national survey study reported a high level of 2.5% of Human Immunodeficiency Virus (HIV) infection prevalence in general population.[1] Among key populations in Togo, studies conducted in 2017 reported HIV prevalence estimated at 22% among Men having Sex with Men (MSM) and 13% among Female Sex Workers (FSW).[2–4] With such high levels of HIV prevalence among these key populations with at high-risk of transmission, test and treat strategies are crucial for the control of the pandemic.[5] No data are currently available in Togo regarding the proportion of key populations receiving an antiretroviral (ARV) treatment.[6] Furthermore, there is no data regarding the proportion of key populations successfully ARV-treated based on HIV serosurveillance data. Additionally, genotypic resistance tests are even less available than viral load (VL) and their evaluation is needed.

Since the highest risk of drug-resistance mutation (DRM) emergence is in the case of viral replication under drug selection pressure, the highest probability to detect DRM will be in the group of People Living With HIV (PLHIV) with a detectable VL in whom plasma drug concentrations are detected. Thus, pharmacological measurements could allow to reduce the number of samples for which a genotypic resistance test would be informative to detect DRM.

The aim of this study was to describe the therapeutic status and the proportion of virological success among HIV-infected FSW and MSM in Togo and to evaluate the ability of an innovative Pharmaco-virological (PK-V) algorithm to determine PLHIV at risk of ARV drug resistance.

2 | MATERIALS AND METHODS

2.1 | Study design, sampling, and recruitment

This cross-sectional study was part of a national bio-behavioral study conducted in eight cities of Togo between August and September 2017 among FSW and MSM key populations.[2–4] Three recruitment sites were in Lomé, the capital city, the others being located in Aného, Kpalimé, Atakpamé, Dapaong, Kara, Sokodé, and Tsevié. Participants were recruited using a respondent-driven sampling (RDS) method. Before the study, a mapping of locations specific to each group (bars, brothels for FSW; associations for MSM) was done in collaboration with community leaders from each key population group. The first "seeds" for each group were identified based on specific characteristics to ensure the representativeness of the sample. Three coupons with unique identification code were given to the first "seeds" in each group (FSW and MSM) to recruit other seeds in their network until the desired sample size for each group was reached. Participants could be included in the study if they were 18 years old or older; were living in Togo for a minimum of 3 months before the study; and had a recruitment coupon. Additional inclusion criteria for MSM were to have had anal and/or oral sex with a man in the previous 12 months and for FSW to have had sex in exchange for money as compensation in the previous 12 months. The exclusion criteria included the inability to respond to the questionnaire and refuse to sign consent to participate in the survey.

2.2 | Study procedures

After obtaining written informed consent, whole blood was collected by trained research staff. A rapid test (SD Bioline HIV/Syphilis Duo; Abbott) was used for HIV screening, which was confirmed with another HIV rapid test (First Response® HIV 1-2-O Card Test). Blood samples were kept in a biobank for other biological tests.

2.3 | HIV VL quantification

Plasma VL was determined using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Molecular Systems) with a limit of quantification (LOQ) of 20 c/mL. In this study, virological success was defined as having a VL below 200 c/mL.

2.4 | Determination of plasma drug concentrations

Plasma drug concentrations were determined by Ultra Performance Liquid Chromatography combined with tandem mass spectrometry (UPLC-MS/MS) (Waters Corporation Milford).[7] Briefly, all ARV were measured simultaneously in 50 µL of human plasma using a sensitive and

rapid UPLC-MS/MS method after a protein precipitation, with at least of 75% recovery and within- and between-day accuracies of at least 85%.

2.5 | Genotyping resistance test

Genotypic resistance tests of HIV protease and reverse transcriptase (RT) regions were performed according to the complete sequencing procedures and primers sequences described at www.hivfrenchresistance.org. Sequences were interpreted using the Agence Nationale de Recherches sur le SIDA|Maladies Infectieuse Emergentes (ANRS|MIE) resistance algorithm (www.hivfrenchresistance.org, October 2021, version 32). All consensus sequences generated in this study have been submitted to NCBI GenBank (accession number OP490377 to OP490551).

2.6 | Statistical analyses

For the sample size calculation, we estimated that the proportion of key population on ARV treatment will be 50% with the precision of the estimate of 7% and the risk of first species α of 0.05. The minimum number of key populations for this study should be at least 216 key populations. Descriptive analyses were performed and presented with frequency and proportions for categorical variables and with median and interquartile range (IQR) for continuous variables.

3 | RESULTS

3.1 | Patients' characteristics

A total of 259 PLHIV were included (n = 123 FSW, n = 136 MSM). Among FSW, 64% were recruited in Lomé, while it was 95% for MSM participants. The median age was 32 years (IQR = 28–38).

3.2 | Description of pharmacological and virological status

Plasma ARV concentrations measurements showed that 61 out of the 123 FSW (50%) and 90 out of the 136 MSM (66%) were receiving ARV treatment (Figure 1). The most frequent ARV treatment received was tenofovir disoproxil fumarate, lamivudine (3TC) and efavirenz (EFV) found in 94% (n = 142/151) of ARV-treated subjects. The proportion of ARV-treated subjects with VL < 200 c/mL was 69% (n = 42/61) for FSW and 80% (n = 72/90) for MSM (Figure 2). Overall 34% (n = 42/123) of the FSW and 53% (n = 72/136) of the MSM had virological suppression and were at low risk of HIV transmission (Table 1).

Among the FSW recruited in the three sites of Lomé and receiving ARV, 83.8% (n = 31/37) had a VL < 200 c/mL. This percentage of successfully ARV-treated FSW was significantly lower in the other recruitment sites with only 45.8% of them with a VL suppression (n = 11/24) (p = 0.02). Overall, HIV VL distribution was as follows: 42.1% (n = 109) had VL below 100 c/mL, 12.0% (n = 31) had VL between 100 and 1000 c/mL, 9.7% (n = 25) between 1000 and 10000 c/mL, 19.7% (n = 51) between 10000 and 100000 c/mL, and 16.6% (n = 43) above 100000 c/mL (Figure 3).

3.3 | PK-V algorithm application

PK-V algorithm sorted out 19 FSW and 18 MSM at risk of drug resistance mutations emergence defined as VL > 200 c/mL while ARV drugs were detectable in plasma (Figure 1). Median VL were 1450 c/mL (IQR = 430–17080) and 965 c/mL (IQR = 385–6775) among the FSW and MSM groups, respectively.

Among the successfully sequenced samples (n = 12 and n = 8 for FSW and MSM, respectively), CRF02_AG was the most prevalent lineage (n = 15). DRM were detected in six (50%) samples among the FSW group and in five (63%) among the MSM group. Nucleosides RT

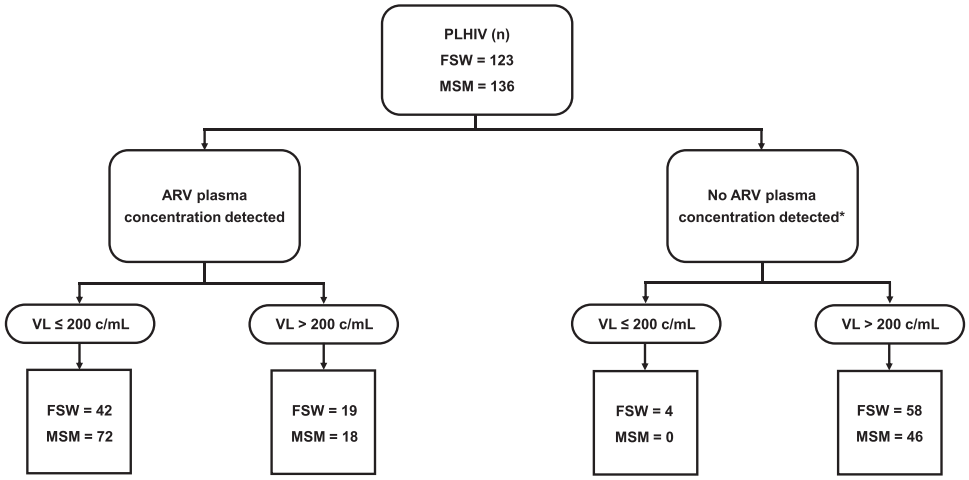

FIGURE 1 Flow-chart of the study participants. *Plasma drug concentration < LOQ. ARV, antiretroviral; FSW, Female Sex Workers; LOQ, limit of quantification; MSM, Men who have Sex with Men; PLHIV, Persons Living with HIV; VL, viral load.

inhibitors (NRTI) and non NRTI (NNRTI) DRM were present in 9 and 10 of the 11 samples, respectively. The most prevalent NRTI DRMs were M184I/V ($n = 6$) and K65R ($n = 2$); the most prevalent NNRTI DRM was K103N ($n = 7$) (Table 2). Overall, three PLHIV exhibited a virus resistant to one ARV of the regimen and seven exhibited a virus resistant to two or three ARV of the regimen.

3.4 | PK-V algorithm evaluation

A concordance between virological and pharmacological results was observed in 119/123 cases (96.7%) in the FSW group and in 100% of the cases in the MSM group. For the four discordant cases in the FSW group, all showed no ARV treatment and VL below 200 c/mL.

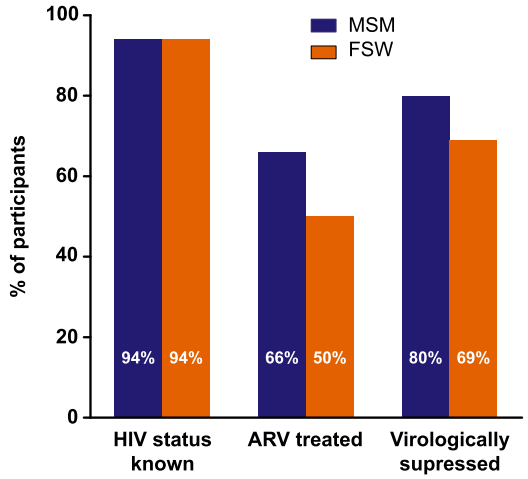

FIGURE 2 Treatment cascade for Men Having Sex With Men (MSM) and Female Sex Workers (FSW). ARV, antiretroviral; HIV, Human Immunodeficiency Virus.

Further investigations showed a confirmation of a positive HIV fourth-generation ELISA test associated with a complete HIV type 1 Western-Blot for all four samples, and one had a detectable PCR signal. An HIV type 2 infection was excluded by a differentiation serological test in all four cases.

To determine how many PLHIV at risk of harboring resistant viruses would have been missed by this PK-V algorithm, a genotypic resistance test was performed among all samples with VL > 200 c/mL without ARV drugs detected ($n = 62$ FSW, $n = 46$ MSM). In the FSW group, 25.0% ($n = 11$) of the 44 successfully amplified samples showed DRM while in the MSM group only 8.8% ($n = 3$) of the 34 successfully amplified samples exhibited DRM. All but one DRM detected in the samples issued from FSW were hallmark of NNRTI resistance (data not shown). Regarding samples issued from MSM, the three DRM detected were K103N alone or associated to another NNRTI DRM.

Overall, drug resistance testing strategy based only on VL would have led to the realization of 77 and 64 genotypic resistance tests for the FSW and MSM groups, respectively. Adding ARV plasma concentration measurements enabled to narrow down to 19 and 18 for FSW and MSM groups, respectively, leading to an overall decrease of 73.8% of the number of genotypic resistance tests. Thus, eligibility to genotypic resistance test defined only on VL would have led to 25.5% of genotypic resistance tests revealing DRM ($n = 25/98$), while the additional data of drug plasma concentrations enabled to enhance resistance testing efficacy to 55.0% of genotypes harboring DRM ($n = 11/20$).

4 | DISCUSSION

In this study, among key populations in Togo, we showed that half of the HIV + FSW and two-third of the HIV + MSM were receiving an ARV treatment and only 34% of the FSW and 53% of the MSM were at low risk of HIV transmission.

TABLE 1 Pharmaco-virological HIV infection status description of the study participants.

	FSW ($n = 123$)	MSM ($n = 136$)
Percentage of participants recruited in Lomé (capital city)	64%	95%
Detectable ARV, *n* (%)	61 (50.0%)	90 (66.2%)
VL ≤ 200 c/mL among ARV-treated subjects, *n/N* (%)	42/61 (69.0%)	72/90 (80.0%)
Virological suppression with low risk of transmission, overall, *n* (%)	42 (34.1%)	72 (53.0%)
Successfully genotyped samples among treated subjects with VL > 200 c/mL, *n/N*	12/19	8/18
DRM among PK-V algorithm selected subjects (ARV-treated subjects with VL > 200 c/mL), *n/N* (%)	6/12 (50.0%)	5/8 (62.5%)
Successfully genotyped samples among nontreated subjects with VL > 200 c/mL, *n/N*	44/62	34/46
DRM among non-treated patients with VL > 200 c/mL, *n/N* (%)	11/44 (25.0%)	3/34 (8.8%)

Abbreviations: ARV, antiretroviral, DRM, drug resistance mutation; FSW, Female Sex Workers; MSM, Men who have Sex with Men; PK-V, Pharmaco-virological; VL, viral load.

Data on the treatment cascade among key populations in sub-Saharan Africa are mitigated. In regard to FSW, in Benin, a study found that of the 60.6% FSW who knew their status, 90.5% were on ARV and 81.8% of them had achieved viral suppression.[8] Similar trends were reported in a cross-sectional national study among FSW in South Africa with 92% known HIV-positive, 87% on ARV treatment and 74% virologically-suppressed.[9] In cross border areas of East Africa as well as in Mozambique, studies have demonstrated similar data and showed that FSW did not reach the 3 × 95 UNAIDS' goals.[10,11] In our study, 94% of the FSW participants indicated having tested for HIV before the survey. However, since the HIV status could change over the period of the time, the proportion who were aware of their HIV status is not certain. At the time of the present survey, only two-third of the FSW were treated. When treatment was initiated, a proportion of virological success similar to the other studies was achieved (80%). A meta-analysis of the HIV treatment cascade among MSM in Africa found that of the pooled proportions of 67.3% knowing their status, the pooled proportion of MSM on treatment was 60.1% and the pooled levels of viral suppression among MSM on ARV treatment were 75.6%.[12] These results are similar to the proportions depicted in our study regarding the MSM participants in terms of treatment access (66%) and efficacy (69% of virological success) while in our work more MSM participants (94%) were aware of their HIV status.

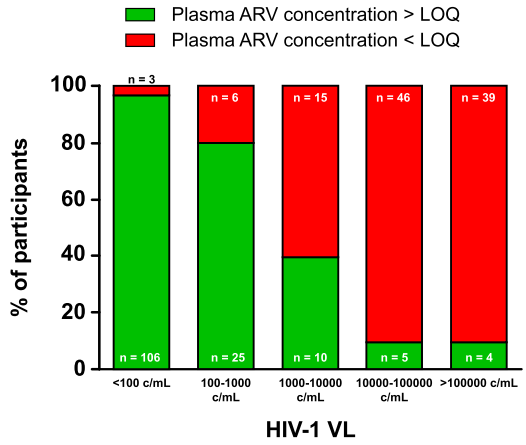

FIGURE 3 Viral load distribution based on antiretroviral (ARV) plasma concentrations among female sex workers and men who have sex with men in Togo. ARV, antiretroviral; LOQ, limit of quantification.

Overall, data from our findings and from studies in the African region indicates that there is an important gap in the achievement of the HIV treatment cascade targets among FSW and MSM. The first challenge is in key populations' linkage to care, with barriers such as stigma and discrimination facilitating low access and avoidance of health care services.[13,14] This raises the question of the relevance of a differentiated model of care specific to key populations, taking into account their unique needs. The second challenge, the third 95 of UNAIDS' goals, is directly linked to ARV treatment adherence to reduce HIV transmission risk. Additional efforts are needed regarding this aspect, notably by supporting the overwhelmed health care system in addition to appropriate training. In the context of sub-Saharan Africa, peer educators and community health workers should be considered in their roles as the bridge between the health care system and the community, particularly in adherence counseling.[15]

In the present study, the proportion of FSW successfully treated with ARV was significantly higher in Lomé than in the others

TABLE 2 Description of the eleven participants selected by the PK-V algorithm (ARV-treated and viral load > 200 c/mL) harboring viruses with drug resistance mutations.

Patient ID	Key population	HIV-1 subtype	NRTI DRM	NNRTI DRM
1	FSW	CRF06_cpx	T69N	None
2	FSW	CRF02_AG	None	V90I-K103N
3	FSW	G	M184V	K103N-P225H
4	FSW	CRF02_AG	M184V	V106A-V179I-G190A-M230I
5	FSW	URF	M41L-L74I-M184V-L210W-T215Y	A98G-K103N-E138Q
6	FSW	URF	K65R	L100I-K103N-E138G
7	MSM	CRF02_AG	M184V	K103N-Y181C
8	MSM	CRF02_AG	M184I	M230I
9	MSM	CRF02_AG	K65R	V90I-K103N-Y181C-G190A-H221Y
10	MSM	CRF02_AG	None	K101E
11	MSM	CRF02_AG	M184V	K103N-Y181C

Abbreviations: DRM, drug resistance mutation; FSW, Female Sex Workers; MSM, Men who have Sex with Men; NNRTI, nonnucleosides reverse transcriptase inhibitors; NRTI, nucleosides reverse transcriptase inhibitors. URF, Unique Recombinant Form.

recruitment sites (84% vs. 46%, respectively, $p = 0.02$). This difference could denote an inequity in terms of access to HIV care and treatment for FSW in the other towns of the country compared to the capital city. Further studies could explore this difference in access to HIV care for FSW in Togo.

A very high level of concordance was observed between virological and pharmacology (PK) results, meaning that detectable VLs were regularly associated with low or absence of plasma drug level.

With the application of the PK-V algorithm genotypic resistance tests were performed only among patients with detectable ARV drugs and VL > 200 c/ml. Targeting the population at high-risk of resistance selection enabled to reduce the number of genotypic resistance tests down to 19 and 18 for FSW and MSM groups, respectively, leading to an overall decrease of 73.8%. Obviously, using this PK-V algorithm prevents to assess transmitted drug resistance or resistance among ARV-treated patients who have discontinued ARV.

The prevalence of ARV drug resistance among patients with no detectable ARV in plasma was less frequent in the MSM than in the FSW population (8.8% vs. 25.0%, respectively). One explanation of this difference between these two key populations can result from the fact that FSW could have acquired resistance over a strategy of prevention of mother-to-child transmission. Indeed, resistance mutations among FSW with undetectable plasma ARV drugs were almost exclusively NNRTI resistance-associated mutations. Another explanation could be that FSW are a very mobile population more likely to discontinue treatment, compared to MSM, who most often are better organized as groups, less mobile, hence might be less likely to discontinue treatment or be lost-to-follow-up Innovative approaches to HIV care and treatment for FSW should take into account the high rate of mobility of FSW and adapt services to ensure their linkage and retention in care.

In limited-resource countries, HIV biological monitoring is a crucial issue while treatment access is increasing. In these settings, VL availability is still very limited and has to be generalized while genotyping resistance tests are currently not feasible. To get around this hindrance, PK may be a decision tool to sort out patients harboring resistant virus in need to ARV switch. One other way to bypass the implementation issues for genotypic resistance tests is to use dried blood spots (DBS) samples. Several studies showed a very good correlation regarding the detection of DRM between plasma and DBS samples, despite a decrease in sensitivity compared to plasma samples.[16] DBS have also been shown to be useful and reliable to measure ARV drug plasma concentrations[17] which are cheaper than genotypic tests.

Of all FSW and MSM, 45.9% had a VL > 1000 c/mL, the current WHO threshold for virological failure. Thus, at least half of HIV-positive FSW and MSM would be considered at high risk of HIV transmission. Access to ARV treatment has changed over the past few years with the seek and treat approach to treatment, which consists in treating HIV patients as soon as diagnosed, regardless of CD4 cells count. For key populations, this timing is all the more critical as there are more opportunities to be lost-to-follow-up from HIV diagnosis to linkage to care. A community-based approach could be applied with the use of peer-educators and community health workers that would guarantee the link between an HIV-positive diagnosis and the time of ARV treatment initiation especially initiate treatment immediately after a positive test.

This study is among the first to explore the 2nd and 3rd goals of 95 UNAIDS in key populations in Togo demonstrating a gap between those objectives and real-life data with half of HIV-positive FSW and two-thirds of MSM were on ARV treatment, 69% and 80% of FSW and MSM, respectively, had a suppressed VL (<200 c/mL). These data strengthen the fact that VL monitoring should be generalized.

It is also one of the first studies in this country to have explored beyond HIV prevalence by suggesting a PK-V algorithm. Cost-effectiveness studies should be also carried out to determine if it could be adapted to low- and middle-income countries.

This study presents several limitations. First, it focuses on key populations of MSM and FSW and cannot be generalized to all the PLHIV of the country. Second, the recruitment methods, RDS, while necessary to reach out the participants in the current setting, may not guarantee a fully fair representation of these populations. Finally, this study was conducted in 2017 and another study nowadays is needed to assess the evolution of HIV care of key populations in Togo.

5 | CONCLUSIONS

In conclusion, this study highlights that although key populations in Togo are tested for HIV, linkage to HIV care and ARV treatment remains a concern. This should be addressed by innovative approach to link and maintain these populations into care. HIV serological screening enables to identify HIV-positive individuals, but it needs to be completed with PLHIV follow-up, taking into account ARV treatment and VL measurements, to identify individuals most at risk of transmission and to break these transmission chains.

AUTHORS CONTRIBUTION

Claver A. Dagnra, Charlotte Charpentier, and Didier K. Ekouevi conceptualized the study. Alexandra M. Bitty-Anderson, Claver A Dagnra, Fifonsi A. Gbeasor-Komlanvi conducted the investigation and sample collection. Valentine M. Ferré, Gilles Peytavin and Minh P. Lê conducted biological analyses. Valentine M. Ferré, Alexandra M. Bitty-Anderson, Charlotte Charpentier, and Didier K. Ekouevi conducted formal analyses and wrote the manuscript. Gilles Peytavin, Claver A. Dagnra, Romain Coppée, Fifonsi A. Gbeasor-Komlanvi, and Diane Descamps reviewed and edited the manuscript. Diane Descamps, Charlotte Charpentier, and Didier K. Ekouevi supervised the study.

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CONFLICT OF INTEREST STATEMENT

V. M. F. received personal fees from Astra Zeneca and congress accommodation from Gilead outside the submitted work. G. P. has received personal fees from MSD, Janssen-Cilag, Gilead, ViiV Healthcare, Theratechnologies, outside the submitted work. D. D. has received personal fees from Gilead-Sciences, ViiV Healthcare, MSD, Janssen-Cilag, and research grants from Gilead-Sciences and ViiV Healthcare, outside the submitted work. The other authors declare having no conflict of interest with the current work. C. C. has received personal fees from MSD, Janssen-Cilag, Gilead, ViiV Healthcare, Theratechnologies, outside the submitted work. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available upon request to the corresponding author.

ETHICS STATEMENT

This study was approved by the Bioethics committee for health research of the Ministry of Health of Togo (No. 19/2017/CBRS) on June, 22, 2017. Participants were informed of the use the blood sample for HIV screening and for a biobank for research purposed, and provided written informed consent. All data was computerized and no personal identifiers and information that could reveal any personal identification were entered into the database.

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