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The Prediction of Integrase Inhibitors Efficacy in Third Line Regimen after First and Second Line Antiretroviral Therapy Failure in Senegal

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Abstract

The optimal efficacy of the INI depends on the backbone of nucleoside inhibitors, which seems to be challenged in a context of late switch and drug resistance mutations accumulations. It is also known that before using the 3rd line regimen, a drug resistance testing is recommended.

This paper aims to predict the efficacy of integrase inhibitors in third line regimen after 1st and 2nd line failure and to describe the HIV-1 genetic diversity. A cross sectional study was conducted in 52 Senegalese HIV-1 infected patients. After viral load (VL) quantification, a drug resistance testing was performed for patients with VL ≥ $3\log_{10}$ copies/ml. ART combinations and DRM for each patient were considered to predict possible future regimens. The phylogenetic analysis was done using Seaview v4.4.2 and Simplot v3.5.1 software's. The medians of virological failure (VL) and treatment follow up duration in 1st and 2nd line ART were respectively 4.09 vs 1.6 $\log_{10}$ copies/ml and 55 vs 32 months. The most common therapeutic combinations were 2 NRTI (D4T/AZT+3TC)+1NNRTI (EFV/NVP) and 2 NRTI (TDF+3TC/FTC)+1 PI (LPVr) respectively at 1st and 2nd line. A number of 29 and 13 in VF (VL ≥ $3\log_{10}$ copies/ml) were genotyped on Protease and partial RT genes at 1st and 2nd line ART; and 12 among the 13 were genotyped in integrase gene. The TAMs (85.5 vs 90.9%), M184V (32.9 vs 27.3%) and K103N (24.2 vs 33.3%) were predominant both for the 1st and 2nd line therapy. No major DRM was found in integrase gene. The phylogenetic analysis shows a predominance of CRF_02AG both in protease-partial RT and integrase genes. Third line regimen including NRTI and new generation of NNRTI is possible only for 6/12 patients failing in second line ART. These findings highlighted the importance to reinforce virological monitoring of HIV-1 infected patients and to consider the drug resistance results for a third line regimen.

Keywords: HIV-1; Drug resistance mutations; Integrase inhibitors; Third line regimen; Senegal

Introduction

Successful antiretroviral therapy (ART) has reduced the morbidity and mortality among HIV-1 infected individuals and turned HIV infection to a chronic disease. The success of HIV-1 treatment has been justified by the long-term viral load suppression and the absence of HIV drug resistance [1] . According to the World Health Organization (WHO), the first line ART in resource limited setting includes two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and one Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI). After first line ART failure, a boosted protease inhibitor (PI), with two NRTIs has been recommended for second line ART [2]. The occurrence of virological failure with the presence of multiple drug resistance mutations (DRM) in HIV-1 infected patients lead to the use of Raltegravir (RAL), the first integrase inhibitor (INI) [3]. One of the first ART initiatives sponsored by an African government was launched in Senegal in 1998 and the Patients were monitored clinically and biologically in different clinical sites and laboratories. At that time some patients began their ART including non-boosted PI mainly Indinavir. These therapeutic regimens were used before WHO recommendations were launched. The clinical follow up has been performed during the monthly examination and the biological follow up includes plasma HIV-1 RNA viral load quantification and CD4 cell counts at the baseline and every 6 months' time intervals for patients in structured cohort [4,5]. On the contrary, for patients followed through the National Program based on public health approach in Senegal as well as in other African countries where HIV-1 RNA viral load tests are not always available, WHO has recommended the use the immunological or clinical criteria to switch the first line ART [6]. Results from these patients showed a high rate of drug resistance mutations and an accumulation of the thymidine analog mutations (TAMs) both for the patients under first and second line ART in Senegal [7]. In this study the main limit was the low number of patients on second line compared to the total of patients under 2nd line biologically followed based on the database at the Bacteriology and Virology Laboratory. These patients needed more attention because in case of 2nd line therapeutic failure, a 3rd line regimen, which includes the still efficient NRTIs, NNRTIs and PIs with the new class of IN inhibitors (INIs) is required. However, before using the 3rd line regimen, a drug resistance testing is recommended [8]. The optimal efficacy of the INI depends on the backbone of the remaining nucleoside inhibitors which seems to be challenged in a context of late switch and drug resistance mutations accumulations. The aim of this study was to predict the efficacy of INI in third line regimen after first and second line failure and to describe the HIV genetic diversity in this study population.

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Methods

Specimen collection, viral load and resistance testing

The patients were enrolled in the Senegalese Antiretroviral Drug Access Initiative (ISAARV) from 2001 to 2013. Patients were eligible for inclusion in this study if they were HIV-1 infected adults, underwent on second line ART regimen containing PI and followed in ISAARV program. Exclusion criteria were: HIV-2 or HIV-1+2 infected patients, unknown ART starting and/or switching date and infants on second line ART regimen. This study was approved by the Senegalese Ethics Committee. A cross sectional study was conducted in the Centre de Traitement Ambulatoire (CTA) where patients were followed. Socio-demographical, clinical, biological and therapeutic regarding patients were collected. The whole Blood was collected in EDTA tubes and plasma samples were isolated and stored at –80°C until their use. The viral load (VL) quantification was performed at the Bacteriology and Virology Laboratory based at the Aristide Le Dantec University Hospital in Dakar, using the Abbott Real Time HIV-1 m2000rt quantitative assay (Abbott Laboratories, Chicago, IL) with VL cut off is 1.6 $\log_{10}$ (40) copies/ml. The virological failure (VF) was defined as a VL ≥ 3$\log_{10}$ copies/ml. In case of VF, drug resistance testing in *pol* gene was done using the available kits either by the ViroSeq HIV-1 Genotyping System v2.0 according to the manufacturer's instructions (Celera Diagnostics, San Francisco, CA) or by the ANRS AC11 resistance study group protocol (http: //www.hivfrenchresistance.org/). The same protocol of the ANRS AC11 was also used to amplify the first 288 amino acids for integrase gene with the primers previously described by Monleau [9]. The PCR products were purified and sequenced on the ABI 3100-*Avant* using the Big Dye Terminator v3.1 technology (Applied Biosystems, Courtaboeuf, France). The generated sequences were edited on SeqMan II from the DNAStar software v.5.08 (Lasergene, Madison, WI, USA). The drug resistance mutations were investigated with the Stanford database v6.2.0 (http://hivdb.stanford.edu/). In order to predict possible future regimens including the NRTI, NNRTI or PI with INI, a transversal analyze was performed based on the HIV-1 drug resistance mutations report obtained from both first and second line generated sequences.

Phylogenetic analysis

The HIV Protease-partial RT and integrase generated sequences were aligned then Neighbor-joining trees with 100 bootstrap replicates were drawn on Seaview software v4.4.2. The Recombinant analysis and Bootscanning were performed with Simplot software v3.5.1. All pure subtypes and Circulating Recombinant Forms (CRFs) in West Africa were included in the phylogenetic analysis.

Results

Patient characteristics

A total of 52 HIV-1 infected patients undergoing second line ART, monitored since their first line ART, were included in this study. At ART initiation the median age was 41 (IQR, 18-78) years, 28 (53.8%) were women and the majority of patients was on WHO clinical stages 2 and 3 (n=37; 71.1%) (Ranged from stage 1 to 4). The medians of CD4-T cells count, viral load and treatment follow up duration in first and second line ART were respectively 128 Cells/mm^3 [n=50, (IQR, 2-566)] vs 153 Cells/mm^3 [n=51, (IQR, 2-566)], 4.09 $\log_{10}$ copies/ml [n=49, (IQR, 1.6-5.82)] vs 1.6 $\log_{10}$ copies/ml [n= 52, (IQR, 1.6-6.07)] and 55 months (IQR, 11-153) vs 32 months (IQR, 3-71). The mostly common therapeutic combinations were 2 NRTI (D4T or AZT+3TC)+1 NNRTI (EFV or NVP) (n=37; 71.2%) and 2 NRTI (3TC or FTC+TDF)+1 PI (LPVr) (n=36; 69.2%) at the first and second line, respectively.

Virological outcomes

Routine VL tests were available for the 49/52 patients at first line. Among them, 43 had VL ≥ 3 $\log_{10}$ copies/ml stratified as follow according to the treatment duration M6-M12 (1/2), M13-M24 (3/3) and >M24 (39/44) with no significant difference (P=0.21).

For patients under second line treatment, all of them had VL determination showing a good rate of viral suppression but 13 (25%) out of them still had a VL higher than 3 $\log_{10}$ copies/ml.

Drug resistance mutations (DRM)

A total of 29 and 13 samples were successfully genotyped on Protease-partial RT genes for the first line and second line ART respectively. Only one patient out of the 29 samples was not sequenced in protease gene but this patient was not exposed to PI containing regimen. Among the patients under second line ART, 12 were genotyped on integrase gene.

Genotypic drug resistance profiles at first line failure

All of the genotyped samples during the first line ART, 29/52 (55.7%) had at least one DRM. Among them, 28/29 (96.5%) had both NRTI and NNRTI-associated DRM. Otherwise, the prevalence's of DRM were 14/29 (48.3%) and 12/29 (41.4%) for the NRTI+PI and NRTI+NNRTI+PI combinations respectively.

During the first line ART, the prevalence's of NRTIs, NNRTIs and PIs-associated DRM were (28/29; 96.5%), (28/29; 96.5%) and (14/29; 48.3%), respectively. The TAMs (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) were found in (65/76; 85.5%). The M184V mutation was found in 25/76 of samples (32.9%). The L74I mutation and T69N insertion were observed in each 2/76 samples (2.6%) and the V75AV at once (1.3%) (Figure 1). The most prevalent mutation associated with resistance to NNRTIs were respectively K103N (16/66; 24.2%), L100I (10/66; 15.2%), V90I (5/66; 7.6%), G190A/S (5/66; 7.6%). The other encountered DRM for NNRTI were scored in Figure 2. Related PIs DRM, two patients harbored the L90M (2/28; 7.1%) and two other the both (I54A/V; V82A/F) in 7.1% each, all were previously exposed to Indinavir (protease inhibitor). Furthermore, the different mutations of polymorphism were described in Figure 3.

Genotypic drug resistance profile at second line failure

For patients in virological failure at second line regimen (n=13), DRM was detected at least in 12/29 (41.4%) cases in Protease-partial RT genes. The high rate of DRM was associated with NRTI+PI combination (n=11/13; 84.6%). For other combinations, the prevalence of NRTI+NNRTI was (n=7/13; 53.8%) and NRTI+NNRTI+PI (n=8/13; 61.5%).

The prevalence of NRTIs, NNRTIs and PIs-associated DRM were (11/13; 84.6%), (7/13; 53.8%) and (11/13; 84.6%) respectively. The TAMs were predominant with 30/33 (90.9%) followed by the M184V mutation (9/33; 27.3%). The details of TAMS mutation and other encountered mutations were given in the Figure 1. For NNRTI, the K103N was found in (7/21; 33.3%). The Figure 2 shows the details of the other NNRTIs-DRM. Different major PIs-DRM was observed in the following proportions: M46I (3/35; 8.6%); I54V, L90M, I84V (2/35; 5.7% each), I50V, V82A/F, L76V (1/35; 2.9% each). In addition, mutations of polymorphism were found and details were showed in Figure 3.

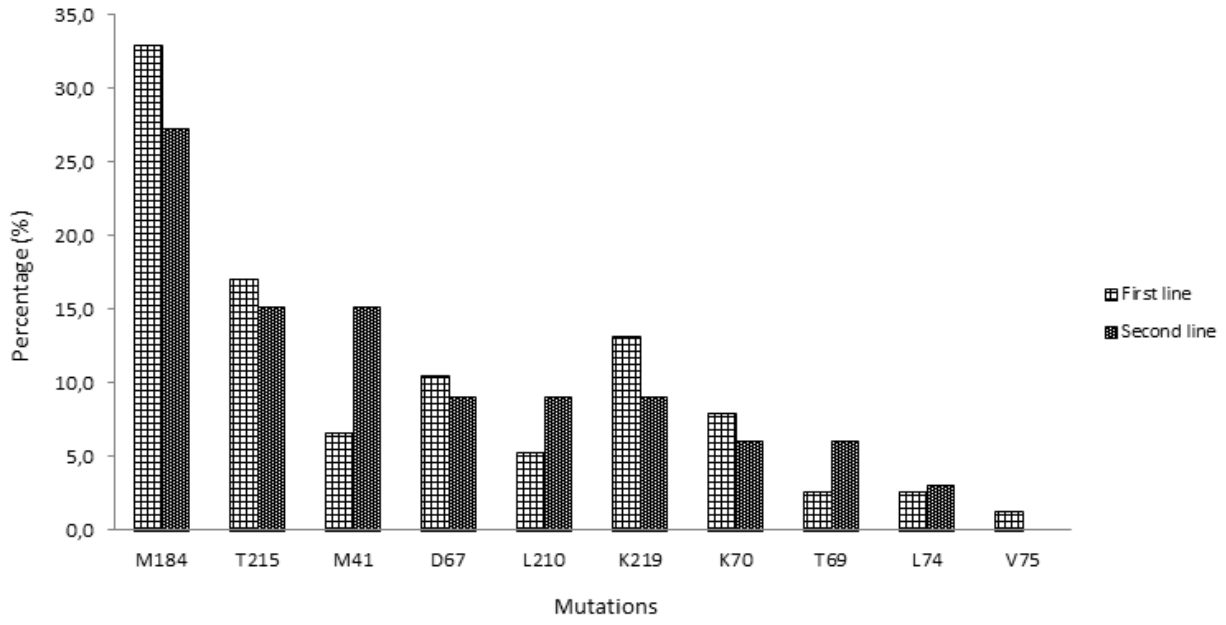

Figure 1: Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTI) associated mutations in first and second line antiretroviral therapy regimen.

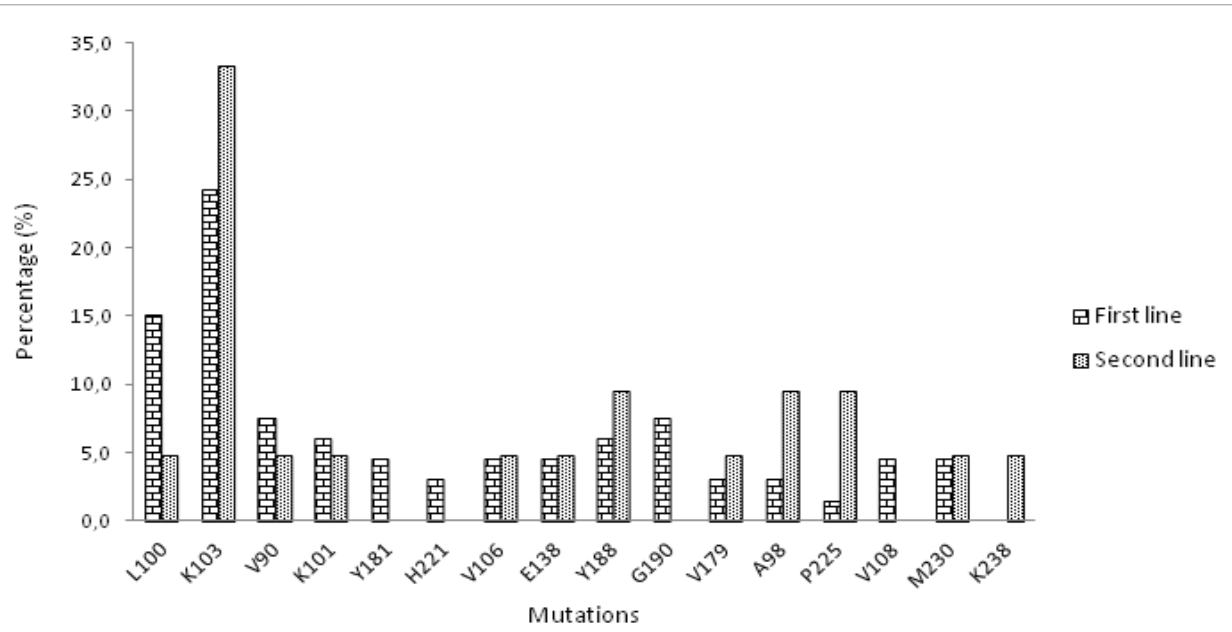

Figure 2: Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI) associated mutations in first and second line antiretroviral therapy regimen.

Resistance to integrase inhibitor and future possible regimens prediction

For the 12 genotyped in integrase gene, no DRM was found and one mutation of polymorphism (L74I) was observed (1/12; 8.3%). However, the future use of INI in third line regimen will be possible only for 6 patients with some NRTIs, NNRTIs second generation and Darunavir still efficient. Among those 6 patients, 4 had viruses still sensitive to NRTIs and 2 to NNRTIs second generation. Among the six remaining patients, there are no efficacy drugs for 2 and for the others a salvage therapy might be possible using co-receptor and fusion inhibitors.

Table 1 summarizes the different efficient drugs that could be used as a third line regimen for each of the 12 patients who had VL ≥ 3 $\log_{10}$ copies/ml at second line.

Phylogenetic analysis

A number of 29 samples were sequenced in full protease and partial reverse transcriptase (RT) genes. Among them, one sample was genotyped only on RT gene and two without overlapping between RT and protease gene. The phylogenetic distribution of these three samples was subtype C^{RT}, CRF02_AG$^{(prot\ \&\ RT)}$ and U/A3^{prot}/A3^{RT} respectively. The most common HIV-1 variant for the 26 overlapping sequences was

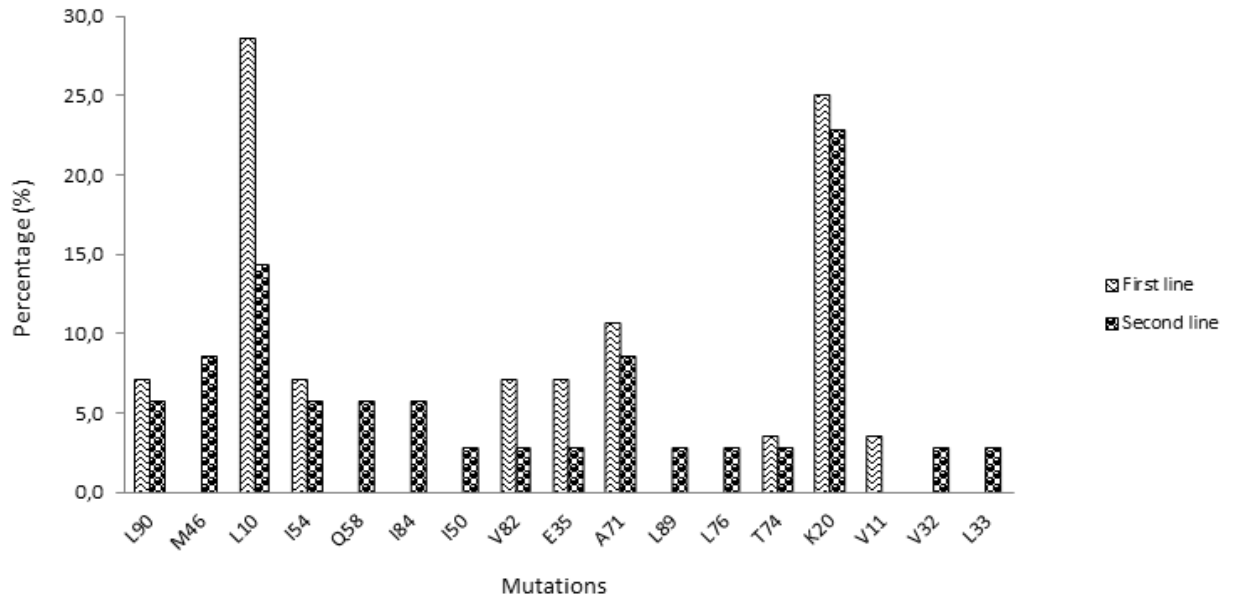

Figure 3: Protease inhibitors (PI) associated mutations in first and second line antiretroviral therapy regimen.

Samples identify	PIs								NRTIs							NNRTIs 1st generation		NNRTIs 2nd generation		Drugs still efficient for a third line regimen with INI
	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r	3TC	ABC	AZT	D4T	DDI	FTC	TDF	NVP	EFV	ETV	RPV	
101_FL	LLR	S	LLR	IR	LLR	HLR	IR	S	HLR	IR	IR	IR	LLR	HLR	S	HLR	HLR	IR	HLR	DRV/r, TPV/r, TDF
101_SL	LLR	S	LLR	IR	LLR	HLR	IR	S	HLR	IR	IR	IR	LLR	HLR	S	HLR	HLR	IR	HLR	
1523_FL	S	S	S	S	S	PLLR	S	S	HLR	IR	IR	IR	IR	HLR	PPLR	HLR	HLR	IR	HLR	ATV/r, DRV/r, FPV/r, LPV/r, NFV
1523_SL	S	S	S	S	S	PLLR	S	S	HLR	LLR	S	S	PLLR	HLR	S	HLR	HLR	IR	HLR	
1837_FL	S	S	S	S	S	S	S	S	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	IR	IR	None efficient drugs
1837_SL	IR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	S	S	S	S	
2107_FL	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NA	NG	NG	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r
2107_SL	S	S	S	S	S	PLLR	S	S	HLR	IR	HLR	HLR	IR	HLR	LLR	HLR	HLR	LLR	LLR	
2401_FL	S	S	S	S	S	S	S	S	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	PLLR	LLR	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r
2401_SL	S	S	S	S	S	PLLR	S	S	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	PLLR	LLR	
478_FL	S	S	S	S	S	PLLR	S	S	HLR	LLR	S	S	PLLR	R	S	HLR	HLR	LLR	IR	DRV/r, AZT, D4T, TDF
478_SL	LLR	S	LLR	LLR	LLR	IR	LLR	IR	S	S		S	S	S	S	S	S	S	S	
2931_FL	S	S	S	S	S	S	S	S	HLR	LLR	S	S	PLLR	HLR	S	HLR	HLR	S	S	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r, AZT, D4T, ETV, RPV
2931_SL	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3259_FL	IR	LLR	IR	IR	IR	HLLR	IR	LLR	HLR	IR	IR	IR	IR	HLR	LLR	S	S	S	S	NVP, EFV, ETV, RPV
3259_SL	HLR	IR	HLR	HLR	HLR	HLR	IR	LLR	HLR	HLR	HLR	IR	HLR	HLR	IR	S	S	S	S	
4039_FL	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HLR	HLR	IR	IR	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r, 3TC, ABC, AZT, D4T, FTC, TDF
4039_SL	S	S	S	S	S	S	S	S	S	S	S	S	PLLR	S	S	S	S	S	S	
8253_FL	S	S	S	S	S	PLLR	S	S	HLR	IR	IR	LLR	LLR	HLR	S	HLR	HLR	S	S	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r, ETV, RPV
8253_SL	S	S	S	S	S	PLLR	S	S	HLR	IR	IR	LLR	LLR	HLR	S	HLR	HLR	S	S	
929_FL	LLR	S	LLR	IR	LLR	HLR	IR	S	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	None efficient drugs
929_SL	HLR	LLR	HLR	HLR	IR	HLR	HLR	IR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	HLR	IR	HLR	
2698_FL	S	S	S	S	S	S	S	S	HLR	LLR	S	S	PLLR	HLR	S	HLR	HLR	LLR	HLR	ATV/r, DRV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r, TPV/r, AZT, D4T, TDF
2698_SL	S	S	S	S	S	PLLR	S	S	HLR	LLR	S	S	PLLR	HLR	S	HLR	HLR	LLR	HLR	

Table 1: Drugs still efficient that could be associated with Integrase Inhibitors (INI) for third line regimen. S: Sensible, LLR: Low level resistance, IR: Intermediate resistance, PLLR: Potential low level resistance, HLR: High level resistance, R: resistance, FS: first line, SL: second line, NG: Not genotyped, PIs: Protease inhibitors, NRTIs: Nucleoside reverse transcriptase inhibitors, NNRTIs: Non-nucleoside reverse transcriptase inhibitors, INI: Integrase inhibitor, 1st: First, 2nd: Second. AZT: Zidovudine, 3TC: Lamivudine, FTC: Emtricitabine, D4T: Stavudine, NVP: Névirapine, EFV: Efavirenz, ETV: Etravirine, RPV: Rilpivirine, DDI: Didanosine, TDF: Tenofovir, ABC: Abacavir, LPV: Lopinavir, ATV: Atazanavir, IDV: Indinavir, r: Ritonavir, TPV: Tripanavir, DRV: Darunavir, FPV: Fosamprenavir, NFV: Nelfinavir, SQV: Saquinavir.

CRF02_AG (n=13; 50%). Many additional variants were identified such as: C (n=5; 19.2%), B (n=2; 7.7%) and one (3.8%) each of the following Circulating Recombinant Forms (CRFs)/subtypes: CRF11_cpx, CRF13_cpx, CRF02_AG/A3, CRF06_cpx/CRF02_AG, U/CRF45_cpx, and D. Overall, the most prevalent variants were CRF02_AG (n=14; 48.2%), C (n= 6; 20.7%) and the Unique Recombinant Forms (URFs) (n=4; 13.8%). Otherwise, the phylogenetic analysis in Protease-partial RT gene sequences obtained from the 12 patients with VL ≥ 3 $\log_{10}$ copies/ml at first and second line, showed the same results both on the first and second line ART. The phylogenetic tree of the 26 overlapping sequences on Protease-partial RT genes is presented in Figure 4 and the Table 2 shows the obtained subtypes/CRFs/URFs for the 12 samples failing both first and second line ART.

For integrase gene, the subtypes and CRFs distribution were as follows CRF02_AG (6/12; 50%), C (2/12; 16.7%) and one (1/12; 8.3%) of each B, D, CRF06_cpx, CRF45_cpx/U. The phylogenetic presented

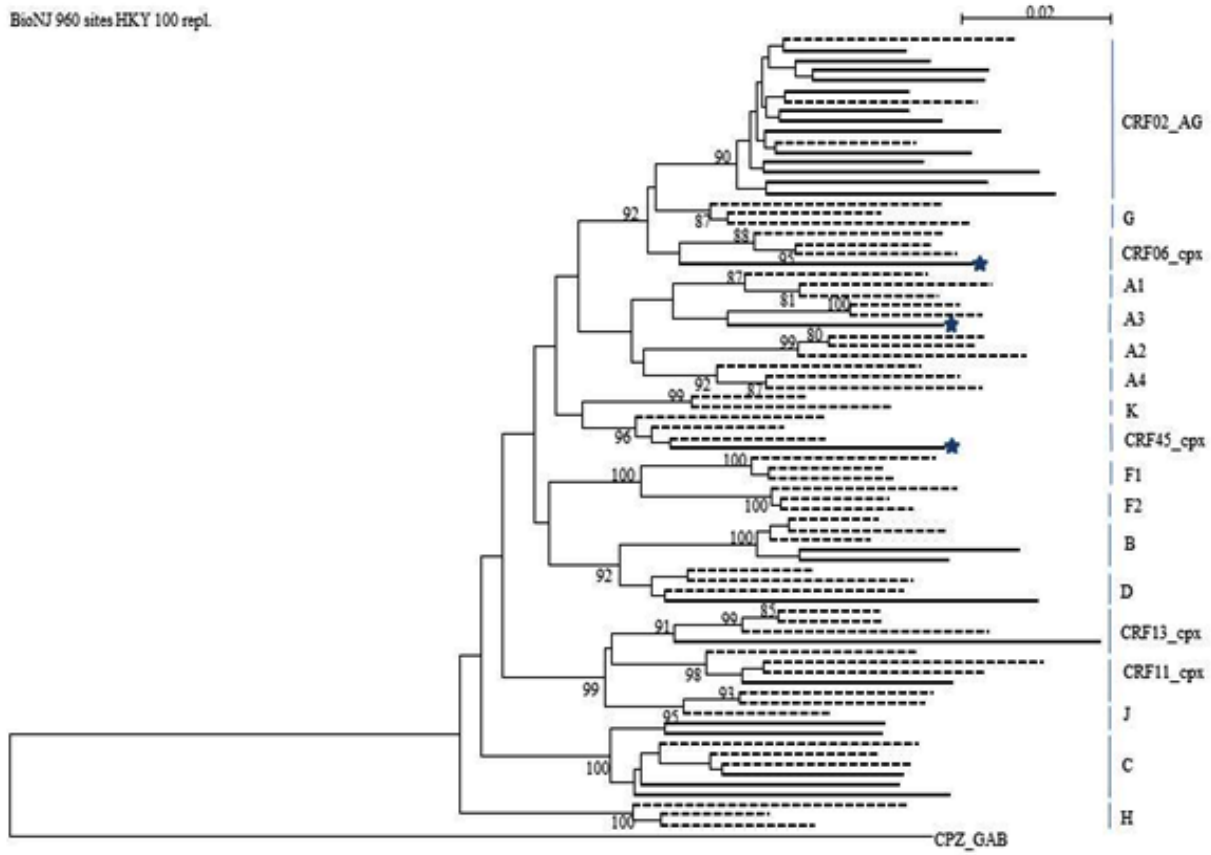

Figure 4: Phylogenetic tree inferred using 26 protease and partial RT sequences alignment (1007pb) showing the relationships between the references sequences (dashed lines) and those of our study (solid lines). Asterisks indicated Unique Recombinant Forms (URFs).

Identify	Protease-partial RT subtype		Integrase subtype
	On first line	On second line	
1523	U/CRF45_cpx	U/CRF45_cpx	CRF45_cpx/U
2401	CRF06_cpx/CRF02_AG	CRF06_cpx/CRF02_AG	CRF06_cpx
2698	CRF02_AG	CRF02_AG	CRF02_AG
2931	C	C	C
3259	D	D	D
929	CRF02_AG	CRF02_AG	CRF02_AG
1837	CRF02_AG	CRF02_AG	CRF02_AG
4039	CRF02_AG	CRF02_AG	CRF02_AG
8253	CRF02_AG/A3	CRF02_AG/A3	CRF02_AG
1393	NG	CRF02_AG	NG
2107	NG	CRF02_AG	CRF02_AG
101	B	B	B
478	C	C	C

NG: Not Genotyped, CRF: Circulating Recombinant Forms, U: Unclassified, RT: Reverse Transcriptase

Table 2: Subtypes comparison of 12 samples in Protease-partial RT and integrase gene.

in Figure 5 showed the subtypes/CRFs/URFS distribution of the 12 integrase gene sequences. Table 2 presents the subtype's distribution of those 12 isolated integrase gene sequences which was concordant between protease-partial RT and integrase genes.

Nucleotide sequence accession numbers

The newly generated sequences: 11 in Protease-partial RT genes and 12 in integrase genes are available in EMBL under the following accession numbers: LM654289, LM654290, LM654291, LM654292, LM654293, LM654294, LM654295, LM654296, LM654297, LM654298, LM654299, LM654300 for the Protease-partial RT gene and LM654301, LM654302, LM654303, LM654304, LM654305, LM654306, LM654307, LM654308, LM654309, LM654310, LM654311, LM654312 for integrase gene. The others 18 Protease-partial RT sequences were previously used and the corresponding accession numbers are: AJ583740, AJ286994, FN599737, FN599691, HE588163, JN673617, JN673570, JN673678, JN673584, JQ855855, JX187611, JX187613, JX187615, KC350002, KC176537, KC350361, KC349986, KC350194.

Discussion

In this study, we reported the prevalence of DRM among 52 HIV-1 infected patients who failed on first line and underwent second line ART; then we documented the HIV-1 genetic diversity and identified the drugs still efficient which could be used in third line regimen.

The majority of patients (71.1%) were on WHO clinical stage 2 and 3 that also seems to be associated with the low median CD4 cell counts both at ART initiation (128 cells/mm^3) and before the second line regimen (153 cells/mm^3). These results suggest that the patients were immune compromised before getting care from the health facilities and could develop opportunistic infections [10]. Thus, strategies and new approaches are needed for an earlier enrollment for care mainly in resources limited-settings (RLS) and it becomes a major challenge for the national ART programs [11,12]. Despite the unavailability of VL in remote areas in Senegal, HIV-1 infected patients were followed using VL testing in the main site in the capital city. Our results shows a high rate of VF (82.7%) during the first line ART as previously described in Nigeria [13], which was the main reason for switching of line regimen

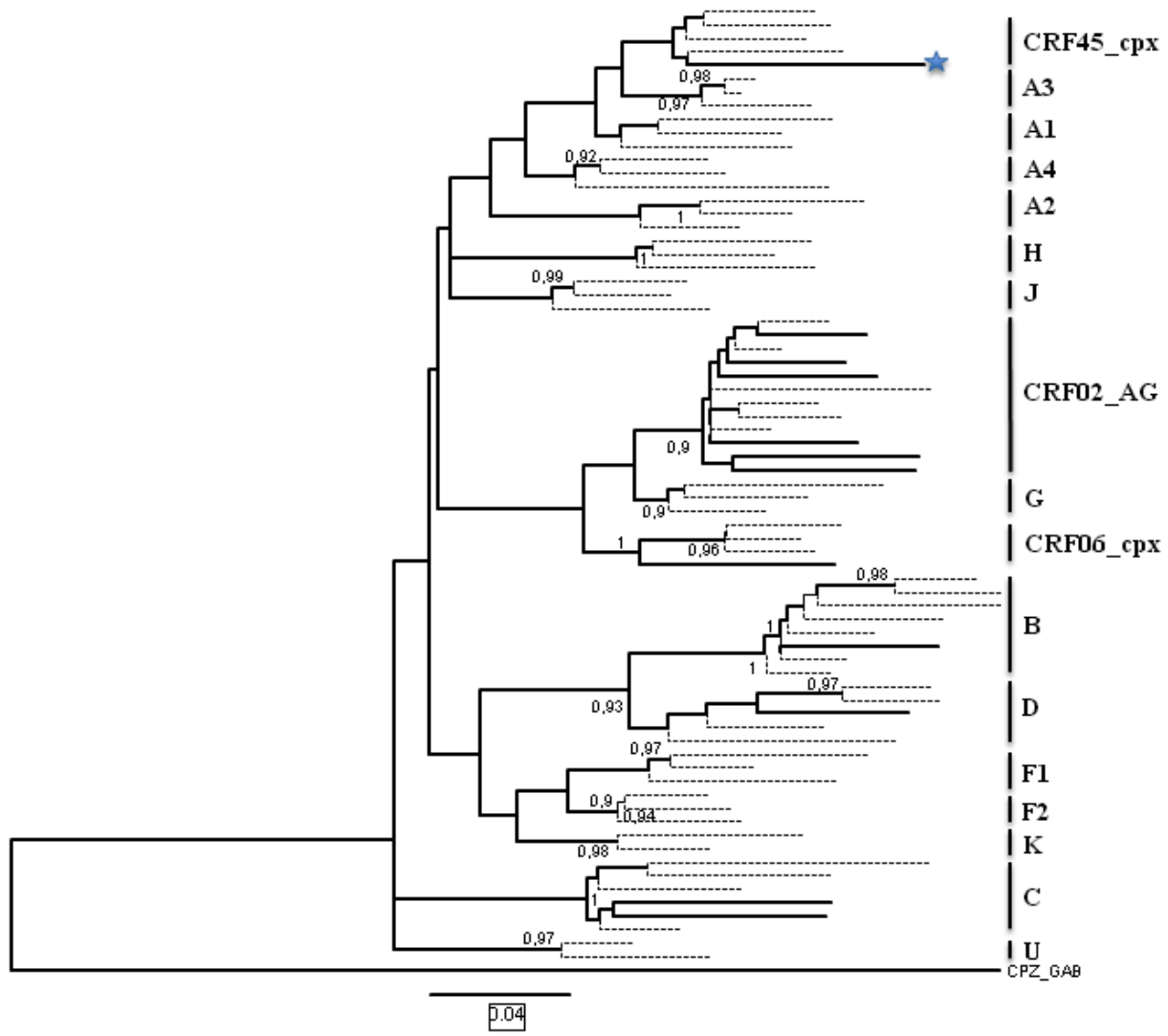

Figure 5: Phylogenetic tree inferred using 12 integrase sequences alignment (865pb) showing the relationships between the references sequences (dashed lines) and those of our study (solid lines).

[14,15]. However, our data showed nine patients switched to second line regimen with three without VL testing and six with plasma viral load below 3 $\log_{10}$ copies/ml. According to virological criteria, these patients were unnecessarily switched to second line therapy. These findings were also highlighted by Sigaloff and colleagues in 13 clinical sites in six African countries. The accuracy of switch based only on clinico-immunological monitoring may be often low [16] and in our context, the turnaround time of the viral load results could be compromised by the procurement of reagents. After 32 months of PIs-exposure median time, 75% of patients achieved virological suppression (VL ≤ 40 copies/ml), which is lower than the proportion reported by Patel and colleagues in India (82% at 12 months treatment duration) [17].

As previously reported on *pol* gene in Senegal [18,19] and on integrase gene in samples from different African Countries [9], the phylogenetic analysis shows a predominance of CRF_02AG in our study (Figures 4 and 5). The second major strain was subtype C in *pol* gene which is the predominant in MSM group in Senegal [20]. The URF were also found in 17.2% cases similarly described in Senegal [19,21].

For 55 months of median ART follow up duration, at least one DRM was found in 55.8% (n=29/52) of patients on first line, which is not significantly different (p=0.07) that previously observed in Senegal [7]. Similar results have been reported in Cameroon [22,23], in Republic of Central Africa [24] and in Republic of South Africa [25]. These observations show an importance to better manage patients undergoing ART by physicians for an earlier suppression of VL. Hence, the proportion of patients who should be switched decreased in order to avoid the irrational use of second line ART, which is more expensive [26]. For the patients under second line ART, 12 among 29 HIV-1 infected patients (41.9%) had at least one DRM after 32 months of median duration. Reported to the study population, the prevalence of DRM was (23.07%; 12/52). This finding is significantly lower than previously reported in Senegal (p<0.01) and Mali (p<0.01) [7,27]. The differences could be explained by the longer second line ART median duration (4 years) for the study conducted in Mali and the limited sample size of the study in Senegal. The rates of DRM for the NRTIs+NNRTIs, NRTIs+PIs and NRTIs+NNRTIs+PIs combinations were respectively 53.8%, 84.6%, and 61.5%. The high rates of NRTIs+NNRTIs-associated DRM (53.8-96.5%) both for the first and second line ART was due to the re-emergence of archived mutations as a result of first-line ART failure. The rates of other combinations with PIs are similar to those obtained by Saravanan in India [28,29].

The major DRMs found on Protease-partial RT genes for patients on first and second line ART as mentioned in the Figures 1 and 2, were respectively TAMs (85.5% vs 90.9%), M184V (32.9% vs 27.3%) for NRTIs; K103N (24.2% vs 33.3%) for NNRTIs. Except the TAMs, the rates of these DRM in our study were lower than those found in a systematic review in RLS with different rates: (5-20%) for TAMs, 65% for M184V and 52% for K103N [30], where the first line regimen includes 2NRTIs+NNRTI and 2NRTIs+PI for the second line. For PIs, the L90M (7.1%) was found in only two patients on first line and 5.7% on second line (Figure 3). While being a prevalent PIs-associated DRM in our study, the rate of L90M mutation is lower than that observed in India [29]. In our study, the M46I (8.6%) was the most prevalent such as in several studies from India [31,32]. For the integrase gene, 12/13 samples of patients at second line VF, successfully genotyped were susceptible to all integrase inhibitors. Despite those patients were not exposed to IN inhibitors, one among them harbored accessory mutation L74I (1/12; 8.3%). This rate was twofold higher that found Monleau in naïve patients in Sub-Sahara [9].

Based on our generated data and in order to improve the therapeutic follow up of patients, the salvage antiretroviral drugs, which may consist of NRTIs, NNRTIs and PIs could be used. As shown in Table 1, the majority of the PIs with a good listing of Darunavir (DRV/r), remain effective to at least nine patients. Some studies have highlighted the efficacy of DRV/r [28,29]. In addition, 4/12 and 6/12 patients show their susceptibility to some NRTIs and NNRTIs second generation respectively. Even if the use of third line therapy with IN inhibitors will be the next possibility, it required to have NRTIS, NNRTIs and PIs still effective. A randomized clinical trial showed the efficacy of association integrase inhibitors with second generation NNRTI and PIs [33]. Two limitation of this study are the small sample size and most of the collected patients were from CTA.

Conclusion

The study showed a high rate of drug resistance mutations for patients under first and second line ART. These findings highlighted the importance to reinforce virological monitoring of HIV-1 infected patients and to consider the drug resistance results for a salvage antiretroviral drug and a third line regimen efficacy prediction including INI as recommended by WHO.

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References

1. Fibriani A, Wisaksana R, Indrati A, Hartantri Y, van de Vijver D, et al. (2013) Virological failure and drug resistance during first line anti-retroviral treatment in Indonesia. J Med Virol 85: 1394-1401.
2. WHO guidelines (2013) The use of antiretroviral for treating and preventing HIV infection. Recommendations for a public heath approach.
3. Stellbrink HJ (2009) Raltegravir in the management of HIV-infected patients. Drug Des Devel Ther 2: 281-288.
4. Laurent C, Diakhaté N, Gueye NF, Touré MA, Sow PS, et al. (2002) The Senegalese government's highly active antiretroviral therapy initiative: an 18-month follow-up study. AIDS 16: 1363-1370.
5. De Beaudrap P, Thiam M, Diouf A, Toure-Kane C, Ngom-Guèye NF, et al. (2013) Risk of virological failure and drug resistance during first and second-line antiretroviral therapy in a 10-year cohort in Senegal: results from the ANRS 1215 cohort. J Acquir Immune Defic Syndr 62: 381-387.
6. WHO (2010) Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach: 2010 revision. World Health Organization, Geneva, Switzerland.
7. Thiam M, Diop-Ndiaye H, Diouf AD, Vidal N, Ndiaye O, et al. (2013) HIV-1 genetic diversity and drug resistance among Senegalese patients in the public health system. J Clin Microbiol 51: 578-584.
8. Mandelbrot L, Berrebi A, Rouzioux C, Partisani M, Faucher P, et al. (2014) [Reproductive options for people living with HIV: 2013 guidelines from the French expert working group]. Gynecol Obstet Fertil 42: 543-550.
9. Monleau M, Aghokeng AF, Nkano BA, Chaix ML, Peeters M; AC11/AC12 ANRS Working Group (2012) Drug resistance mutations of HIV type 1 non-B viruses to integrase inhibitors in treatment-naive patients from sub-saharan countries and discordant interpretations. AIDS Res Hum Retroviruses 28: 1157-1160.
10. Ogouyemi-Hounto A, Zannou D, Metodakou D, Lafia B, Gomez V, et al. (2010) [Laboratory testing including CD4 T-cell count and determination of viral load to evaluate the impact of first line antiretroviral treatment at 6 months in adults in Benin]. Med Trop (Mars) 70: 100.
11. Keiser O, Chi BH, Gsponer T, Boulle A, Orrell C, et al. (2011) Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. AIDS 25: 1761-1769.

12. Harries AD, Zachariah R, van Oosterhout JJ, Reid SD, Hosseinipour MC, et al. (2010) Diagnosis and management of antiretroviral-therapy failure in resource-limited settings in sub-Saharan Africa: challenges and perspectives. Lancet Infect Dis 10: 60-65.

13. Onyedum CC, Iroezindu MO, Chukwuka CJ, Anyaene CE, Obi FI, et al. (2013) Profile of HIV-infected patients receiving second-line antiretroviral therapy in a resource-limited setting in Nigeria. Trans R Soc Trop Med Hyg 107: 608-614.

14. Fox MP, Shearer K, Maskew M, Macleod W, Majuba P, et al. (2012) Treatment outcomes after 7 years of public-sector HIV treatment. AIDS 26: 1823-1828.

15. Hosseinipour MC, Kumwenda JJ, Weigel R, Brown LB, Mzinganjira D, et al. (2010) Second-line treatment in the Malawi antiretroviral programme: high early mortality, but good outcomes in survivors, despite extensive drug resistance at baseline. HIV Med 11: 510-518.

16. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, et al. (2011) Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr 58: 23-31.

17. Patel D, Desai M, Shah AN, Dikshit RK (2013) Early outcome of second line antiretroviral therapy in treatment-experienced human immunodeficiency virus positive patients. Perspect Clin Res 4: 215-220.

18. Toure-Kane C, Montavon C, Faye MA, Gueye PM, Sow PS, et al. (2000) Identification of all HIV type 1 group M subtypes in Senegal, a country with low and stable seroprevalence. AIDS Res Hum Retroviruses 16: 603-609.

19. Diouara AA, Diop-Ndiaye H, Kebe-Fall K, Tchiakpè E, Ndiaye O, et al. (2014) Dried blood spots for HIV-1 drug resistance genotyping in decentralized settings in Senegal. J Med Virol 86: 45-51.

20. Ndiaye HD, Tchiakpe E, Vidal N, Ndiaye O, Diop AK, et al. (2013) HIV type 1 subtype C remains the predominant subtype in men having sex with men in Senegal. AIDS Res Hum Retroviruses 29: 1265-1272.

21. Kebe K, Thiam M, Diagne Gueye NR, Diop H, Dia A, et al. (2013) High rate of antiretroviral drug resistance mutations in HIV type 1-infected Senegalese children in virological failure on first-line treatment according to the World Health Organization guidelines. AIDS Res Hum Retroviruses 29: 242-249.

22. Aghokeng AF, Kouanfack C, Eymard-Duvernay S, Butel C, Edoul GE, et al. (2013) Virological outcome and patterns of HIV-1 drug resistance in patients with 36 months' antiretroviral therapy experience in Cameroon. J Int AIDS Soc 16: 18004.

23. Kouanfack C, Montavon C, Laurent C, Aghokeng A, Kenfack A, et al. (2009) Low levels of antiretroviral-resistant HIV infection in a routine clinic in Cameroon that uses the World Health Organization (WHO) public health approach to monitor antiretroviral treatment and adequacy with the WHO recommendation for second-line treatment. Clin Infect Dis 48: 1318-22.

24. Péré H, Charpentier C, Mbelesso P, Dandy M, Matta M, et al. (2012) Virological response and resistance profiles after 24 months of first-line antiretroviral treatment in adults living in Bangui, Central African Republic. AIDS Res Hum Retroviruses 28: 315-323.

25. El-Khatib Z, Ekstrom AM, Ledwaba J, Mohapi L, Laher F, et al. (2010) Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. AIDS 24: 1679-1687.

26. Long L, Fox M, Sanne I, Rosen S (2010) The high cost of second-line antiretroviral therapy for HIV/AIDS in South Africa. AIDS 24: 915-919.

27. Maiga AI, Fofana DB, Cisse M, Diallo F, Maiga MY, et al. (2012) Characterization of HIV-1 antiretroviral drug resistance after second-line treatment failure in Mali, a limited-resources setting. J Antimicrob Chemother 67: 2943-2948.

28. Saravanan S, Vidya M, Balakrishnan P, Kantor R, Solomon SS, et al. (2012) Viremia and HIV-1 drug resistance mutations among patients receiving second-line highly active antiretroviral therapy in Chennai, Southern India. Clin Infect Dis 54: 995-1000.

29. Saravanan S, Madhavan V, Balakrishnan P, Smith DM, Solomon SS, et al. (2013) Darunavir is a good third-line antiretroviral agent for HIV type 1-infected patients failing second-line protease inhibitor-based regimens in South India. AIDS Res Hum Retroviruses 29: 630-632.

30. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM (2010) Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis 10: 155-166.

31. Kandathil AJ, Kannangai R, Verghese VP, Pulimood SA, Rupali P, et al. (2009) Drug resistant mutations detected by genotypic drug resistance testing in patients failing therapy in clade C HIV-1 infected individuals from India. Indian J Med Microbiol 27: 231-236.

32. Gupta A, Saple DG, Nadkarni G, Shah B, Vaidya S, et al. (2010) One-, two-, and three-class resistance among HIV-infected patients on antiretroviral therapy in private care clinics: Mumbai, India. AIDS Res Hum Retroviruses 26: 25-31.

33. Yazdanpanah Y, Fagard C, Descamps D, Taburet AM, Colin C, et al. (2009) High rate of virologic suppression with raltegravir plus etravirine and darunavir/ritonavir among treatment-experienced patients infected with multidrug-resistant HIV: results of the ANRS 139 TRIO trial. Clin Infect Dis 49: 1441-1449.