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High levels of HIV-1 drug resistance in children who acquired HIV infection through mother to child transmission in the era of Option B+, Haiti, 2013–2014

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Abstract

Background: The main objective of this study was to determine the frequency and patterns of HIVDR-associated mutations among children <18 months old born to HIV-1-positive mothers enrolled in the prevention of mother-to-child transmission (PMTCT) services in Haiti.

Methods: Between January 1, 2013 and December 31, 2014, HIV-positive remnant dried blood spots (DBS) collected from children under 18 months old for Early Infant Diagnosis (EID) at the National Public Health Laboratory were used for HIV-1 genotyping. HIVDR mutations were analyzed using the Stanford Drug Resistance HIVdb program.

Results: Of the 3,555 DBS collected for EID, 360 (10.1%) were HIV-positive and 355 were available for genotyping. Of these, 304 (85.6%) were successfully genotyped and 217 (71.4%) had ≥ one DR mutation. Mutations conferring resistance to NRTIs and NNRTIs were present in 40.5% (123) and 69.1% (210), respectively. The most frequent mutations were K103N/S (48.0%), M184V (37.5%), and G190A/S (15.1%), and Y181C/G/V (14.1%). Predicted drug resistance

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analysis revealed that 68.8% of the children had high-level resistance to NNRTIs and 11.5% had intermediate to high-level resistance to abacavir.

Conclusions: This study showed high rates of resistance to NRTIs and NNRTIs among newly HIV-diagnosed children in Haiti, suggesting that in the era of "option B+" (initiation of lifelong combination antiretroviral therapy to pregnant women with HIV), the majority of children who acquire HIV infection through MTCT have resistant HIV. These results have led the National HIV Program to revise the pediatric guidelines to include protease inhibitors in first-line regimens for all HIV-positive newborns.

Keywords

Children; HIV; prevention of mother-to-child transmission; drug resistance

In early 2012, Haiti adopted the Option B+ strategy for preventing mother-to-child transmission of HIV (PMTCT) based on the World Health Organization (WHO) recommendation to initiate lifelong antiretroviral therapy (ART) for all HIV-positive pregnant and breastfeeding women.[1] The proportion of HIV-positive pregnant and breastfeeding women identified and placed on ART has increased from 40% in 2009 to 90% in 2017[2, 3] and the MTCT rate through 12 months of age has decreased from 8.9% in 2009 to 5.8% in 2017.[4, 5]

Those infants who become infected despite their mothers receiving ART through Option B+ programs will be fewer in number but may have a higher likelihood of carrying drug-resistant virus.[6–10] HIV-exposed infants may become infected with resistant HIV in three ways: transmission of a predominantly resistant virus from their mothers, transmission of a minor resistant variant that is ultimately selected under suboptimal antiretroviral prophylaxis with nevirapine or zidovudine, and they may become infected initially with a wild-type virus that go on to develop resistance with suboptimal prophylaxis. It is thus important as national programs scale up Option B+ approaches, pediatric HIV drug resistance (HIVDR) surveillance systems are also put in place to monitor patterns of HIVDR that can guide timely and appropriate adjustments to infant antiretroviral (ARV) drug regimens.[11] The objective of this study was to evaluate the frequency and patterns of HIVDR in children newly diagnosed with HIV through the EID program in the era of the Option B+ program.

METHODS

Study setting, patient population, and specimens

From early 2012, all HIV-positive pregnant women started a triple-drug ART regimen of tenofovir disoproxil fumarate (TDF) or zidovudine plus lamivudine plus one of the following: efavirenz or abacavir or lopinavir/ritonavir (LPV/r). The Haiti National HIV guidelines recommend that all HIV-exposed infants receive prophylaxis consisting of zidovudine or nevirapine from birth until either the first PCR test or until six weeks and referring such infants for EID as early as six weeks after birth.[12] The pediatric HIV guidelines recommend initiating ART in all HIV-infected infants. At the time of the study, the first-line regimens were zidovudine or abacavir + lamivudine + nevirapine for children with no or unknown exposure to ART, whereas for children with known exposure to

nevirapine or efavirenz, the recommended first-line regimens were zidovudine or abacavir + lamivudine + LVP/r.

Two laboratories provide EID testing services in Haiti: the Laboratoire National de Santé Publique (LNSP) and the Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections – Institute of Infectious Diseases and Reproductive Health (GHESKIO-IMIS) Rodolphe Mérieux Laboratory.

For EID, whole blood was collected by finger, heel, or toe pricks from HIV-exposed infants. A dried blood spot (DBS) was prepared by spotting a drop of blood onto one of the five circles on a Whatman 903 grade filter paper cards (Whatman Inc., Piscataway, NJ), dried overnight at ambient temperature at the health facility, packaged in humidity-free bags with desiccants, and sent to one of the two EID laboratories in Port-au-Prince for PCR testing. For this study, we included remnant DBS samples collected from children <18 months of age and newly diagnosed with HIV infection by EID at either LNSP or GHESKIO-IMIS between January 1, 2013 and December 31, 2014. The PCR test was performed using the Roche Amplicor HIV DNA test version 1.5 test (Roche Diagnostics, Indianapolis, IN).

HIV genotypic drug resistance testing

HIV genotyping was conducted on the remnant EID DBS specimens at the WHO-designated Specialized Drug Resistance Laboratory at the International Laboratory Branch at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, using a broadly sensitive in-house genotyping assay.[13, 14] A 1,084 base-pair segment of the 5' region of the *pol* gene encompassing the protease and 5' segment of the reverse transcriptase (RT) region was generated by RT-PCR and nested PCR. The purified PCR products were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and analyzed on the ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). The customized ReCALL software program was used to edit the raw sequences and generate consensus sequences[15] and sequence quality assurance was performed on each newly obtained sequence using MEGA.[16] HIVDR mutations and drug susceptibility profiles were determined using the HIVdb algorithm (version 8.4) deployed at the Stanford University Drug Resistance Database (http://hivdb.stanford.edu). Drug susceptibility profiles were interpreted such that the presence of any drug resistance mutation that causes low-level, intermediate, or high-level of drug resistance was defined as resistance; those with susceptible or potential low-level of resistance were designated as susceptible. HIV-1 subtypes were determined using the REGA HIV subtyping tool.[17]

Statistical analyses

The data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC) and Epi Info 3.5.4 (CDC, Atlanta, 2013). Frequencies and chi-square tests were used to summarize categorical demographic data and mutation prevalence data while median and interquartile range [IQR] was reported for age. All graphics were produced using Microsoft Excel (Microsoft Corp., Redmond, WA, 2007).

Ethical considerations

The study protocol was reviewed and approved by the Haiti National Bioethics Committee and the Office of the Associate Director of Science in the Center for Global Health at the Centers for Disease Control and Prevention. The study was determined to be not human subjects research. Upon receiving the HIVDR results, the National HIV Program shared them with clinicians for patient management.

RESULTS

Geographic distribution and demographic characteristics of participants in the study

Between January 1, 2013 and December 31, 2014, DBS samples collected from 3,555 HIV-exposed children from all 10 of Haiti's geographic departments were submitted to the LNSP for EID by PCR (Figure 1). Of these, 360 (10.1%) were PCR-positive. Among the 360 HIV-positive DBS specimens, 355 had sufficient residual DBS sample for inclusion in the study. Of the specimens submitted for genotyping, 304 (85.6%) were successfully genotyped, including 139 DBS samples collected in 2013 and 165 collected in 2014 (Figure 1). The mean age of the children tested in 2013 was 6.8 months (standard deviation, SD 5.3 months), whereas the mean age of the children tested in 2014 was 6.2 months (S.D. 5.1 months); 243 (79.9%) of the children were under 6 months of age.

Prevalence of HIV-1 drug resistance mutations

Among the 304 children for whom genotyping results were obtained, 217 (71.4%) had at least one DR mutation (Table 1), with 123 (40.5%) children having at least one DR mutation conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and 210 (69.1%) having at least one DR mutation conferring resistance to non-NRTIs (NNRTIs). Moreover, 121 (39.8%) children harbored viruses with DR mutations conferring resistance to both NRTIs and NNRTIs, and 122 (40.1%) had two or more NNRTI mutations. Twenty-nine (9.5%) of the children had additional NNRTI mutations (A98G, E138A/G/K/Q, H221Y, and M230L) that confer resistance to second generation NNRTI drugs etravirine and rilpivirine. Forty-four (14.5%) of the children had one thymidine analogue mutation (TAM) and 28 (9.2%) had two or more TAMs. The most frequent mutations observed were K103N/S (48.0%), M184V (37.5%), G190A/S/E/Q/R (15.1%), and Y181C/G/V (13.8%) (Table 1).

The frequency of HIVDR mutations increased from 44.6% (62/139) in 2013 to 93.9% (155/165) in 2014 ($P < 0.0001$). Similarly, the frequency of mutations conferring resistance to NRTIs increased from 8.6% (12/139) in 2013 to 67.3% (111/165) in 2014 ($P < 0.0001$), whereas the frequency of mutations conferring resistance to NNRTIs increased from 41.0% in 2013 to 92.7% in 2014 ($P < 0.0001$).

Ten (3.3%) of the children had at least one mutation associated with protease inhibitor (PI) resistance. One child had a major M46I PI mutation, which causes low level of resistance to all PIs except darunavir and tipranavir, and another had the I50L major PI mutation, which causes high level resistance to atazanavir (Table 1). The other eight children had accessory PI mutations (L10F, L33F, K43T, and Q58E).

The numbers of children with low-, intermediate-, and high-level drug resistance mutations are shown in Figure 2. Among the 119 children with resistance mutations to abacavir, 25 (21.0%) had mutations conferring high-level resistance, 10 (8.4%) had mutations conferring intermediate resistance, and 84 (70.6%) had mutations resulting in low-level resistance. Forty-two children had resistance mutations to zidovudine: 16 (38.1%) had high-level resistance, 19 (45.2%) had intermediate-level resistance, and 9 (16.7%) low-level resistance. Twenty-seven children had resistance to tenofovir: 5 had high-level resistance (18.5%), 9 had intermediate-level resistance (33.3%), and 13 had low-level resistance (48.1%). All of the 209 children with resistance to efavirenz/nevirapine harbored virus with intermediate- to high-level mutations. As for newer generation NNRTIs, 71 children had resistance mutations to etravirine: 10 (14.1%) high-level, 57 (80.3%) intermediate, and 4 (5.6%) low-level and 110 had resistance mutations to rilpivirine: 48 high-level (43.6%), 28 intermediate (25.5%), and 34 (30.9%) low-level resistance.

Predicted resistance to abacavir, zidovudine, and lamivudine were 39.1%, 14.1%, and 37.8%, respectively, among the children whereas predicted resistance to tenofovir was detected in 9.2% of the children (Figure 2). Predicted resistance was detected to nevirapine and efavirenz in 68.8% of children, whereas predicted resistance to the second generation of NNRTIs etravirine and rilpivirine were detected in 23.4% and 36.2%, respectively.

The main HIV-1 subtype circulating in this population was B or B-like in 267 children (87.8%). Other variants included recombinant forms of B/D (22; 7.2%), B/F1 (3; 1.0%) and others (9; 4.0%).

DISCUSSION

This is the first study to examine the frequency and patterns of HIVDR in newly HIV-diagnosed children less than 18 months of age in the era of Option B+ in Haiti. Our results show high rates of resistance to NNRTIs in children under 18 months old. We found that 79% of the children had at least one HIVDR mutation associated with resistance to drugs in use in Haiti at the time of study. In addition, the proportion of HIV-infected infants with HIVDR increased significantly from 2013 to 2014, suggesting that with the scaling up of option B+, the infants could have developed resistance as a result of exposure to suboptimal levels of mothers' cART prenatally or through breastfeeding and/or the infants' prophylaxis.

Overall, 45 (14.8%) of the children had at least one TAM and 8.6% had two TAMs or more, indicating that NRTI drugs may prove less effective in these children. Indeed, predicted resistance to NRTI drugs were high in our study, ranging from 9.2% for tenofovir to 38.8% for abacavir and lamivudine, respectively (Figure 2). This is concerning in light of the barriers faced when initiating PI-based antiretroviral regimens in children, including high cost, unpleasant taste, and drug- interactions.[18] Children receiving incompletely effective regimens are at high risk for virologic failure and may go on to accumulate additional HIVDR mutations.[19–21] Options for second-line and third-line drug regimens are still limited in Haiti and few ARV clinics have the drugs routinely available.

Our study results are in line with what had been observed in similar, low-resource settings, where NNRTI-based ART is used in the first-line regimens for pregnant or breastfeeding women. For instance, a drug resistance survey in Togo found that 60% of children diagnosed with HIV infection through an EID program had at least one mutation conferring resistance to NRTI drugs, and 71% of the children had NNRTI mutations.[9] Similarly, studies from sub Saharan Africa report high rates of NNRTI drug resistance in children exposed to ARV drugs through PMTCT, ranging from 56.8% in South Africa[7] to 74.7% in Zimbabwe.[22] We observed high rates of resistance to NNRTIs in our study, with marked increases between 2013 and 2014.

Our results highlight the need to improve adherence counselling and support programs among pregnant and breastfeeding women receiving ART. Indeed, although ART coverage for pregnant and breastfeeding women has expanded in the last five years in Haiti, adherence in pregnant and breastfeeding women remains inadequate: one quarter of women enrolled in option B+ program dropped out within three months of initiating ART.[23] Our findings also support the inclusion of HIV-positive pregnant women as a key group for which viral load testing should be prioritized and results made available rapidly in order to ensure complete suppression and decreased transmission and drug resistance. HIV drug resistance testing is not yet routinely available in Haiti but there are efforts under way to give pregnant women and children access in order to determine if specific DR mutations were passed from mother to child.

Rates of resistance to PIs were low in our study as have been observed in other countries. This can potentially be explained by the low PI coverage in Haiti where only 5% of patients receiving ART are receiving a PI-based regimen.[24] Additionally, since PIs have a much higher barrier to resistance, patients receiving PI-based regimens are less likely to develop resistance.

Given the mean ages of 6.8 months in 2013 and 6.2 months in 2014 at testing, it appears that most children were diagnosed after the 6 weeks recommended by the National Pediatric HIV guidelines[12]. Greater efforts are needed to ensure that all HIV-exposed infants are diagnosed within the recommended six weeks after birth. Earlier diagnosis of HIV in infants would result in shorter exposure to prophylaxis that would lower the risk for HIVDR and would enable earlier linkage to ART.

Our study has some limitations. Due to nature of the study design, we did not have access to data for the ARV exposure status for the mothers and children and, as a result, we could not assess the children’s breastfeeding status and exposure status to antiretroviral drugs. These limitations prevented us from analyzing specific factors associated with HIVDR in the children. These aspects will be the focus of future studies.

In conclusion, our study contributes important information on HIVDR rates in newly HIV-diagnosed infants under 18 months in Haiti. The results support the decision in the National HIV/AIDS Program pediatric guidelines to introduce a PI-based antiretroviral first line regimen for all HIV-positive newborns.[25] As the program moves toward the elimination of vertical transmission of HIV, it will be vital to not only strengthen strategies to find women

who give birth outside of healthcare facilities but also to improve PMTCT retention and viral load suppression in women enrolled in option B+. Finally, it will also be important to focus on early viral load monitoring of HIV-positive infants receiving ART and to continue HIVDR surveillance among children under 18 months old in order to inform future guideline decisions.

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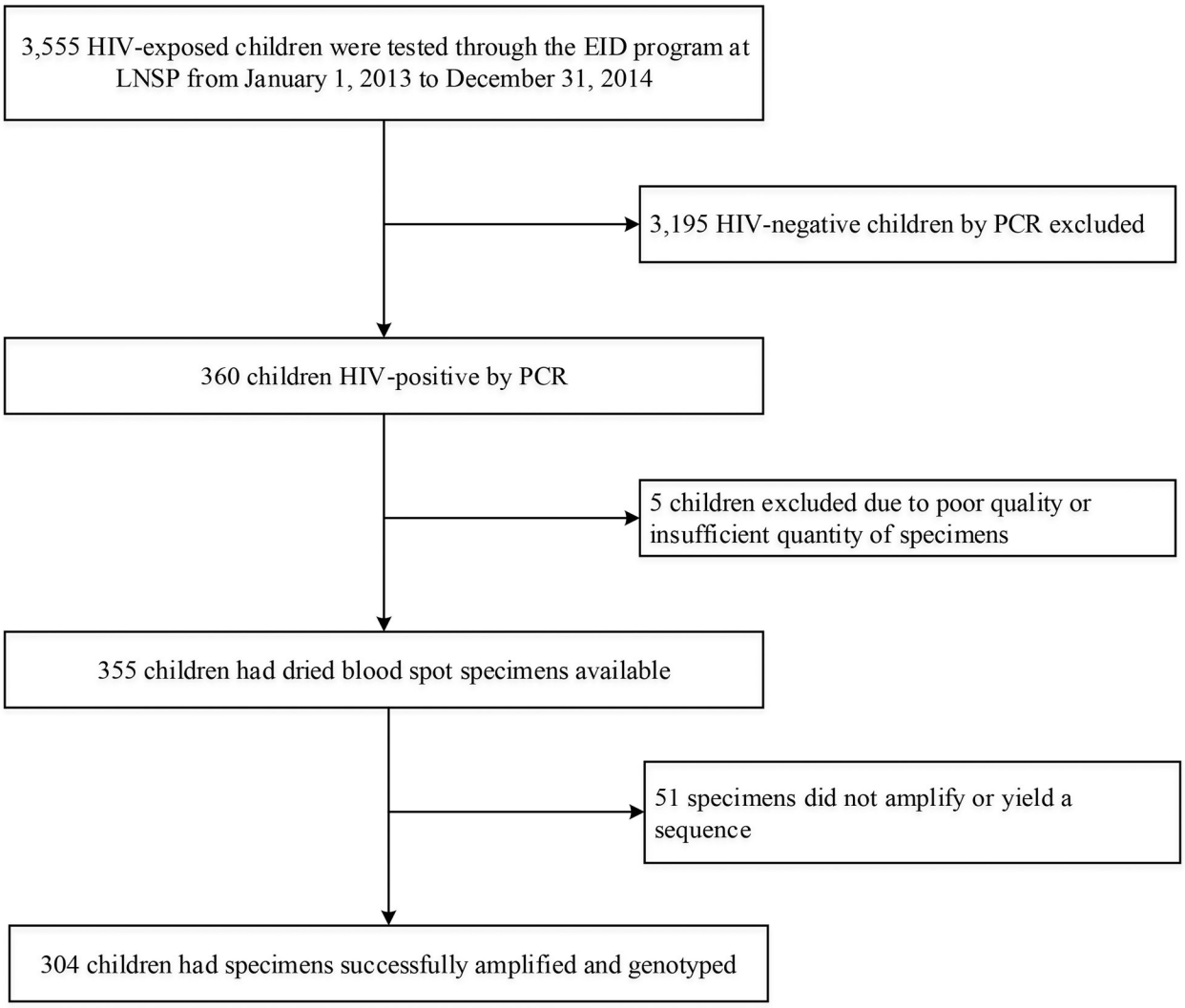

Figure 1.
Description of the study population

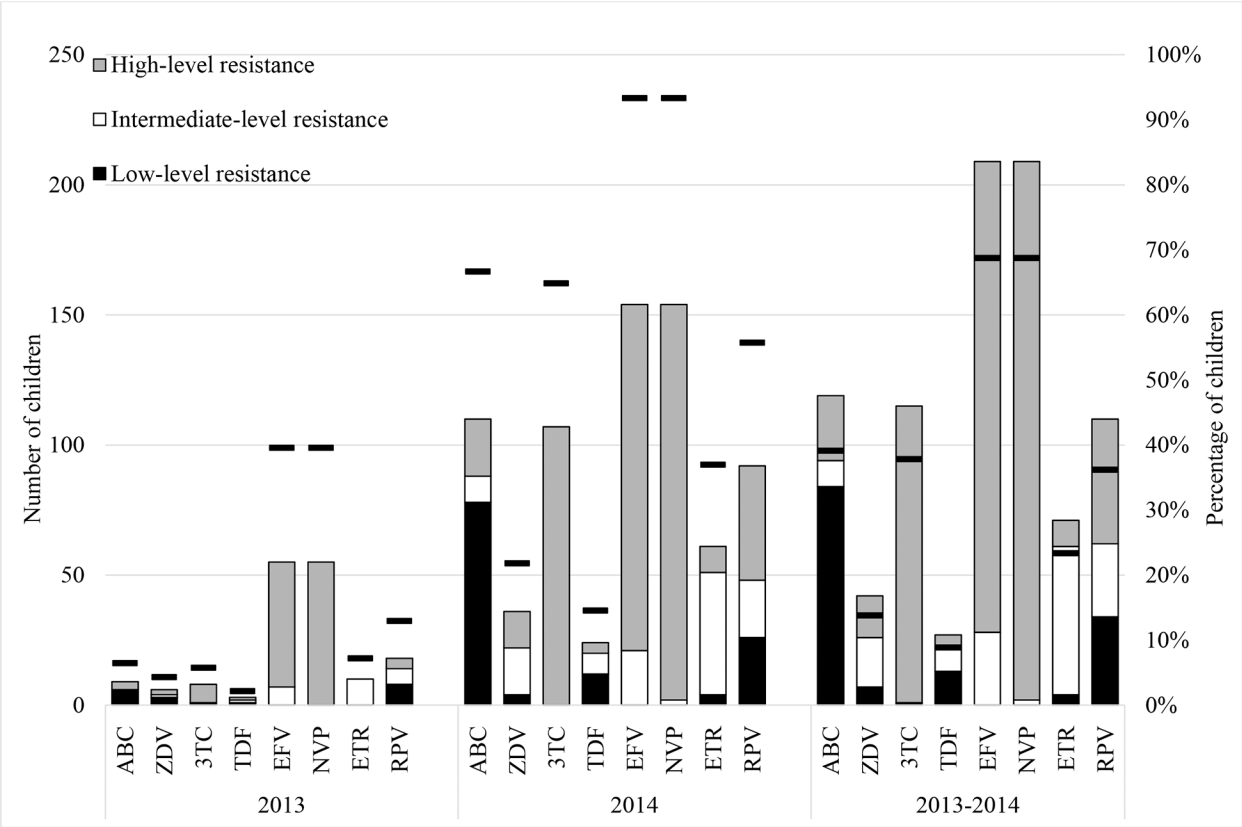

Figure 2.

Levels of HIVDR mutations and predicted resistance to NRTI and NNRTI drugs in 304 HIV-positive children younger than 18 months of age diagnosed through early infant diagnosis, Haiti, 2013–2014.

Drug resistance is defined as any drug resistance mutations that cause low-level, intermediate or high-level of resistance to the relevant antiretroviral drugs. ABC = abacavir; ZDV = zidovudine; 3TC = lamivudine; TDF = tenofovir; EFV = efavirenz; NVP = nevirapine; ETR = etravirine; RPV = rilpivirine

Table 1.

Frequency of HIV drug resistance mutations observed in 304 newly HIV-diagnosed children, Haiti, 2013–2014

Mutation[§]	2013	2014	2013–2014
	N = 139	N = 165	N = 304
	n (%)	n (%)	n (%)
Any mutation	62 (44.6)	155 (93.9)	217 (71.4)
NRTIs	12 (8.6)	111 (67.3)	123 (40.5)
NNRTIs	57 (41.0)	153 (92.7)	210 (69.1)
≥ 2 NNRTIs	16(11.5)	106 (64.2)	122 (40.1)
NRTIs and NNRTIs	11 (7.9)	110 (66.7)	121 (39.8)
Any thymidine analogue mutation (TAM [*])	5 (3.6)	39 (23.6)	44 (14.5)
≥ 2 TAMs	3 (0.7)	25 (15.2)	28 (9.2)
Any PI mutation	4 (2.9)	6 (3.6)	10 (3.3)
NRTI mutations			
M41L	3 (2.2)	16 (9.7)	19 (5.6)
K65R	0 (0.0)	1 (0.6)	1 (0.3)
D67N/G	1 (0.7)	16 (9.7)	17 (5.6)
K70T/R	1 (0.7)	14 (8.5)	15 (4.9)
L74I/V	2 (1.4)	10 (6.1)	12 (3.9)
Y115F	0 (0)	1 (0.6)	1 (0.3)
M184V	7 (5.0)	107 (64.8)	114 (37.5)
L210W	2 (1.4)	7 (4.2)	9 (3.0)
T215A/I/F/Y/N/S	5 (3.6)	28 (17.0)	33 (10.9)
K219Q/E	0 (0.0)	11 (6.7)	11 (3.6)
NNRTI mutations			
A98G	0 (0)	3 (1.8)	3 (1.0)
L100I	2 (1.4)	3 (1.8)	5 (1.6)
K101E/H/N/Q	0 (0)	23 (13.9)	23 (7.6)
K103N/S	44 (31.7)	102 (61.8)	146 (48.0)
V106A/I	1 (0.7)	8 (4.8)	9 (3.0)
V108I	3 (2.2)	29 (17.6)	32 (10.5)
E138A/G/K/Q	2 (1.4)	6 (3.6)	8 (2.6)
Y181C/G/V	8 (5.8)	35 (21.2)	43 (14.1)
Y188C/F/L	1 (0.7)	7 (4.2)	8 (2.6)
G190A/S	6 (4.3)	40 (24.2)	46 (15.1)
H221Y	2 (1.4)	15 (9.1)	17 (5.6)
P225H	8 (5.8)	21 (12.7)	29 (9.5)
F227L	1 (0.7)	11 (6.7)	12 (3.9)

Mutation[§]	2013	2014	2013–2014
	N = 139	N = 165	N = 304
	n (%)	n (%)	n (%)
M230L	0 (0)	2 (1.2)	2 (0.6)
K238NT	0 (0)	10 (6.1)	10 (3.3)
PI mutations			
L10F	1 (0.7)	0 (0)	1 (0.3)
L33F	0 (0)	1 (0.6)	1 (0.3)
K43T	0 (0)	1 (0.6)	1 (0.3)
M46I	1 (0.7)	0 (0)	1 (0.3)
I50L	0 (0)	1 (0.6)	1 (0.3)
Q58E	2 (1.4)	3 (1.8)	5 (1.6)

§: HIV drug resistance mutations were identified using the Stanford University HIVdb algorithm.

**:* TAMs are defined as M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E; NRTIs: nucleoside reserve transcriptase inhibitors; NNRTIs: Non-nucleoside reserve transcriptase inhibitors; PI: protease inhibitors.