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ANTIRETROVIRAL RESISTANCE IN HIV-INFECTED PERSONS AT VIROLOGICAL FAILURE IN CÔTE D'IVOIRE, WEST AFRICA

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ABSTRACT: *The selection of resistance mutations is one of the main consequences of virological failure. The objective of our study was to determine the resistance profile of HIV-1 to ARVs and molecular phylogeny in patients treated with virological failure in Côte d'Ivoire. The genotypic resistance testing (GRT) were performed and interpreted according to the ANRS algorithm (www.hivfrenchresistance.org). Phylogenetic trees were created using BioEdit v7 and Mega7 software. Phylogenetic analysis showed that CRF02_AG (83%) was the most represented. Also noted are the circulation of subtype A (9%), B (2%), C (1%), D (1%) and complex recombinants CRF02/A1 (1%), CRF02/CRF09 (1%), CRF09_cpx (2%), CRF06_cpx (2%). The frequency of resistance to at least one ARV molecule was 74%. It was 87% for nucleoside reverse transcriptase inhibitors (NRTIs), 81% for non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 37% for protease inhibitors (PIs). The resistance frequency at two classes was 45% and 12% respectively at NRTIs/NNRTIs and NRTIs/PIs. The frequency of resistance to all three classes (NRTIs /NNRTIs/PIs) was 24%. Frequently encountered resistance mutations were for NRTIs: M184V (88%), "thymidine analogue mutations" (23%); for NNRTIs: K103N (65%). As for PIs, resistance to lopinavir/ritonavir, atazanavir/ritonavir and darunavir/ritonavir were 78%, 57% and 14% of PIs resistance, respectively. The analyses showed a high prevalence of resistance in patients in therapeutic failure followed routinely. These data support more accessible monitoring for the viral load and GRT in subjects treated for therapeutic failure.*

KEYWORDS: HIV-1, ARV Resistance, Molecular Phylogeny, Côte d'Ivoire.

INTRODUCTION

Since treatment intensification began in the 2000s, the level of resistance of the human immunodeficiency virus (HIV) to antiretrovirals (ARVs) has gradually increased (WHO, 2012; Gupta *et al.*, 2012) and resistance has been identified as one of the main consequences compromising the effectiveness of commonly used therapeutic regimens (Hamers *et al.*, 2012; Wittkop *et al.*, 2011).

The adoption of the « Test and Treat All » strategy to achieve the 90-90-90 targets has resulted in a considerable increase in people receiving antiretroviral treatment (ART) and requiring follow-up. But this situation could result in an increase in resistance in the population, which would be at the root of not achieving one of UNAIDS' objectives, which is to have 90% of people on ART with a viral load suppressed (UNAIDS, 2014).

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This increase in resistance would probably come from difficulties in accessing ARVs and monitoring response to treatment. Indeed, these strategies should involve virological tests such as viral load and possibly genotypic resistance testing (GRT). However, access to viral load in Côte d'Ivoire is still limited to certain centres and GRT to reference centres.

Thus, therapeutic regimens are generally initiated or modified without taking into account the level of viral replication and resistance developed by the virus. In addition, HIV-1 subtypes and recombinants have natural resistance to certain molecules (Wainberg and Brenner, 2012 ; Santos and Soares, 2010).

Therefore, monitoring of resistance in people on ART is essential, in a context where therapeutic combination choices remain limited and the number of people on treatment is increasing.

The aim of our study is to determine the resistance profile of HIV-1 to ARVs and molecular phylogeny in patients treated for at least 12 months in Abidjan (Côte d'Ivoire).

MATERIALS AND METHODS

Study population

This is an analytical, descriptive study that analyzed the plasmas of patients with an indication of HIV-1 GRT and followed routinely from June 2015 to July 2017 at the Abidjan Integrated Bioclinical Research Center (CIRBA). The study was approved by the National Ethics Committee for life and health sciences (CNESVS).

Determination of HIV-1 resistance patterns to ARVs

HIV-1 resistance profiles to ARVs have been determined using techniques developed by the ANRS AC11 (http://www.hivfrenchresistance.org/). To do this, plasma aliquots of at least 1 mL obtained from samples taken from Ethylene-Diamine-Tetra-Acetic (EDTA) tubes were stored at -80°C until the GRT were obtained. Viral RNA was extracted from plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). The Titan One Tube RT-PCR System (Roche Diagnostics, Manheim, Germany) was used for the first PCR and the Expand™ High Fidelity PCR System (Roche Diagnostics, Manheim, Germany) for the second PCR. The amplicons obtained were purified with the QIAquick PCR Purification Kit (Qiagen, Germany). The sequencing of protease and reverse transcriptase genes was performed with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Courtaboeuf, France) and the Genetic Analyser 3130 sequencer (Applied Biosystem, Courtaboeuf, France) according to the manufacturer's recommendations (Applied Biosystem, Courtaboeuf, France). The sequences obtained were aligned with the HIV-1 reference (HIV-1 HXB2, accession number GenBank : K03455) using SeqScape software 3.0 (Applied Biosystem, Courtaboeuf, France). The list of defined mutations is that of the International Aids Society (IAS, http://www.iasusa.org). The interpretation was made using the ANRS algorithm (http://www.hivfrenchresistance.org/, September 2017, Version 27).

Phylogenetic analyses

In order to specify the viral subtypes, the consensus sequences obtained were aligned with the reference sequences available in the GenBank (http://hiv-web.lanl.gov/). The sequences were

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aligned with Clustal W (Thompson *et al.*, 1994) software implemented in the BioEdit v7 program (Hall, 1999). The phylogenetic trees were made with Mega 7 software (Kumar *et al.*, 2016).

Data processing and statistical analysis

The SPSS Statistics 17.0.1 software was used for statistical analyses.

RESULTS

Study Patient Characteristics

One hundred eighty-two (N=182) patient plasmas were analyzed over the period June 2015 to July 2017. Patients had a median age of 43 years (18-75). Female gender represented 53% (n = 96/182).

Overall distribution of viral subtypes and recombinants

The synthesis of the different phylogenetic analyses showed that CRF02_AG was the most represented 83% (n= 151/182). Also noted were circulation of subtype A (9%; n= 16/182), B (2%; n= 3/182), C (1%; n= 1/182), D (1%; n= 1/182) and complex recombinants CRF02/A1 (1%; n= 1/182), CRF02/CRF09 (1%; n= 1/182), CRF09_cpx (2%; n= 4/182) and CRF06_cpx (2%; n= 4/182) (Fig. 1).

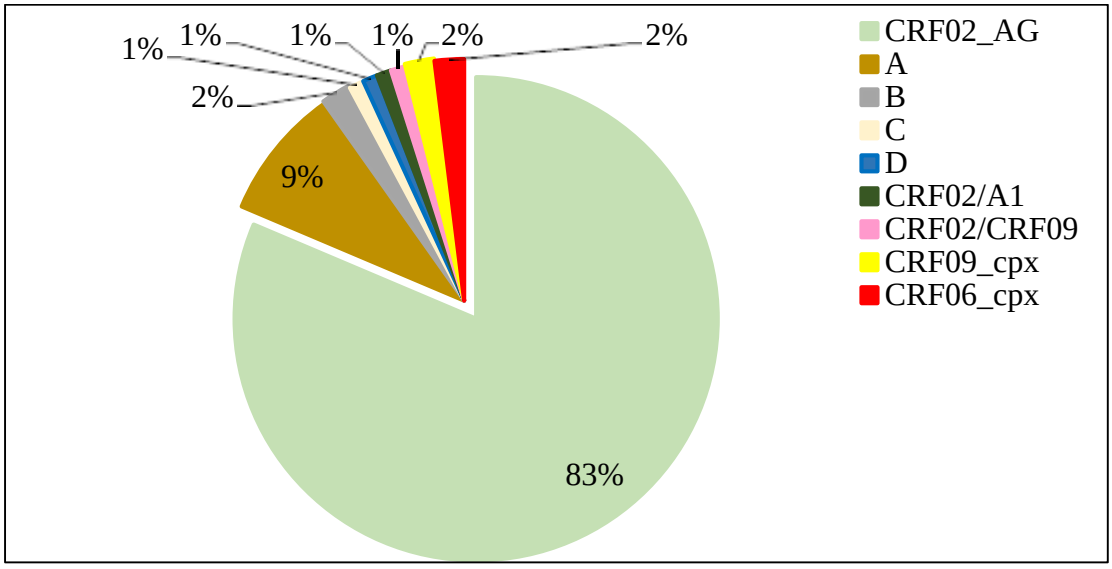

Figure 1 : Global distribution of subtypes and viral recombinants of 182 HIV-1 patients routinely monitored from June 2015 to July 2017 at CIRBA obtained from phylogenetic trees produced using BioEdit v7 and Mega7 software. The consensus sequences were aligned and compared with the reference sequences: subtype A (92UG-UGO37, 94SE-SE7523, and 94KE-Q23), subtype B (90US-WEAU160, 86US-JFRL, and 83FR-HXB2), subtype C (86ET-ETH2220, 96BW-0502, and 95IN-21068), subtype D (94UG-114, 83CD-NDK, and 99TCMN011), F1 subtype (93BR-20, 93BE-VI850, and 93FI-FIN9363), F2 subtype (95CM-MP255, CM-MP257, and CM53657), G subtype (93SE-SE61615, 93FI-HH8793,

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and 96DE-DRCBL), H subtype (90CF-CF056, BE.VI991, and BE.VI997), subtype J (93SE-SE92809 and 93SE-SE91733), subtype K (97CD-EQTB11 and 96CM-MP535), CRF02_AG (IBNG, DJ263, and 98SE-MP1211), CRF06_cpx (BFP90 and 95ML-84), and CRF09 (96GH2911, 95SN1795, and 95SN7808).

Frequency of resistance to different classes of ARVs

The frequency of resistance to at least one ARV molecule was 74% (n= 134/182). It was 87% (n= 117/134) for nucleoside reverse transcriptase inhibitors (NRTIs), 81% (n= 108/134) for non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 37% (n= 49/134) for protease inhibitors (PIs). The frequency of resistance to two classes was 45% (60/134) at NRTIs/NNRTIs and 12% (16/134) at NRTIs/PIs. The frequency of resistance to all three classes (NRTIs/NNRTIs/PIs) was 24% (32/134) (Fig. 2).

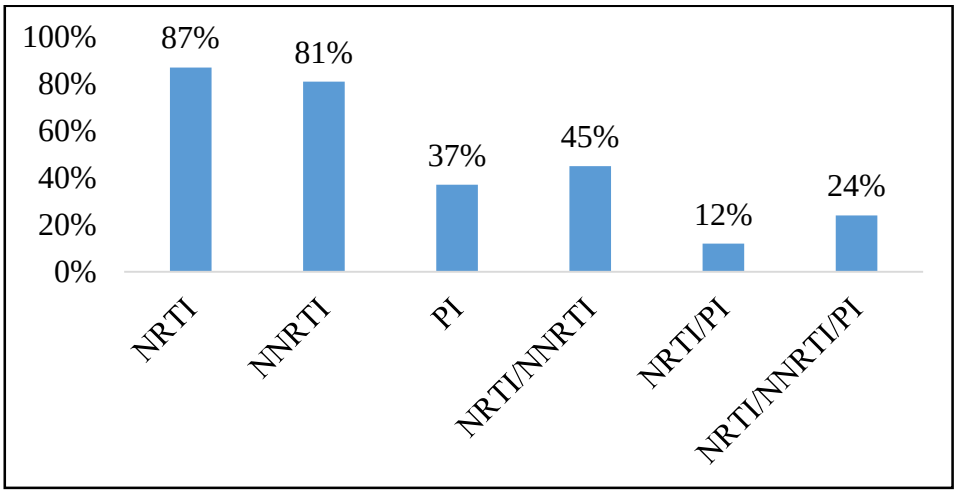

Figure 2: Frequency of resistance of 134 patients to different classes of ARVs out of 182 HIV-1 patients routinely followed from June 2015 to July 2017 at CIRBA. NRTI : nucleoside reverse transcriptase inhibitor ; NNRTI : non-nucleoside reverse transcriptase inhibitor ; PI : protease inhibitor.

Frequency of resistance to NRTIs

The resistance frequency at 3TC/FTC was 93% (n= 109/117), 62% (n= 73/117) at d4T, 58% (n= 68/117) at ZDV, 50% (n= 59/117) at ABC, 27% (32/117) at ddI, and 23% (28/117) at TDF (Fig. 3).

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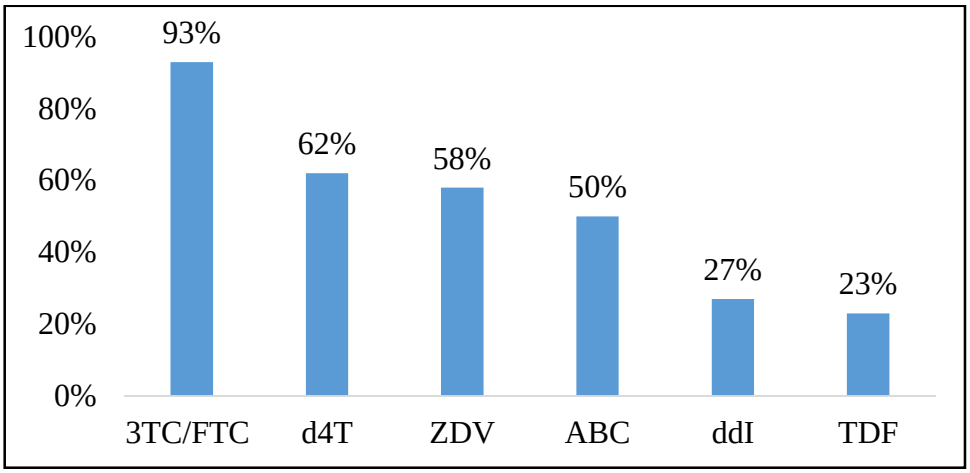

Figure 3: Frequency of resistance of 117 patients to NRTIs out of 134 HIV-1 patients resistant to at least one ARV molecule routinely monitored from June 2015 to July 2017 at CIRBA. 3TC: lamivudine ; FTC: emtricitabine ; d4T: stavudine ; ZDV: zidovudine ; ABC: abacavir, ddI: didanosine ; TDF: tenofovir.

Frequency of resistance to NNRTIs

The frequency of resistance to EFV and NVP was 94% (n= 102/108 and n= 101/108 respectively). The RPV was 63% (n= 22/108) and 20% ETR (Fig. 4).

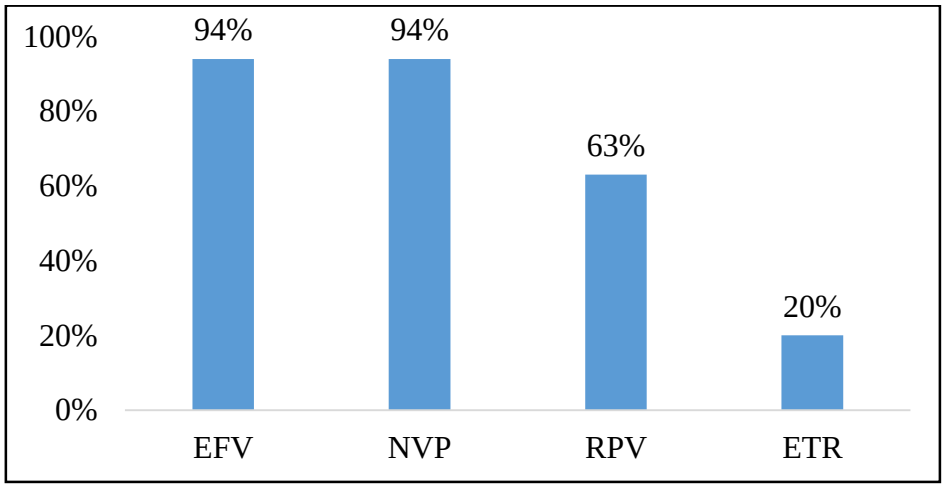

Figure 4: Frequency of resistance of 108 patients to NNRTIs out of 134 HIV-1 patients resistant to at least one ARV molecule routinely monitored from June 2015 to July 2017 at CIRBA. EFV: efavirenz ; NVP: nevirapine ; RPV: Rilpivirine ; ETR: Etravirine.

PIs resistance frequency

The frequency of resistance to IDV was 84% (n= 41/49), 78% (n= 38/49) to LPV/r, 63% (n= 31/49) respectively to SQV/r and fosAPV/r, 61% (n= 30/49) to NFV, 57% (n= 28/49) to ATV/r, 43% (n= 21/49) to TPV/r and 14% (n= 7/49) to DRV/r (Fig. 5).

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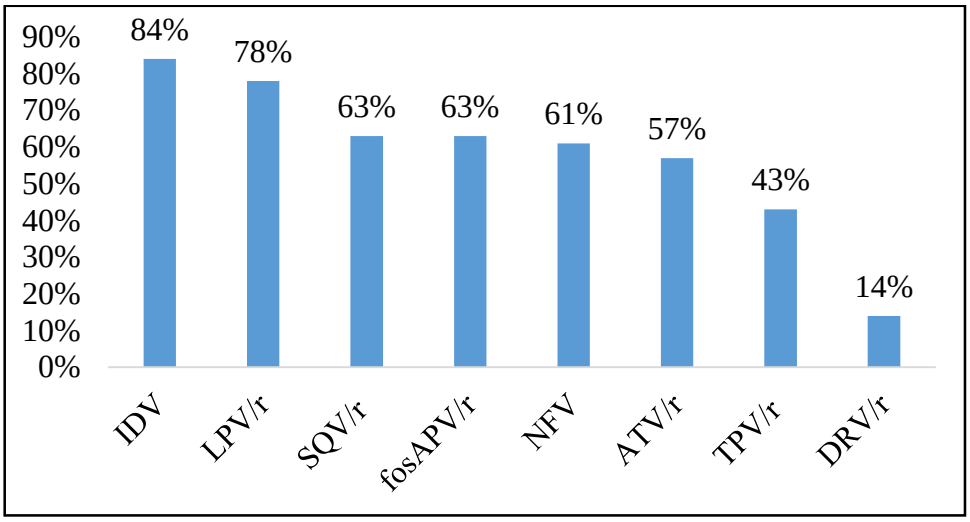

Figure 5: Frequency of resistance of 49 patients to PIs out of 134 HIV-1 patients resistant to at least one ARV molecule routinely monitored from June 2015 to July 2017 at CIRBA. IDV: indinavir ; LPV/r: lopinavir/ ritonavir ; SQV/r: saquinavir/ ritonavir ; fosAPV/r: fosamprenavir/ritonavir ; NFV: nelfinavir ; ATV/r: atazanavir/ ritonavir ; TPV/r: tipranavir/ ritonavir ; DRV/r: Darunavir/ritonavir.

Frequency of resistance mutations in different classes of ARVs

Frequently encountered resistance mutations were M184V (88%) and "thymidine analogue mutations (TAMS)" (T215Y/F, M41L, L210W, D67N, K70R and K219Q/E) (23%) for NRTIs. For NNRTIs, K103N (65%) was frequently encountered. Concerning PIs, the most common were H69K (84%), K20I (73%) and M36I (71%) for minor mutations and M46I (67%) for major mutations.

Distribution of primary mutations responsible for resistance to a wide range of ARVs

Primary mutations responsible for resistance to a wide range of ARVs have been found in all classes of ARVs. Thus, resistance to a wide range of the 7 ARVs in the NRTIs class was found in K65R (71%; n= 5/7) and Q151M (57%; n= 4/7) in 1 and 5 patients, respectively. Resistance to all NNRTIs was observed in 23 patients. It was due to Y181C (100%; n= 4/4). Also, 75% (n= 3/4) of ARV resistance in this class was due to K101E, E138K or M230L. These mutations were found in 5, 1 and 2 patients respectively. In the PIs class, the major mutations responsible for the 22% resistance (n=2/9) of ARVs were I84V and N88S found in 20 and 2 patients respectively (Table 1).

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Table 1: Distribution of ARV resistance obtained in 134 HIV-1 patients out of a total of 182 routinely followed from June 2015 to July 2017 at CIRBA

Mutations (ARVs affected by mutations)	**Number of patients (%)**
Mutations : NRTI (N= 117)	
M184V/I (3TC/FTC)	106 (91)
T215I/N/V/Y/F (ZDV, d4T)	53 (45)
Q151M (ZDV, ddI, d4T, ABC)	1 (1)
K65R (3TC/FTC, ddI, d4T, ABC, TDF)	5 (4)
L74V/I (ddi, ABC)	25 (9)
V75M/T (d4T)	5 (4)
Y115F (ABC)	7 (6)
K70E (TDF)	9 (8)
Mutations : NNRTI (N= 108)	
K103N/S (EFV, NVP)	72 (67)
L100I (EFV, NVP)	8 (7)
K101E (EFV, NVP, RPV)	5 (5)
K101P (RPV)	2 (2)
V106M (EFV, NVP)	1 (1)
E138K (EFV, RPV, ETR)	1 (1)
E138A/G/Q (RPV)	20 (19)
Y181C (EFV, NVP, RPV, ETR)	23 (21)
Y181V (RPV, ETR)	1 (1)
Y188C/L (EFV, NVP)	13 (12)
Y188L (RPV)	11 (10)
G190A/S (EFV, NVP)	17 (16)
P225H (EFV)	22 (20)
M230L (EFV, NVP, RPV)	2 (2)
A98S (NVP)	2 (2)
H221Y (RPV, ETR)	13 (12)
Mutations : PI (N= 49)	
M46I/L (IDV)	34 (69)
V82A/F/T (IDV)	18 (37)
I84V (IDV, NFV)	20 (41)
G48V (SQV/r)	2 (4)
N88S (NFV, ATV/r)	2 (4)
L90M (NFV)	4 (8)
I50V (fosAPV)	49 (2)
I47A (LPV/r)	2 (4)
L76V (LPV/r)	20 (41)

N : Number of persons with a mutation ; NRTI : nucleoside reverse transcriptase inhibitor ; NNRTI : non-nucleoside reverse transcriptase inhibitor ; PI : protease inhibitor ; M : Méthionine ; V : Valine ; T : Thréonine ; Y : Tyrosine ; F : Phénylalanine ; L : Leucine ; N : Asparagine ; K : Lysine ; Q : Glutamine; E : Acide glutamique ; R : Arginine ; I : Isoleucine ; S : Sérine ; C : Cystéine; P : Proline ; H : Histidine ; A : Alanine ; G : Glycine ; 3TC: lamivudine ; FTC: emtricitabine ; d4T: stavudine ; ZDV: zidovudine ; ABC: abacavir, ddI: didanosine ; TDF: tenofovir ; EFV: efavirenz ; NVP: nevirapine ; RPV: Rilpivirine ; ETR:

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Etravirine ; IDV: indinavir ; LPV/r: lopinavir/ ritonavir ; SQV/r: saquinavir/ ritonavir ; fosAPV/r: fosamprenavir/ritonavir ; NFV: nelfinavir ; ATV/r: atazanavir/ ritonavir ; TPV/r: tipranavir/ ritonavir ; DRV/r: Darunavir/ritonavir.

DISCUSSION

In this study, we determined the phylogenetic and resistance profiles of HIV-1 to ARVs in patients treated for at least 12 months in Abidjan (Côte d'Ivoire).

We found that 26% of patients on treatment for at least 12 months, who had an indication for GRT, had no resistance mutation. This proportion is approximately equal to that obtained in Rwanda (27%) in patients in virological failure without ARV resistance mutations (Ndahimana *et al.*, 2016).

Therefore, one of UNAIDS' goals of having 90% of people on ART with viral load suppressed (UNAIDS, 2014) is not yet achieved. Health authorities must make efforts in this direction to achieve this objective by 2020.

Almost three quarters of patients (74%) had viruses resistant to at least one ARV molecule. This prevalence is close to that obtained in Senegal and Guinea Conakry (Diouara et al., 2014). The proportion of patients with mono-class resistant viruses were 87%, 81% and 37% respectively at NRTIs, NNRTIs and PIs. It is substantially equal to that obtained in Mali and Kenya with regard to NRTIs and NNRTIs (Fofana *et al.*, 2014 ; Kantor *et al.*, 2014). As for PIs, it varies between 0% and 50% in resource-limited countries (Hosseinipour *et al.*, 2013). Prevalences of 18% and 22% were observed in the same region (Ajose *et al.*, 2012 ; Boender *et al.*, 2016). Our study confirms this trend.

Viruses resistant to dual classes were identified in 45% and 12% of patients for NRTIs/NNRTIs and NRTIs/PIs respectively. For the NRTIs/NNRTIs association, the prevalence is estimated at 47% in Zambia and 52% in Cameroon and Guatemala respectively (WHO, 2017). These values are similar to those of our study. Regarding the NRTIs/PIs association, the prevalence noted in our study is lower than that obtained by other authors (22%) (Boender *et al.*, 2016).

In 24% of cases, patients had viruses resistant to all three classes (NRTIs/NNRTIs/PIs). This challenges us on the need for virological monitoring including GRT for treatment optimization.

Our study thus confirms a high prevalence of resistance to different classes of ARVs in Côte d'Ivoire.

The resistance profiles of ARVs analysed individually revealed lower resistance to ddI (27%) and TDF (23%) in the NRTIs class. In NNRTIs, only 6% of patients had viruses susceptible to EFV and NVP respectively. Moderate resistance to ETR (20%) was found in contrast to RPV (63%). For PIs, 86% of patients had a virus susceptible to DRV/r and 57% to TPV/r.

Our study confirmed the resistance of viruses to the second generation of NNRTIs (ETR and RPV). Our results corroborate those obtained in Togo with roughly equal proportions, 21.5% and 64.4% respectively for ETR and RPV (Konou *et al.*, 2015).

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The resistance mutations frequently encountered in our study were M184V (88%) and TAMS (23%) for NRTIs. For NNRTIs, we had K103N (65%). For PIs, H69K (84%), K20I (73%) and M36I (71%) for minor mutations and M46I (67%) for major mutations.

The high prevalence of these mutations in our study has been confirmed by other authors.

As regards mutations at NRTIs, our results are close to those obtained in Togo (M184V (88%) and the TAMS frequently encountered were M41L, D67N/D, K70R, K219E/Q and T215Y/F) (Dagnra *et al.*, 2016), Nigeria (M184V (89%) and TAMS (27%)) (Chaplin *et al.*, 2011) and Mali (M184V (76%) and the TAMS found were M41L, D67N, L210W and T215Y/F/N) (Fofana *et al.*, 2014).

Concerning mutations at NNRTIs, our observations are not very far from those obtained in South Africa (K103N (50%)) (Etta *et al.*, 2017) and Mauritania (K103N (43%))(Fall-Malick *et al.*, 2014).

A study in India revealed that the mutations frequently observed conferring resistance to NNRTIs and NNRTIs respectively were M184V (90%) and K103N (45%) (Kandathil *et al.*, 2009).

As far as major mutations to PIs are concerned, our results are in agreement with those of a study conducted in sub-Saharan Africa which indicates that M46I (86%) is frequently encountered (Boender *et al.*, 2016). The same finding has been made in patients in India (Kandathil *et al.*, 2009). The minor mutations in our study were identified as part of the minor mutations frequently found in patients in Nigeria (M36I (93%), K20I (77%) and H69K (86%)) (Okopi *et al.*, 2013). This same observation was made in patients who had CRF02_AG in common (Anejo-Okopi *et al.*, 2014).

Mutations that can induce major resistance as a function of ART are K65R and Q151M for NRTIs, Y181C for NNRTIs and I84V and N88S for PIs.

Indeed, the administration of d4T is associated with the emergence of Q151M and K65R mutations which confer cross-resistance to NRTIs (Nouhin *et al.*, 2013). The selection of such mutations is a critical issue in Côte d'Ivoire where NRTIs molecules available for second-line treatment remain limited. Although the main reasons why World Health Organization (WHO) recommended in 2010 to reduce or discontinue the use of d4T in first-line ARV treatment in resource-limited countries are related to excessive toxicity (WHO, 2009), our observations support this direction because of the association with mutations that make the virus resistant to most or all of the NRTIs available in Côte d'Ivoire.

Many countries with limited resources, such as Côte d'Ivoire, have adopted and adapted to their contexts the 2013 and 2014 recommendations of the WHO (PNLS, 2015; WHO, 2014; WHO, 2013). Thus, the NNRTI-based treatment protocol is widely used as an initial regimen for adult and adolescent patients who have never received HIV-1 treatment (PNLS, 2015). But the problem with the first generation of NNRTIs is its low genetic barrier and broad cross-resistance (Teeranaipong et al., 2016). Thus, a mutation such as Y181C leads to high level resistance to the first generation of NNRTIs (NVP and EFV) and intermediate level resistance to the second generation (RPV and ETR) (Teeranaipong *et al.*, 2016; Diphoko *et al.*, 2018) not yet available in Côte d'Ivoire (PNLS, 2015). In addition, the association of Y181C with other mutations may synergistically exacerbate resistance to ETR and RPV (Teeranaipong *et al.*,

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2016). Therefore, early detection of such a mutation would be essential to preserve the activity of future therapeutic regimens including NNRTIs.

PIs resistance is the consequence of the accumulation of amino acid substitutions in the protease (Baxter *et al.*, 2016). Thus I84V and N88S are non-polymorphic mutations leading to resistance to IDV and NFV for the former and to ATV/r and NFV for the latter (http://www.hivfrenchresistance.org/, September 2017, Version 27). The panel on antiretroviral guidelines for adults and adolescents, recommends the non-use of certain ARVs including IDV and NFV due to suboptimal antiviral potency, unacceptable toxicities, high pill load, or pharmacological concerns (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2018). Our observations corroborate this orientation because of their associations with mutations that make the virus resistant to certain PIs including ATV/r which is the preferred option in second line schemes in Côte d'Ivoire (PNLS, 2015).

In light of the evidence, there is a real and growing need for increased access to third line drug options, ideally guided by genotypic resistance testing, to ensure successful HIV-1 treatment in Côte d'Ivoire. In anticipation, increased efforts are needed to reduce the prices of currently unaffordable third line drugs, including DRV/r and Raltegravir (RAL) inhibitors.

The synthesis of the different phylogenetic analyses showed that CRF02_AG (83%) was the most represented. Circulation was also noted for subtype A (9%), B (2%), C (1%), D (1%) and complex recombinants CRF02/A1 (1%), CRF02/CRF09 (1%), CRF09_cpx (2%) and CRF06_cpx (2%).

The predominance of CRF02_AG and the evolution of the genetic diversity of circulating strains has been observed in several West African countries (Dagnra *et al.*, 2016 ; Anejo-Okopi *et al.*, 2014; Loubet *et al.*, 2015). Our study thus confirms this trend and the need to establish monitoring of the dynamics of these strains.

CONCLUSIONS

This study confirmed the predominance of CRF02_AG but showed an evolution in the genetic diversity of circulating strains. The analyses showed a high prevalence of resistance in patients in therapeutic failure followed routinely for at least 12 months. These data support more accessible monitoring for viral load and GRT in subjects treated for therapeutic failure.

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