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 ≥ 3log10 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 log10 copies/ml and 55 vs 32 months. The most common therapeutic combinations were 2 NRTI (D4T/3TC+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 ≥ 3log10 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 data 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 log10 (40) copies/ml. In case of VL, 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/mm3 [n=50, (IQR, 2-566)] vs 32 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 log10 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 log10 copies/ml.

Drug resistance mutations (DRM)

A total of 29 and 13 samples were successfully genotyped on Protease-partial RT genes for the first 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 were (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 L190M (2/28; 7.1%) and two other the both (15A4V; 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 profiles 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’s 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 (28/29; 96.5%) and 12/29 (41.4%) for the NRTI+PI and NRTI+NNRTI+PI combinations respectively.

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Volume 6(3): 127-134 (2014) - 128

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 log10 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\textsuperscript{r}, CRF02\_AG\textsuperscript{mnr04004} and U/A3\textsuperscript{mr}/A3\textsuperscript{r} 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.

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 log₁₀ 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).](image)

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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


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).
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 clinic 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_02_AG 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 regime includes 2NRTIs+NNRTI and 2NRTIs+Pis 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 naive 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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