Genotypic diversity and mutation profile of HIV-1 strains in antiretroviral treatment (ART) -Naïve Ghanaian patients and implications for antiretroviral treatment (ART)

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Forty samples collected from HIV-1 AIDS and asymptomatic patients in 2002 and 2003 before the initiation of antiretroviral treatment (ART), most in poorly investigated regions of Ghana were retrospectively examined. For baseline data on genetic diversity before therapy, env, gag and pol phylogenetic analysis was generated. pol was further analysed for potential resistance to antiretroviral drugs. Plasma viral load was also quantified. HIV-1 subtypes A1 and G constituted a minority, while simple and/or complex recombinant strains dominated by CRF02_AG and including CRF06_cpx, CRF09 and CRF10 was observed. Major mutations, protease (PR) L33F and reverse transcriptase (RT) M41L, associated with high levels of phenotypic resistance or clinical evidence for reduced virological response were identified. In the protease-coding domain, positions 10 and 11 were mutated in 21% (5/24) of patients examined, carrying putative resistance to PIs. In the reverse transcriptase-coding domain, seven mutations were detected in 6/35 patients (17%). Of these, 8.5% (3/35) had either double or single mutations possibly affecting NRTIs or NNRTIs respectively. Within PR and RT regions, the mutation rate ranged between 2.6 and 7.0%. Position 10 had 57% mutation frequency. The mutational profile of our non-B HIV-1 strains were identical with subtype B strains at documented DR-related positions. Therefore, drug resistance (DR) pathways of non-B strains were presumed similar to subtype B viruses. Although antiretroviral therapy was expected to be successful, patients presenting resistance-related mutations prior to the initiation of ART could develop clinically relevant resistance earlier than wild-type strains.

Key words: HIV-1 genotyping, antiretroviral therapy, drug-naive HIV-1 patients, drug resistance.

INTRODUCTION

HIV-1 which accounted for AIDS disease in North America, Europe and Asia, was identified in Ghana in the early 90s (Mitchell et al., 1991). HIV-1 has since become predominant, significantly obscuring HIV-2 (Bonney et al., 2008; HSS Report, 2008, 2009). In 2010, the median prevalence of single HIV-1 and HIV-2 was 96.0 and 1.4% of AIDS cases, respectively, while dual infection occurred in 2.6% (HSS Report, 2011). HIV-1 subtypes A and G were subsequently reported in southern Ghana (Ishikakwa et al., 1996; Brandful et al., 1998), after which Ghana’s HIV landscape evolved with the identification of simple and/or complex recombinant strains, dominated by HIV-1 CRF02_AG (Fischetti et al., 2004; Brandful et al., 2007). HIV-1 CRF06_cpx (Brandful et al., 2007; Montavon et al., 2002) and unique recombinant forms

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URFs (Sagoe et al., 2007; Delgado et al., 2008) were also observed.

In Ghana, HIV Sentinel Survey (HSS) among pregnant women at selected antenatal clinics (ANC) has served as a proxy of the spread of HIV infection in the population. Since 2004, HSS data has been calibrated with Demographic and Health Survey (DHS) data and other indicators to estimate the National HIV Prevalence (NACP, GHS, 2011). Ghana’s median HIV prevalence (MHP) was thus estimated at 2.1% in 2011 and 2.0% in 2010, constituting an adult population of ~198,000 (HSS Report 2011). From 2000, the MHP has fluctuated between 2.2% in 2008, constituting an HIV population of 236,000 and 3.6% in 2003 representing a population of 392,000. Generally, Ghana’s HIV prevalence level has slowly declined, evidenced by linear trend analysis (HSS Report, 2011). Anti-retroviral therapy (ART) commenced in June 2003 at five sites in southern Ghana, including the Eastern Region with the highest HIV median prevalence of 3.2% (HSS Report, 2010). ART followed guidelines specifying first and second line treatment drugs, each line with two choices and two options, constituting a triplet of nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Ghana Health Service GHS, 2008). In 2010, the HIV population was ~198,000. Treatment sites numbered 150 countrywide under a scale-up programme providing ART to 33,418 AIDS patients out of 94,791 patients who needed therapy. This amounted to 34.9% coverage (HSS Report 2010).

With the co-circulation of HIV-1 subtypes A1 and G, simple and/or complex recombinant strains dominated by CRF02_AG and URFs in southern Ghana, scant data on genotypic diversity of HIV-1 in other regions of the country, as well as the absence of ART, 40 samples obtained from HIV-1 ARV-naive patients in 2002/3, most in mid and northern Ghana were used for a retrospective exploratory study to generate baseline data on HIV-1 molecular epidemiology and prevalence of drug resistance (DR) strains circulating prior to the initiation of ART.

PATIENTS AND METHODS

Forty patients with AIDS-like clinical presentations were recruited after informed consent between 2002 and 2003, from Tamale Regional and Nandom District Hospitals, both located in the Northern Region (prefix ‘NT’ and ‘NNA’ respectively). Others were from Sunyani Regional Hospital in the Brong Ahafo Region (‘NSU’), Komfo Anokye Teaching Hospital, Kumasi in the Ashanti Region (‘NKS’) in the middle belt. Four (10%) samples from 37 Military Hospital in Accra, southern Ghana, were included for comparison. All patients were ART naive.

Plasma isolation and HIV serology

Briefly, 7 ml of blood was drawn from the patients into K2-EDTA tubes, kept at 4°C and transported to the Virology Department, Noguchi Memorial Institute for Medical Research (NMIMR) within 24 hours. Plasma was harvested and 1ml aliquots were prepared for serology. The rest was kept at -40°C until needed. Serological screening was performed with HIV-1/HIV-2 InstantScreen® (GAIFAR, Potsdam, Germany) and Determine HIV-1/2 (Abbott Diagnostics, USA) according to the manufacturers’ instructions. Confirmatory testing and typing were done with the immunoblot, PeptLav I/II (Diagnostic Pasteur, Marnes-la-Cochette, France).

Genomic RNA extraction and quantification

In brief, 50 µl total RNA was eluted using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Manheim, Germany) as instructed and quantified by real-time (RT)-PCR MX-3000® Q-PCR system (Stratagene, La Jolla, CA, USA) as previously reported (Fischetti et al., 2004).

Amplification of gag, env and pol

RNA was reverse-transcribed with 10 U AMV RT (50 U/µL) (Promega Corporation, Madison, USA) and amplified in the p7 and p24 coding domains of gag, primed with 10 pmols each of Hig1777 (5′- TCACCTAGACTTGAATGCAATGGG-3′) (HXB2 1231-1255) and HIP202 (5′- TTAATCATTATCTACGTCCTGT-3′) (HXB2 2328-2352) in 10X buffer, 5 mM MgCl2 (25 mM), 0.4 mM dNTPs (20 mM) and 2.5 U Super-Therm DNA polymerase (5 U/µL) (Southern Cross Biotechnologies) in 50 µL. The PCR reaction was carried out as follows: one cycle of 94°C, 5 min preceded 35 cycles of 94°C 30 s, 55°C 30 s, 72°C 1.5 min and 72°C for 10 min. Round 2 PCR was performed with a reduced annealing temperature of 50°C, 2 – 3 µL of Round 1 product and final concentrations of 10 pmols each of Hig1584 (5′- AAAAGATGGGATAATCTGG-3′) (HXB21577-1595) and g17 (5′- TCCACATTCACACCCCTTTTTT-3′) (HXB2 1977-2040), generating approximately a 463 bp fragment.

The env gp41 transmembrane (TM) domain was amplified as previously described (Cilliers et al., 2005), yielding approximately a 700 bp product. For the pol gene cDNA was generated with 50U of Expand RT (50 U/µL) Roche and amplified as described by Steegen et al. (2006), with modifications in primer sets. Amplification of PR and RT-coding domains was primed with PR1 (5′- ATGATGAGAGAGCCATT-3′) (HXB2 1918-1937) and 3RT (5′- TATTCTGCTATTAGTGCTTTTGATGGGTA-3′) (HXB2 3506-3536) and an inner primer set, PoFr2 (5′- CRGARCCAAAAAGCCCCACCDG-3′) (HXB2 2147-2167) and RT3303 (5′- TAATTTTGTATRCTGAC-3′) (HXB2 3309-3329). One round of 94°C for 4 min was followed by 10 cycles each at 94°C for 30 s; 45°C for 30 s; 68°C for 1 min and 94°C for 30 s; 50°C for 30 s; 68°C for 2 min. Finally, 15 cycles of 94°C for 30 s; 55°C for 30 s; 68°C for 3 min and a single cycle of 68°C for 10 min were performed, yielding approximately a 1.18 kb fragment.

Purification of PCR amplicons and sequencing

PCR amplicons were gel-purified with QIAquick Gel Extraction Kit (Qiagen, Germany) as directed. The ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Kit, version 1.0 (Applied Biosystems Inc., CA, USA) was used as instructed, the amplified DNA was isopropanol precipitated and sequenced with the BigDye Terminator System in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Inc., CA, USA).

Sequence analyses and phylogenetic tree construction

ABI files were edited with Sequence Navigator and Fasta (word)
formatted. Multiple sequence alignments were done against reference sets from Los Alamos gene bank (http://www.hiv-web.lanl.gov/content/index) with Clustal W for McVector. The alignment files were edited in Se-Al v2.0. Bootstrap analysis was done with PAUP using 1000 re-samplings for gag, env and pol phylogenetic trees. Unclassified and/or unique recombinant strains were subjected to web-based tools namely NCBI HIV-1 Subtyping Tool http://www.ncbi.nlm.nih.gov/projects/genotyping/form page1.cgi and REGA HIV-1 Subtyping Tool Version 2.0 (http://www.Bioafrika.net/ virus-genotype/ html/).

Mutational analysis

Genetic variability was investigated by documenting nucleotide base changes in the PR and RT-coding domains of pol. DR related mutations documented for HIV-1 subtype B strains, on which ARVs were designed (Hirsh et al., 2008), were used as reference for comparison with mutations observed in our non-B samples.

RESULTS

Patient information

The patients’ information is documented in Table 1. Eighteen (45%) were from Tamale (NT) and Nandom (NNA), 9 each (22.5%) from Kumasi (NKS) and Sunyani (NSU), while 4 (10%) were from Accra (MHE) for comparison. All patients were for ARV naive and aged 25 to 60 years, except a baby of 6 months (NT34) born to a seropositive mother (NT33). Thirty eight or 95% were seropositive for HIV-1 infection and 2 patients (5%) were dually seroreactive for HIV-1 and HIV-2. Thirty three or 83% had AIDS-defining symptoms, including unexplained weight loss, chronic diarrhoea, persistent cough and fever of 39°C or higher. Additionally, 7 or 17% were asymptomatic.

Patient viral load

Thirty eight (95%) of 40 patients had a median viral load (MVL) of 3.10E+06 IU/ml (range: 3.10E+04 to 7.30E+06 IU/ml, SD=1.24E+09 IU/ml) (Table 1). Of these, 84% (32/38) symptomatic and 16% (6/38) asymptomatic patients respectively had MVL of 3.30E+06 IU/ml (range: 4.00E+05 to 7.30E+09 IU/ml, SD=1.93E+09 IU/ml) and 4.50E+05 IU/ml (range: 3.10E+04 to 3.20E+06 IU/ml, SD=1.18E+06 IU/ml). The difference between the symptomatic and asymptomatic groups was not statistically significant (p=0.414).

Genotypic diversity, phylogenetic analysis and subtype viral load

Genotyping of 36 strains was done from relatively short informative sequences of ~450 nts. Thirty one (86%) were subtyped in 2 or 3 genomic regions and five (14%) (NT23, NT49, NT50, NSU27, MHE43) were subtyped in one genomic region. Four (NT09, NT43, MHE42, MHE45) each gave different designations (unt ypable). Of the 36 subtyped samples, twenty three (64%) were CRF02_AG, 6 (17%) (NT15, NT17, NT22, NT42, NSU28, MHE19) were CRF06_cpx, 3 (8%) (NSU21, NSU27, NKS54) were A1 and one each (3%) was CRF09 (NSU26), CRF10 (MHE43) or subtype G (NKS25). Seventeen (42%) strains comprising 15 CRF02_AG and 2 CRF06_cpx constituted complete concordant phylogenetic clusters in all three genomic regions examined, that is “pure recombinants” (Table 1).

Figure 1 is a gag bootstrap tree of 36 samples. NT09 and NT43 were genotyped as G/CRF06-like grouping most closely with the G/CRF06 cluster and strongly supported by 85% bootstrap replicates. Bootstrap trees of 35 env gp41 and 36 pol (RT) sequences are shown in Figures 2 and 3, respectively. In env gp41, NT09 and NT43 conclusively clustered with CRF06_cpx and CRF02_AG respectively (Figure 2; Table 1) while in pol, NT09 was classified as CRF02_AG and NT43 as CRF06_cpx (Figure 3; Table 1). MVL for the subtypes identified were: 4.40E+06 IU/ml for twenty-one CRF02_AG strains, 2.30E+06 IU/ml for seven CRF06_cpx strains and 8.55E+06 IU/ml for two A1 strains. One each was either subtype G or CRF09 recombinant with a viral load of 1.30E+06 IU/ml and 1.40E+06 IU/ml respectively. Between CRF02_AG and CRF06_cpx, the difference in MVL was statistically significant (p=0.029) but not between CRF02_AG and the other subtypes (A1, G and CRF09) with P values of 0.186, 0.325 and 0.312, respectively.

Mutational profile and sample genotype

PR-coding domain

Five mutations potentially affecting protease inhibitors (PIs) were seen in 15% (6/40) patients. L10I was identified in two (5%) patients (NSU25, NSU26) and L10V and L10M in one patient (2.5%) each, which is NT15 and NSU28, respectively. At position 11, two (5%) patients (NT34, NSU28) had V11I mutation, while one (2.5%) (NSU22) had L33I. In NSU28, we detected L10I and V11I simultaneously (Table 1). Three (7.5%) patients (NT34, NSU22, NSU25) with PI-directed mutations were ‘pure’ CRF02_AG strains. Two (5%) (NSU28, NT15) were infected with CRF06_cpx, while NSU26 alone had CRF09 infection.

RT-coding domain

Seven mutations were observed among 15% (6/40) patients at HIV-1 subtype B DR positions 41, 67, 69 70, 179 and 188 (Table 1). Five double mutations in three patients, potentially affecting NRTIs were M41L/T69P (MHE42), D67G/K70W (NKS25) and D67V/T69P (NSU29).
Table 1. Patient information.

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</table>
Mutations potentially affecting NNRTIs were detected also in three (7.5%) patients, namely NKSS4 (V179F) and NKSS2 and NSU23 (Y188F) (Table 1). Three of the six patients (7.5%) (NSU23, NSU29, NKSS2) with RT-directed mutations were infected with ‘pure’ CRF02_AG strains. NKSS25 and NKSS4 were respectively infected with subtype G and A1 strains. MHE42 was infected with different strains in each gene investigated and was therefore untypable. (Table 1).

**DISCUSSION**

Forty patients, most from mid and northern Ghana, were recruited. Incomplete patient information from non-availability of clinical notes affected data-gathering slightly although 63 and 75% of patients had information regarding age and gender respectively, (Table 1). Except for NSU27, designated as HIV-1 subtype A1, all 40 samples were recombinant in one or more of the key genes analyzed. Apart from the ‘pure’ CRF02_AG or CRF06_cpx strains, specific cases of complex mosaic forms observed included MHE42 which clustered with consensus HIV-1 subtype D in gag, CRF02_AG in env gp41 and CRF10 in pol (D/AG/CRF10) and NT24 which was C_CRF07 genotype in pol and CRF02_AG in gag and gp41 that is C_CRF07/AG. NSU26 and NKS02 were AG/CRF09 recombinant, while URFs were exemplified by NKS25, NSU26, NKS02, NT17, NT22 and others. Previously analyzed pol sequences of samples collected in 2003 and 2004 also recorded a high prevalence of URFs in Ghana (Delgado et al., 2008). Thus, over approximately 5 years, the HIV profile saw the emergence of recombinants despite the low median adult prevalence of less than 5% (HSS Reports 2005, 2008, 2009, 2010, 2011).

Various reasons presumably contributed to the apparent shift from pure strains to recombinant and other mosaic forms in Ghana. Pre-existing epidemiologic and genetic conditions at the start of this study were permissive for recombination and evolution of new HIV-1 variants. These included the co-circulation of diverse HIV-1 subtypes (Brandful et al., 1998; Fischetti et al., 2004), the characteristically high replication rate and re-infections (Requejo, 2006) and higher transmission efficiency of recombinants (Njai et al., 2006; Konings et al., 2006) as reported for CRF02_AG which possessed a higher replicative capacity than its parental subtypes A and G (Konings et al., 2006). A high viral load also reportedly promoted transmission (Fischetti et al., 2004). Our CRF02_AG strains had a high MVL of 4.40E + 06 IU/ml (SD=5.43E + 08 IU/ml), similar to CRF06_cpx and CRF09, as well as the parental subtypes A1 and G, all of 6 logs magnitude, at least. Moreover, CRF02_AG, CRF06 and CRF09 which were all identified in this study, each

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Table 1. Contd.

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a, NT34 was a 6-month old baby of patient NT33; S, symptomatic; A, asymptomatic, nk: not known, na: not available. CRF06 = Complex HIV-1 recombinant CRF06_cpx; AG = CRF02_AG recombinant; ^CRF09 = complex recombinant comprising genomic fragments of HIV-1 subtypes A, G.
Figure 1. Bootstrap tree of 36 Ghanaian HIV-1 specimens (shaded diamonds) aligned with reference strains (open black diamonds) in gag gene.
comprising genomic segments of HIV-1 subtypes A and G, were related (McCutchan et al., 2004) and had a low genetic barrier between them which facilitated recombination (Magiorkinis et al., 2003). It is possible, however, that recombinants prevailed before or around the time of sample collection for this study because in
Figure 3. Bootstrap tree of 36 Ghanaian HIV-1 specimens (black diamonds) aligned with reference strains (open black circles) on 450 nucleotides of the pol/RT gene.
1994/1996, the prototype IBNG strain of CRF02_AG had been described (Howard et al., 1994; Gao et al., 1996). Similarly CRF06_cpx was described in 2002/2003 (Mamadou et al., 2002, 2003) and CRF10 in 2001 (Koulinska et al., 2001) in West Africa.

NT09 and NT43 were untypeable in gag and impossible to discriminate between subtype C and CRF07 in NT24 designated as C/CRF07 in pol (RT). Subtyping was still unresolved with NCBI which compared a query sequence with a reference sequence set using BLAST for subtyping and REGA which uses phylogeny and recombinant analysis to identify a subtype (de Oliveira et al., 2005). From the phylogeny, NT09 and NT43 were probably HIV-1 subtype G in gag, and NT24 subtype C. Longer genomic segments were needed to clarify the genotypes since subtyping ideally required full genome sequences (Geretti, 2006). Furthermore, sequences of 800 nts or more were required for recombination analysis (personal communication-Tulio de Oliveira, BioAfrica). Our sequences for phylogenetic analysis were ~450 nts only. The designation of ‘recombinants’ was therefore deduced from the cluster profiles of our samples with reference sets in the bootstrap trees generated. However, the simultaneous genotyping in gag, env and pol with relatively short informative sequences underscored the diversity of our strains.

When analyzing mutations potentially interfering with ARVs, subtype B amino acid sequences served as a reference. However, these substitutions were confronted with non-B sequences particularly of CRF02_AG and CRF06_cpx from this study. Where polymorphism was observed, including any assumed interfering mutation, it was not considered a resistance mutation. However, polymorphisms can hold invaluable information regarding drug resistance.

New DR surveillance mutation updates now include mutations at polymorphic sites that contribute substantially to the emergence of resistance e.g. PR V82A/T/F/S/C/M/L to PI resistance and V179F, V106A/M to commonly used or non-used NRTIs (Bennett et al., 2009). PR L33F was identified in patient NSU22, with CRF02_AG infection and also recorded in subtype A and CRF01_AE patients at 0.5% to 1% prevalence (Shafer et al., 2007). L33F is a major non-polymorphic PI-resistance mutation when present with other mutations and reduced susceptibility to PIs (Stanford University, 2007). L33F was detected in a drug-inexperienced person, suggesting the probable transmission of a potential resistance mutation through CRF02_AG strains prior to initiation of ART in Ghana. 10% treatment naïve HIV-1 infected persons reportedly carried resistant strains to current available therapeutic agents (Jacobs et al., 2008). In six CRF02_AG-infected patients, PI-resistance associated positions were mutated as L10M, L10I or L10V and V11I (Table 1) (Baxter et al., 2006; Flor-Parra et al., 2011). L10I conferred resistance to saquinavir and was less prevalent in drug-naïve individuals (Derache et al., 2008).

L10I/V however occurred in about 10% of treatment naïve persons (Holguin et al., 2006), reduced susceptibility to tipranavir (Kinomoto et al., 2005) and to most PIs like nevirapin, lopinavir, indinavir, tipranavir and saquinavir. L10I/V was additionally identified in HIV-1 subtypes A, B, C, D, F as well as CRF01_AE strain (Baxter et al., 2006). In Ghana where CRF02_AG predominates, these PR position 10 mutations could potentially affect the susceptibility of PIs among drug naïve persons.

Indeed, we reported that CRF02_AG strains of drug naïve persons from southern Ghana were significantly less susceptible to nevirapin in particular and exhibited differential susceptibilities to PIs in general (Kinomoto et al., 2005). Using our study group as proxy, PR position 10 with 57% frequency of all the PR mutations constituted a ‘hot spot’ most likely to mutate. Patient NSU28, infected with CRF06, had both PR L10M and V11I mutations. L10M was reported in CRF02_AG, CRF09 and CRF18_cpx-infected untreated individuals from Mali (Stanford University, 2008). Its significance is unclear (Flor-Parra et al., 2011; Holguin et al., 2006). The effect of V11I on NSU28 phenotype regarding PI resistance could not be predicted either. However, V11I is a minor PR resistance-associated mutation directed against darunavir (DRV) and fosamprenavir (FPV), not currently used in Ghana. PR V11I was also observed in CRF02_AG-infected patient (NT34). This study partly aimed at generating DR data to optimize therapy. This case exemplifies the need to avoid DRV and FPV in patients carrying V11I mutation. Since individuals presenting resistance-related mutations prior to ART will generally develop clinically relevant resistance earlier than those with WT strains, it is relevant to identify such potentially transmissible resistant strains and design efficacious patient-specific treatment options rather than investigate DR mutations after treatment failure.

RT M41L and T69P mutations were simultaneously recorded in patient MHE42, infected with CRF10 in pol RT coding domain. RT M41L has also been reported at 1.2% in HIV-1 subtype D strains (Bennett et al., 2009). This major mutation, by itself, associated with high levels of phenotypic resistance or clinical evidence for reduced virological response to several NRTIs including abacavir, didanosine, tenoflovir. It also resulted in clinical evidence of reduced virological response to stavudine and zidovudine. Even though position 69 is a recognized DR associated site in HIV-1 subtype B, the phenotypic significance of the substitution from threonine to proline was unclear and in combination with M41L it was uncertain how this patient would respond to NRTIs. Similarly, the combination of D67V/T69P in NSU29 and D67G/K70W in NKS25 could not be phenotypically predicted. While D67G is indicated to be a treatment-selected mutation, patient NKS25 was treatment-naïve. At position RT 179, valine was substituted by phenylalanine (V179F) in NKS54 with subtype A1.
infection although isoleucine (I) was occasionally present as WT residue at this position in other A1 strains, indicating polymorphism.

The effect of V179F mutation on drug resistance could not be established but was frequently selected by Efavirine (ETR), reducing ETR susceptibility by more than 100-fold when in combination with Y181C/I/V (Bennett et al., 2009). This combination also caused low-level resistance to Efavirenz (EFV) (Flor-Parrà et al., 2011). Others observed the PR mutations seen in this study including L10I/V and V11I (Sagoe et al., 2007). Minor resistance mutations, which by themselves did not induce DR, including K20I, M36I (Holguin et al., 2006; Konings et al., 2004), H69K and L89M occurring at HIV-1 subtype B DR-associated sites were also identified by us (data not shown). Our inability to conduct phenotypic studies limited the interpretation of the diverse nucleotide changes observed and their significance to resistance emergence or ARV susceptibility. The mutational profile at DR-associated sites in our non-B HIV-1 strain was largely identical with those seen at corresponding positions in subtype B strains at both PR and RT-coding domains. Also, since RT and PR in non-B and HIV-1 subtype B strains were reported to be structurally similar, resistance pathways targeted against ARVs were presumed to be broadly comparable in both viruses. However, non-B specific mutations result in DR by a markedly different pathway from subtype B strains (Abecasis et al., 2005), weakening the perception of the usage of primarily similar pathways for resistance emergence (Kantor et al., 2005).

Conclusion

In this study, we showed the genetic diversity of HIV-1 in poorly investigated parts of Ghana where ART was unavailable and identified DR-related mutations in drug-inexperienced patients which constitutes important new information that can have much relevance for ART. Overall, ART was expected to be successful in persons infected with these non-B HIV-1 strains in the short-to-medium term from the baseline cross sectional data currently generated. Continuous monitoring of the HIV genotypic profile, and a wider surveillance among treatment-naïve and ART patients will be important in identifying new baseline and emerging resistance mutations to achieve the most appropriate and optimal drug combinations for ART.

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