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HIV Genetic Diversity, Virological Failure, and Drug Resistance in Libreville, Capital of Gabon, before a Total Dolutegravir-Based Regimen Transition

Berthold Bivigou-Mboumba[1*], Berthe Amélie Iroungou[1], Pamela Moussavou-Boundzanga[1], Laurette Guignali Mangouka[2], Falone Larissa Akombi[1], Aurore Bouassa-Bouassa[1], Sandrine Francois-Souquière[3], Jean Raymond Nzenze[2]

[1]Research Mixed Unit between the International Center for Medical Research of Franceville and the Military Health Service (UMR CIRMF-SSM), Libreville, Gabon
[2]Department of Internal Medicine, Omar Bongo Ondimba Army Training Hospital (HIA-OBO), Libreville, Gabon
[3]Department of Hepato Gastroenterology, Centre Hospitalier Universitaire de Limoges, Libreville, Gabon
Email: *bivigou.berthold@gmail.com

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Abstract

Context: The Human Immunodeficiency Virus (HIV) continues to be the main public health challenge in Gabon. The latest studies highlight a high rate of virological failure and HIV drug resistance in semi-rural Gabon. In Libreville, virological failure data is sparse, data on HIV drug resistance for the former first line and new first-line regimen is lacking. **Methods:** Between January 28th, 2019, and January 31st, 2020, we received patient living with HIV (PLWHA) for CD4 counts, HIV-1 viral load, and/or genotyping of HIV-1 mutation drug resistance. We used the BD FACSPresto for CD4 count, the Biocentric Generic HIV viral load test for HIV-1 quantification, and the HIV-1 drug resistance mutation genotyping (ARNS protocol). **Results:** A total of 1129 HIV-1 patients have been enrolled for this study. The median age was 46 years old and the median of CD4 was 386 cells per cubic millimeter. The virological suppression success was observed at 62.7% of patients on the former first line regimen and 70.6% of the patient on DBR. We successfully amplified and analyzed 76 sequences and noticed the presence of the nineteen different subtypes with the predominance of the subtypes CRF02-AG (37.95%), followed by subtype A (22.3%). For HIV drug resistance analyses, 108 (65.1%) had resistance mutation to nucleoside reverse transcriptase inhibitors (NRTIs); of these, 91 (84%) present M184V/I. When looking for NNRTI mutations, 119 (71.7%) sequences had at least one mutation. Of these, 82 had K103N (68.9%), representing the main NNRTI mutations. The

pattern showing the high level of resistance (HLR) in all molecules of NRTIs and NNRTIs, except for the TDF (intermediate resistance) was M41L-E44DL74I-M184-L210W-T215Y-K101P-K103N-V106I. **Conclusion:** This report paints a picture of a relatively female-dominated HIV-infected Gabonese population with a low level of immunity. The level of drug resistance with the former first-line regimen suggests the need to monitor the drug Dolutegravir resistance.

Keywords

HIV Infection, HIV Drug Resistance, HIV Genetic Diversity, Dolutegravir, Gabon

1. Introduction

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), the global number of people living with HIV/AIDS (PLWHA) was around 37.6 million in 2020 (UNAIDS 2021). Of this number, 67.3% (25.3 million) were from Africa, suggesting a continual transmission despite efforts on increasing access to treatment, diagnostic, and prevention. In 2014, WHO and UNAIDS set an ambitious program to end the AIDS epidemic as a public health problem by 2030. As a strategy to attend this target: the wild access to antiretroviral therapy (ART) and the main 95-95-95 spot for 2025, mean 90% of people diagnosed, 90% of diagnosed have to be under ART and 90% of people under ART have to suppress their HIV viral load (HIV VL < 1000 copies/mL (UNAIDS 2021)). Based on WHO recommendations, the "test, and treat" method has been adopted by many African countries and particularly by Gabon in 2019. This decision targets the treatment coverage of 99% of PLHIV. To accelerate the achievement of the 90-90-90 targets, the dolutegravir-based regimen (DBR) in the first line has been recommended by UNAIDS. In 2019, Gabon officially introduces DBR as a first-line treatment, according to the national program against HIV and sexual infectious diseases: a therapeutic DBR transition is underway in Gabon.

Previous investigations have found high genetic diversity (subtypes A, G, F, H and D) with a dominance of recombinant forms circulating CRF02-AG [1] [2] [3]. A more recent study in south-eastern Gabon in Haut-Ogooué province (250,799 inhabitants, about 14% of the Gabonese population) found a high rate (42.9%) of virological failure linked to 67.2% antiretroviral resistance mutation rates in patients on the old first-line regimen [4].

Moreover, currently, we do not have a profile of HIV drug resistance prior to switching to DBR. This missing point could have a negative impact on the success of DBR, due to exposure to the dolutergravir monotherapy regimen.

In Libreville, Gabon's capital where 80% of people living with HIV are located, virological failure data is sparse, data on HIV drug resistance for the former first line and new first-line regimen is lacking. Since 2015, after the vulgari-

zation by public laboratories and in 2019 after the creation of the "*Unite Mixte de Recherche entre le Centre International de Recherches Medicales de Franceville et le Service de Sante Militaire*" (UMR CIRMF-SSM), access to viral load tests and genotyping of resistance mutations has become easier. At this level, it is important to verify and describe the virological success of the two first lines currently used and to describe the currently circulating HIV strain.

2. Methods

This study investigates the immunological and HIV-1 genetic diversity of patients living with HIV (PLHIV) and previously diagnosis in all HIV care centers of Gabon. According to the national and international recommendations, PLHIVs under ART were submitted to CD4 count, HIV-1 viral load, and HIV-1 drug resistance mutation assay.

2.1. Study Site

The mixed unit between the international center for medical research (CIRMF) and the healthy military service (SSM) from Gabon was established in 2018. Laboratories activity started on January 28th, 2019. This unit (UMR CIRM-SSM) is located in OMAR BONGO ONDIMBA Military hospital in Libreville, kilometric point 9 (PK 9), working on HIV and associated infectious diseases.

2.2. Recruitment

Patients were directly enrolling in the UMR CIRMF-SSM when they come for HIV viral load or CD4 cells count exams. Children were excluding for this study. Patient came with their laboratory test voucher form from HIV/AIDS care centers of the capital and the inners regions of Gabon.

2.3. Study Design

We conducted a cross-sectional and analytical study between January 28th, 2019, and January 31st, 2020. We received PLHIV for CD4 counts, HIV-1 viral load, and/or genotyping of HIV-1 mutation drug resistance (MDR). All PLHIV came from HIV-treatment centers approved by the national program against HIV and provides antiretroviral therapy (ART).

2.4. Absolute CD4 Counts, % of Functional CD4, and Hemoglobin Concentration

We used the BD FACSPresto (Becton-Dickinson, Bioscience, USA) point-of-care (POC) system for CD4 count, CD4% as recommended by the manufacturer. Briefly, 25 µL of total blood samples have been transferred to the BD FACSPresto labeled cartridges. The cartridge cap was closed and placed on the POC BD FACSPresto working station for 18 minutes' incubation. After this incubation, the test strip was removed and the cartridge was inserted into the POC BD FACSPresto analyzer to read the result for about 4 minutes. The absolute CD4

counts, %CD4 results are displayed on the screen and printed automatically.

2.5. Plasmatic HIV-1 Viral Load

The plasmatic HIV-1 viral load was determined with the Generic HIV viral load test (Biocentric, 276 Chemin de Roumpinas-83150 Bandol, France). Amplification and data acquisition was carried out using the Fluorocycler Detection System (Hain Life science GmbH Hardwiesenstr.1, 72147 Nehren Deutschland) with a detection cut-off value of 50 HIV-1 RNA copies/mL. After RNA extraction by the NA Extraction kit (DiaSorin, LTF Labortechnik GmbH & Co. Wasserburg, Germany), a volume of 10 µL of RNA extract from 1 mL of plasma was added to 10 µL the master mix of the Generic HIV viral load. The cycling conditions consisted of 50˚C for 10 min and 95˚C for 5 min, followed by 50 cycles of 95˚C for 15 s and 60˚C for 1 min.

The patients with viral loads higher than 3 log copies/mL were considered to present virological failure, as recommended by WHO. However, patients with viral loads below 3 log copies/mL will be considered virologically successful, as recommended by WHO.

2.6. HIV-1 Molecular and Phylogenetic Analysis

The HIV-1 subtype and resistance-associated mutation (RAM) of the mains ARVs were defined by amplification of the reverse transcriptase (RT) and protease (P) region according to the AC-11 Resistance Group recommendation of the French National Agency for AIDS Research (ARNS). Using RNA extract from the NA arrow extraction kit, we performed a reverse-transcription polymerase chain reaction (RT-PCR) targeting fragments of 463 bp of protease gene and 887 bp of reverse transcriptase gene. An RT-PCR using the one-step superscript IV Invitrogen kit (Thermo Fisher Scientific, 168 Third Avenue Waltham, MA USA) and nested PCR using *Taq* recombinant kit (Thermo Fisher Scientific, 168 Third Avenue Waltham, MA USA) was performed to amplify these genes. The expected product size was verified with 1.3% agarose gel electrophoresis, stained with Gel Red, and revealed under ultraviolet transillumination. PCR amplification products were sent to Macrogen for the sequencing reaction. The AB1 files were analyzed and sequences aligned using DNASTAR Software for life science (version 17.0.1.83). The obtained local alignment of partial *pol* sequences was submitted to the online Stanford University HIV database program for analysis and interpretation of HIV-1 drug resistance mutations (https://hivdb.stanford.edu/hivdb/by-sequences/).

2.7. GeneBank Accession Numbers

We submitted our 76 sequences to the NIH genetic sequence database, through the submission portal using the Bankit tool with wizards to guide the submission process. The accession numbers attributed for our sequences granges from OK636237 to OK636402.

2.8. Statistical Analysis

Statistical analysis was performed using R (R Studio Version 1.2.5042) available online as an R project for statistical analysis (https://www.r-project.org/). Percentages (with 95% confidence intervals) were used to describe baseline characteristics of the studied population.

3. Results

3.1. Sociodemographic Characteristics of the Studied Population

A total of 1129 HIV-1 infected individuals have been enrolled for this study from February 2019 to February 2020 with 1048 (92.8%) of Gabonese and 7.2% of non-Gabonese. All of these patients come from different HIV-treatment centers, with 989 (87.6%) from public structures and 140 (12.4%) private structures. Of the 1129 recruited patients, 733 (64.9%) were from Libreville, Gabon's capital and 396 (35.1%) came from the inner country. The dominance of gender was female with 671 (59.4%) versus male 458 (49.6%). The median age of all patients was 46 years old with a range age from 16 to 61-year-old (IQR: 38 - 54 years old). The median of CD4 was 386 cells per cubic millimeter with a minimum of 1 and a maximum of 1681. The majority of patients, 68.7% (38.3% for less than 5 years and 30.4% between 5 and less than 10 years) was under treatment for less than 10 years and 76.7% of patients were under the older first-line recommended by WHO and approved by the national program against HIV:

- Former first line: TDF-FTC/3TC-EFV
- Former second line: TDF/ABD-3TC-ATV/r/LPV/r
- Former third line: ATV/r/LPV/r-Darunavir
- New first line: TDF-FTC/3TC-DTG

A total of 698 patients (62.0%) were on virological suppression success. The rate of virological success was higher in patients over 35 years of age (96/187; 62.8%) than in patient less than 35 years of age (91/187; 51.3%). P patients on the former first line, 62.7% had virological suppression success. A great majority of patients (70.6%) on the new first line *i.e.*, DRB (TDF-3TC-DTG) had virological suppression success (**Table 1**).

3.2. HIV Genetic Diversity

We successfully amplified and analyzed 76 sequences of the HIV M group from the RT region. We notice the presence of the nineteen different subtypes. The predominant subtypes in this population were CRF02-AG 63/166 (37.95%), followed by subtype A, 37/166 (22.3%), subtype A+G/I (19: 10.84), and G (13: 7.83%). Three sequences (1.8%) corresponded to unknown subtypes according to the Stanford algorithm (**Figure 1**).

When submitting sequences for phylogenetic tree building in PhyML (**Figure 2**) the 3 unknown sequences (2070_Gab, G13_Gab, and G61_Gab) from the Standford algorithm cluster with other sequences described in this study. The 2070_Gab cluster with the reference sequence MN337382 (subtype M/O), the

G13_Gab is in the cluster with the 1669_Gab in the HIV subtype A and the G61_Gab is in the same cluster with 1445_Gab in the cluster of CRF02_AG.

Table 1. Treatment and virological status of the studied population, according to WHO recommendations.

		Virological success (HIV VL < 3 log copies/mL)		Virological Failure (HIV VL > 3 log copies/mL)		P-value
		n	%	n	%	
Gender	Total	698	**62.0**	431	38.0	<0.001
	Male	234	63.4	135	36.6	
	Female	464	61.1	296	38.9	
Age	[16 - 25[	13	46.4	15	53.6	0.004
	[25 - 35[	83	52.2	76	47.8	
	[35 - 45[	204	59.5	139	40.5	
	[35 - 45[	300	66.5	151	33.5	
	60 more	97	65.5	51	34.5	
Treatment Duration (TD) by years	TD <5 years	271	62.7	161	37.3	0.19
	TD ≥ 5 years	465	66.5	234	33.5	
	[5 - 10[	215	66.7	128	37.3	
	[10 - 15[	146	62.7	89	37.9	
	[15 - 20[	97	62.1	44	31.2	
	TD ≥ 20 years	7	43.8	9	56.3	
Treatment regimen	Naive	0	0	3	**100**	0.11
	Old first line	543	**62.7**	323	37.3	
	DTG based	12	**70.6**	5	29.4	
	2nd line	142	58.7	100	41.3	
	3rd line	1	**100**	0	0	

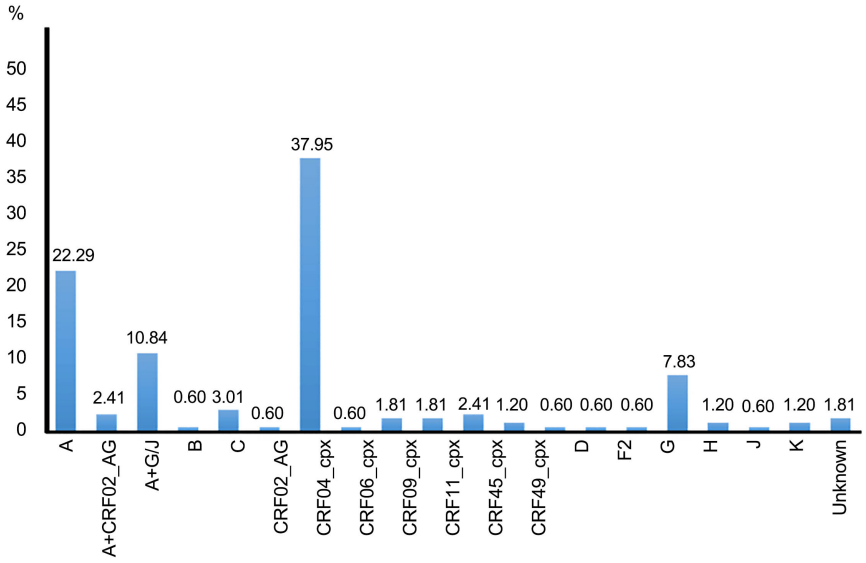

Figure 1. Subtypes prevalence from the 76 sequences submitted. The dominant subtypes are the CRF02_AG and the A.

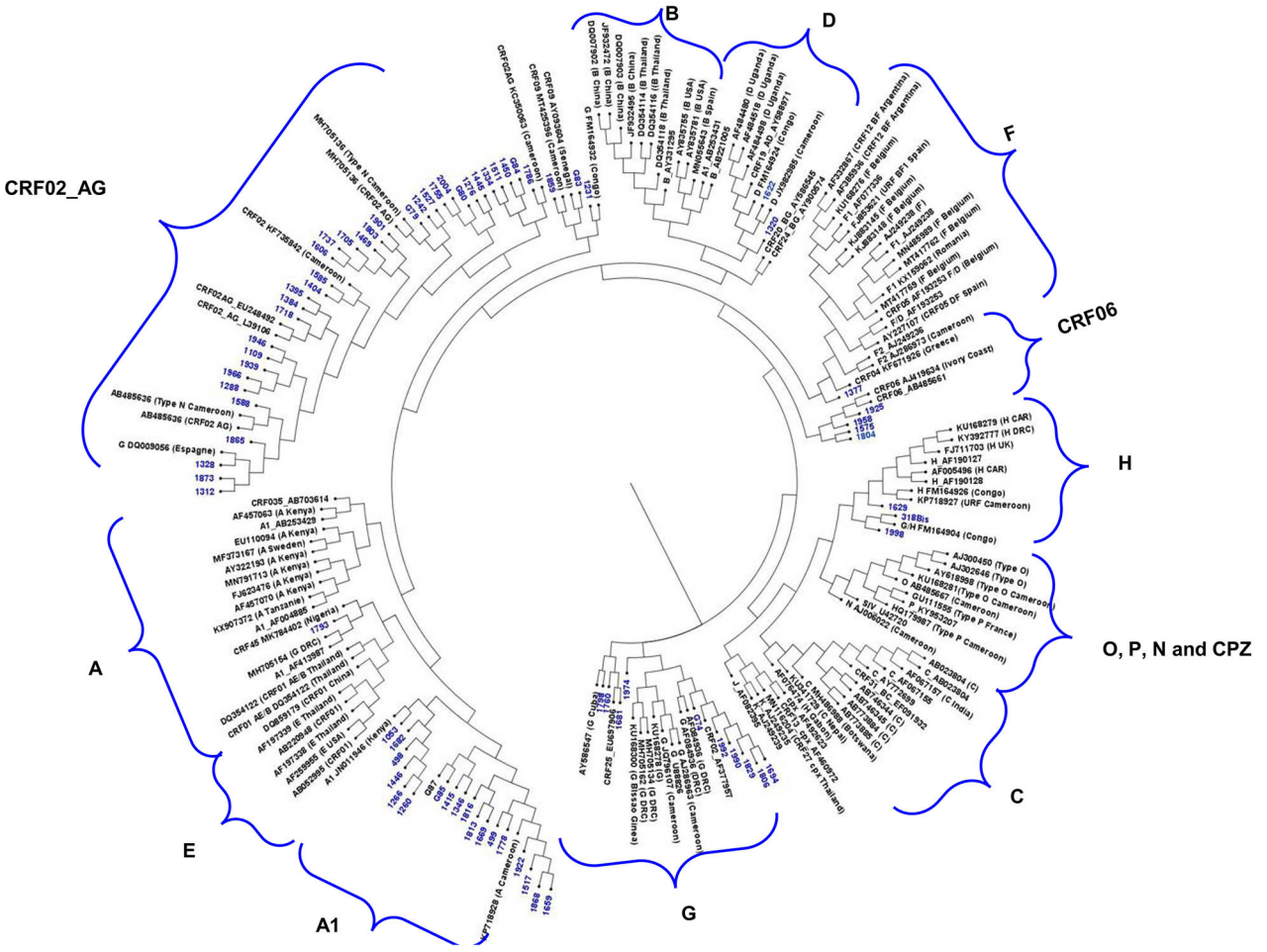

Figure 2. Phylogenetic tree of 166 HIV-1 isolates from patients (in blue color) in Libreville and the inner country (Gabon). Sequences were aligned using Clustal W and analysis was performed using the neighbor-joining method in the Molecular Evolutionary Genetics Analysis Version X (MEGA X).

The tree subtype was also submitted to the online REGA HIV-1 Subtyping Tool-Version 3.0. and we obtained three independent trees (Supplementary data).

In the phylogenetic tree, the 318_Gab sequence appears to be near the type O, N, or P. Looking for the sequence from the Standford algorithm (REGA HIV-1 subtyping analysis), this sequence appears to cluster with a non-subtype B (GenBank accession number AF005496).

3.3. Reverse Transcriptase Resistance Mutations (RTRM)

From the 76 sequences obtained for HIV drug resistance analyses, 108 (65.1%) had resistance mutation to nucleoside reverse transcriptase inhibitors (NRTIs). Of these, 91 (84%) present M184V/I conferring a high level of resistance (Standford algorithm) to 3TC and FTC, a low level of resistance to ABC; 43 (39.8%) present K219E/Q, an accessory mutation resulting to a combination of thymidine analog mutation (TAMs) occur in combination with other TAMs; 39 present K70R (36.1%), and 38 present T215Y/F (35.2%) representing the four

major NRTI mutation. When looking for NNRTI mutations, 119 (71.7%) sequences had at least one mutation. Of these, 82 had K103N (68.9%), 32 had Y179L (26.9%), and 30 had P225H (25.2%), representing the three main NNRTI mutations. The pattern showing the high level of resistance (HLR) in all molecules of NRTIs and NNRTIs, except for the TDF (intermediate resistance) was M41L-E44D-L74I-M184-L210W-T215Y-K101P-K103N-V106I.

4. Discussion

In this study, we try to describe the virological profile of HIV-infected individuals living in Gabon (64.6% from the capital and 35.1% from the inner of the country), before the total dolutegravir-based regimen transition. The previous studies discussing HIV genetic diversity, virological failure, and drug resistance were performed in semirural and rural areas [1] [4] [5]. In Libreville, access to viral load and genotyping tests was difficult for patients, because of the cost and availability of a specialized laboratory. The establishment of the International Center of Medical Research of Franceville (CIRMF) in the capital of Gabon, specifically for this issue of monitoring people living with HIV, has allowed, with the military health service of Gabon, to generate these updated data on the virological diversity of HIV and the level of resistance to antiretroviral drugs.

Our study is characterized by a population under treatment for less than 10 years (68.7%) with the dominance of HIV-infected women. This confirms that HIV infection remains a public health challenge and a major burden carried by women from sub-Saharan Africa [6]. This disproportion could be explained by socio-economic, cultural, and genetic factors that remind fully undescribed in Africa. We also highlight that young adults seems to be in virological failure than older adults (more than 35 years of age). This founding has been already described in studies [7] [8] [9]. The reason highlight was the poor adherence because of stigma-related problems like secrecy and other sociological factors. In Gabon the stigmatization continues to be a problem in young adult.

The mean CD4 cell count was fewer than 500 cells per cubic millimeter, showing a low response to CD4 restoration. Patients who have been on treatment for more than 5 years and who have not managed to exceed 500 CD4 cells per cubic millimeter are usually started on treatment with CD4 below 200 cells per cubic millimeter [10]. At this level, immune restoration is less efficient. Also, this population is more representative of people over 45 years old [11] [12]. That is consistent with our studied population with a median age of 46 years old.

The genetic diversity of this studied population is consistent with the previous studies in Gabon [2] [3] and Central Africa [13] [14]. However, one of our sequences appears to cluster with the recombinant M (subtype D) and O subtype brings us to describe for future analysis the full-length or near full-length sequence of this isolate. Indeed, the subtype O described in Cameroon [15] [16] was far away (the year 1996) described in Gabon but just for three sequences of 900 base-pair fragment in strains isolated from the year 1988 to the year 1993

[17]. We need to perform a large-scale HIV genetic diversity study to best understand the circulating dynamic of HIV in Central Africa.

This studied population mostly in the old first-line regimen (TDF-FTC/3TC-EFV) shows a virological success of 62%, far away from the 95-95-95 towards achieving UNAIDS endpoints. Many factors could be targeted in this context: the non-adherence of the patient, the psychological breakdown due to stigma and discrimination, the management of co-morbidity tuberculosis, and viral hepatitis which are still not effective and not efficient. It is worth mentioning here that most of the buildings that house the HIV-care centers are isolated from the hospital services, which indicates exclusive access for PLWHA, thereby increasing the stigma. Evidence on the impact of the use of new technologies (point-of-care) on the care of by the Community Health Workers (CHWs) has been gathered in several studies [18] [19] [20]. In Gabon, these technologies are not sufficiently well developed and the CHWs are not sufficiently trained and well equipped. The CHWs should be trained and provided with the necessary resources to do their work better.

In 2019 the UNAIDS established the 90-90-90 endpoint. To accelerate the third 90 (90% of PLWHA under treatment should have an HIV viral load suppressed), the Dolutegravir-based regimen has been recommended for the first-line treatment. In Gabon, this DBR has been initiated in January 2019. Our studied population is composed of 1.5% of patients in DBR and which show a virological success of 70.6%, nearer to the objectives than the former first line than the old first-line regimen. This assessment confirms the implementation of the DBR in Gabon as shown by other scientific research [21] [22] [23]. However, it would be wise to monitor the emergence of resistance to DTG, as patients are currently on a three-molecule regimen, two of which have already shown resistance in the past; patients run the risk of being on bi- or mono-therapy if the strains they are harboring are resistant to one or both molecules of this DBR.

Furthermore, patients undergoing third-line treatment in this study had a 100% virological suppression success rate. Patients failing the second line of treatment are introduced to a 3rd line committee. This committee is composed of the senior physicians of the centers for the care of patients living with HIV and of a biologist and a pharmacist. Other countries adopted national programs for third-line treatment and covered virological success of 95.5% for Zimbabwe [24], 70% for adolescents in Thailand [25]. Further studies are needed to assess factors associated with this failure.

Globally, HIV drug resistance remained a stumbling block to the achievement of the 90-90-9 goals. Some studies show the impact of HIV genetic diversity in virological success. Our studied population is characterized by the dominance of the circulating recombinant form (CRF) subtype. This CRF was previously described as a dominant subtype by previous publications in Gabon [3] [26] and Cameroon [27] [28]. A more recently conducted study in the southeast of Gabon (Franceville, provincial capital of Haut-Ogooué) shows the dominance of HIV-1

subtype "A" followed by "CRF" [4], in agreement with the previous studies conducted in the Republic of Congo, neighboring the province of Haut-Ogooué whose provincial capital is Franceville [29] [30]. The unknown subtypes describe in this study and the previous one [4] highlights the high genetic diversity of HIV in Gabon and call more studies on HIV genetic diversity, using next-generation sequencing technology in this part of the African continent.

Reverse transcriptase resistance mutations described in this study are in concordance with the previous study in Gabon [4] and Cameroon [27]. This study confirms that TDF has a high genetic barrier and therefore a low level of resistance as described in the literature [27] [31]. However, the high level of 3TC/FTC resistance of the old first-line regimen could expose to DTG monotherapy on the new first line (TDF-3TC/FTC-DTG) treatment. Indeed, it may be that the rapid implementation of the DTG-based regimen did not account for the level of resistance to 3TC and FTC in the treatment switch. A prospective study focusing on the surveillance of emergence DTG resistance is expected in Gabon.

The limitation of this study is the omission of protease inhibitor resistance study and the second-line treatment failure.

5. Conclusion

This report paints a picture of a relatively female-dominated HIV-infected Gabonese population with a low level of immunity, the bulk of whom have been on HIV treatment for more than 5 years. The continuing increase in genetic diversity shows a steady intermingling of the population in Central Africa. The level of drug resistance in the old first-line regimen suggests the need to monitor the appearance of drug resistance to Dolutegravir, the based molecule of the new first-line regimen.

Ethics Statement

Participants were asked to give their written consent to use their sociodemographic and biological data to participate in this study. The national ethic Committee ("Comite Nationla d'Ethique pour la Recherche du Gabon") approves the study on HIV infected individual and gives the number PROT N° 0021/2013/SG/CNE. The study was conducted by our institution ("Centre International de Recherches Medicales de Franceville") following the recommendations of the Convention of Helsinki.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this

paper.

References

[1] Pandrea, I., *et al.* (2002) Analysis of Partial *pol* and *env* Sequences Indicates a High Prevalence of HIV Type 1 Recombinant Strains Circulating in Gabon. *AIDS Research and Human Retroviruses*, **18**, 1103-1116. https://doi.org/10.1089/088922202320567842

[2] Mintsa-Ndong, A., *et al.* (2009) High HIV Type 1 Prevalence and Wide Genetic Diversity with Dominance of Recombinant Strains But Low Level of Antiretroviral Drug-Resistance Mutations in Untreated Patients in Northeast Gabon, Central Africa. *AIDS Research and Human Retroviruses*, **25**, 411-418. https://doi.org/10.1089/aid.2008.0223

[3] Caron, M., *et al.* (2012) Prevalence, Genetic Diversity and Antiretroviral Drugs Resistance-Associated Mutations among Untreated HIV-1-Infected Pregnant Women in Gabon, Central Africa. *BMC Infectious Diseases*, **12**, Article No. 64. https://doi.org/10.1186/1471-2334-12-64

[4] Engone-Ondo, J.D., *et al.* (2021) High Rate of Virological Failure and HIV Drug Resistance in Semi-Rural Gabon and Implications for Dolutegravir-Based Regimen Efficacy. *Journal of Antimicrobial Chemotherapy*, **76**, 1051-1056. https://doi.org/10.1093/jac/dkaa537

[5] Liegeois, F., *et al.* (2012) Virological Failure Rates and HIV-1 Drug Resistance Patterns in Patients on First-Line Antiretroviral Treatment in Semirural and Rural Gabon. *Journal of the International AIDS Society*, **15**, Article ID: 17985. https://doi.org/10.7448/IAS.15.2.17985

[6] Griesbeck, M., Scully, E. and Altfeld, M. (2016) Sex and Gender Differences in HIV-1 Infection. *Clinical Science*, **130**, 1435-1451. https://doi.org/10.1042/CS20160112

[7] Weijsenfeld, A.M., *et al.* (2019) Longitudinal Virological Outcomes and Factors Associated with Virological Failure in Behaviorally HIV-Infected Young Adults on Combination Antiretroviral Treatment in the Netherlands, 2000 to 2015. *Medicine*, **98**, Article ID: e16357. https://doi.org/10.1097/MD.0000000000016357

[8] Kahana, S.Y., *et al.* (2015) Rates and Correlates of Antiretroviral Therapy Use and Virologic Suppression among Perinatally and Behaviorally HIV-Infected Youth Linked to Care in the United States. *Journal of Acquired Immune Deficiency Syndromes*, **68**, 169-177. https://doi.org/10.1097/QAI.0000000000000408

[9] Beer, L., *et al.* (2016) Receipt of Clinical and Prevention Services, Clinical Outcomes, and Sexual Risk Behaviors among HIV-Infected Young Adults in Care in the United States. *AIDS Care*, **28**, 1166-1170. https://doi.org/10.1080/09540121.2016.1160028

[10] Le Moing, V., *et al.* (2007) Long-Term Evolution of CD4 Count in Patients with a Plasma HIV RNA Persistently <500 Copies/mL during Treatment with Antiretroviral Drugs. *HIV Medicine*, **8**, 156-163. https://doi.org/10.1111/j.1468-1293.2007.00446.x

[11] Kaufmann, G.R., *et al.* (2002) The Extent of HIV-1-Related Immunodeficiency and Age Predict the Long-Term CD4 T Lymphocyte Response to Potent Antiretroviral Therapy. *AIDS*, **16**, 359-637. https://doi.org/10.1097/00002030-200202150-00007

[12] Bahemana, E., *et al.* (2020) Impact of Age on CD4 Recovery and Viral Suppression over Time among Adults Living with HIV Who Initiated Antiretroviral Therapy in the African Cohort Study. *AIDS Research and Therapy*, **17**, Article No. 66.

https://doi.org/10.1186/s12981-020-00323-x

[13] Rubio-Garrido, M., *et al.* (2020) Current and Historic HIV-1 Molecular Epidemiology in Paediatric and Adult Population from Kinshasa in the Democratic Republic of Congo. *Scientific Reports*, **10**, Article No. 18461. https://doi.org/10.1038/s41598-020-74558-z

[14] Fokam, J., *et al.* (2020) Pre-Treatment Drug Resistance and HIV-1 Genetic Diversity in the Rural and Urban Settings of Northwest-Cameroon. *PLOS ONE*, **15**, Article ID: e0235958. https://doi.org/10.1371/journal.pone.0235958

[15] Yamaguchi, J., *et al.* (2004) HIV Infections in Northwestern Cameroon: Identification of HIV Type 1 Group O and Dual HIV Type 1 Group M and Group O Infections. *AIDS Research and Human Retroviruses*, **20**, 944-957. https://doi.org/10.1089/aid.2004.20.944

[16] Villabona-Arenas, C.J., *et al.* (2015) HIV-1 Group O Infection in Cameroon from 2006 to 2013: Prevalence, Genetic Diversity, Evolution and Public Health Challenges. *Infection, Genetics and Evolution*, **36**, 210-216. https://doi.org/10.1016/j.meegid.2015.09.006

[17] Delaporte, E., *et al.* (1996) Epidemiological and Molecular Characteristics of HIV Infection in Gabon, 1986-1994. *AIDS*, **10**, 903-910. https://doi.org/10.1097/00002030-199607000-00014

[18] Mwai, G.W., *et al.* (2013) Role and Outcomes of Community Health Workers in HIV Care in Sub-Saharan Africa: A Systematic Review. *Journal of the International AIDS Society*, **16**, Article ID: 18586. https://doi.org/10.7448/IAS.16.1.18586

[19] Khumalo, G.E., *et al.* (2020) Barriers and Facilitators of Rendering HIV Services by Community Health Workers in Sub-Saharan Africa: A Meta-Synthesis Protocol. *BMJ Open*, **10**, Article ID: e037876. https://doi.org/10.1136/bmjopen-2020-037876

[20] Drain, P.K., *et al.* (2019) Point-of-Care HIV Viral Load Testing: An Essential Tool for a Sustainable Global HIV/AIDS Response. *Clinical Microbiology Reviews*, **32**, e00097-18. https://doi.org/10.1128/CMR.00097-18

[21] Venter, W.D.F., *et al.* (2020) 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, Non-Inferiority Trial. *Lancet HIV*, **7**, e666-e676. https://doi.org/10.1016/S2352-3018(20)30241-1

[22] Venter, W.D.F., *et al.* (2019) Dolutegravir plus Two Different Prodrugs of Tenofovir to Treat HIV. *New England Journal of Medicine*, **381**, 803-815. https://doi.org/10.1056/NEJMoa1902824

[23] Lockman, S., *et al.* (2021) Efficacy and Safety of Dolutegravir with Emtricitabine and Tenofovir Alafenamide Fumarate or Tenofovir Disoproxil Fumarate, and Efavirenz, Emtricitabine, and Tenofovir Disoproxil Fumarate HIV Antiretroviral Therapy Regimens Started in Pregnancy (IMPAACT 2010/VESTED): A Multicentre, Open-Label, Randomised, Controlled, Phase 3 Trial. *Lancet*, **397**, 1276-1292. https://doi.org/10.1016/S0140-6736(21)00314-7

[24] Chimbetete, C., Shamu, T. and Keiser, O. (2020) Zimbabwe's National Third-Line Antiretroviral Therapy Program: Cohort Description and Treatment Outcomes. *PLOS ONE*, **15**, Article ID: e0228601. https://doi.org/10.1371/journal.pone.0228601

[25] Prasitsuebsai, W., *et al.* (2017) Treatment Outcomes of Third-Line Antiretroviral Regimens in HIV-Infected Thai Adolescents. *Pediatric Infectious Disease Journal*, **36**, 967-972. https://doi.org/10.1097/INF.0000000000001638

[26] Mouinga-Ondeme, A., *et al.* (2014) Significant Impact of Non-B HIV-1 Variants

Genetic Diversity in Gabon on Plasma HIV-1 RNA Quantitation. *Journal of Medical Virology*, **86**, 52-57. https://doi.org/10.1002/jmv.23770

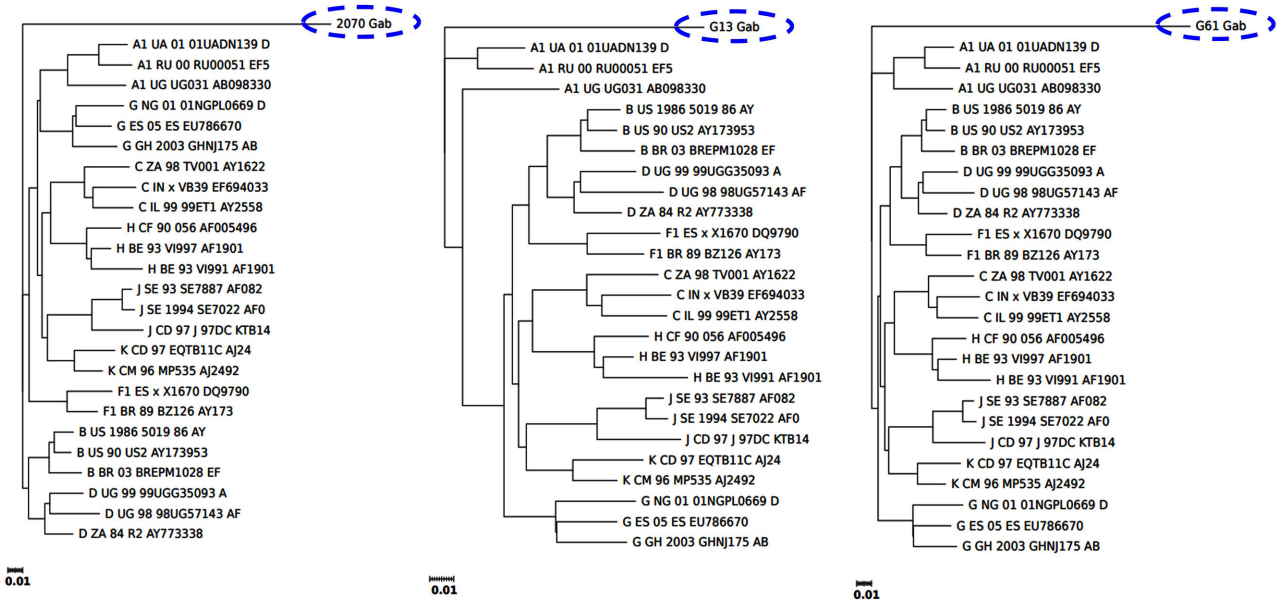
[27] Meriki, H.D., *et al.* (2019) Genetic Diversity and Antiretroviral Resistance-Associated Mutation Profile of Treated and Naive HIV-1 Infected Patients from the Northwest and Southwest Regions of Cameroon. *PLOS ONE*, **14**, Article ID: e0225575. https://doi.org/10.1371/journal.pone.0225575

[28] Abongwa, L.E., *et al.* (2019) Human Immunodeficiency Virus Type 1 ((HIV-1) Subtypes in the Northwest Region, Cameroon. *Virology Journal*, **16**, Article No. 103. https://doi.org/10.1186/s12985-019-1209-6

[29] Rodgers, M.A., *et al.* (2017) Sensitive Next-Generation Sequencing Method Reveals Deep Genetic Diversity of HIV-1 in the Democratic Republic of the Congo. *Journal of Virology*, **91**, e01841-16. https://doi.org/10.1128/JVI.01841-16

[30] Niama, F.R., *et al.* (2017) HIV-1 Genetic Diversity and Primary Drug Resistance Mutations before Large-Scale Access to Antiretroviral Therapy, Republic of Congo. *BMC Res Notes*, **10**, Article No. 243. https://doi.org/10.1186/s13104-017-2550-8

[31] Brooks, K., *et al.* (2016) Treatment Failure and Drug Resistance in HIV-Positive Patients on Tenofovir-Based First-Line Antiretroviral Therapy in Western Kenya. *Journal of the International AIDS Society*, **19**, Article ID: 20798. https://doi.org/10.7448/IAS.19.1.20798



Supplementary Data 1. Phylogenetic tree of 3 HIV-1 isolates from patients in Libreville and the inner country (Gabon), described as unknown subtype by the Stanford algorithm. All phylogenetic tree was performed by the online REGA HIV-1 Subtyping Tool Version 3.0. **Ref:** **Title:** Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: Performance evaluation of the new REGA version 3 and seven other tools. **Authors:** Peña ACP, Faria NR, Imbrechts S, Libin P, Abecasis AB, Deforche K, Gomez A, Camacho RJ, de Oliveira T, Vandamme A-M. **Journal:** Infectious Genetics and Evolution 2013; 19:337-48. doi: 10.1016/j.meegid.2013.04.032.