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Minority HIV-1 Drug-Resistant Mutations and Prevention of Mother-to-Child Transmission: Perspectives for Resource-Limited Countries

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Abstract

The detection and clinical significance of HIV-1 minority drug-resistant variants is a major topic of current HIV research. Whereas much attention has been placed on the clinical impact of minority drug-resistant variants in patients initiating antiretroviral therapy, their possible influence on the effectiveness of antiretroviral therapy following prevention of mother-to-child transmission strategies in resource-limited settings remains largely unexplored. This review outlines the clinical significance and detection of minority drug-resistant variants, focusing primarily on studies of minority variants in the context of prevention of mother-to-child transmission and their possible influence on current regimens, especially those available in resource-limited countries.

The clinical impact of minority nevirapine-resistant variants that arise in the context of prevention of mother-to-child transmission, for example, is an important factor to consider when these women initiate antiretroviral therapy that may include nevirapine or efavirenz. Minority nonnucleoside reverse transcriptase inhibitor-resistant variants have been associated with treatment failure in women exposed to single-dose nevirapine. In countries like South Africa, with its longstanding use of single-dose nevirapine, this question is relevant as it is for other resource-limited countries where single-dose nevirapine is used. In the same context, various other minority drug-resistant variants (e.g. Y181C, K65R and thymidine analogue mutations etc.) are discussed.

The field of next generation sequencing is very dynamic, with rapid improvements on present technologies and the introduction of novel technologies as discussed in this review. As the impact of minority drug-resistant variants in the setting of prevention of mother-to-child transmission becomes more evident, guidelines for this, especially in resource-limited countries, will need revision in order to optimize the clinical benefit from future antiretroviral therapy. **(AIDS Rev. 2014;16:187-98)**

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Introduction

The detection and clinical significance of HIV-1 minority drug-resistant variants (MDRV) is a major topic of current HIV research. Genotypic resistance testing using viral population sequencing only detects viral variants present in at least 15-20% of the HIV quasispecies[1-3]. This underestimates the true burden of resistance, which has potential implications for clinical management and HIV resistance surveillance. Detection of MDRVs is now technically possible through the so-called "ultrasensitive", "ultra-deep", or "deep" HIV genotyping. Ultrasensitive genotyping can be performed by point-mutation real-time PCR assays (allele-specific PCR, or AS-PCR) or with different next-generation sequencing platforms. The latter were originally designed for high-throughput genomics, but can also be used to sequence short viral genomes with high redundancy, thus enabling a quantitative estimate of the variants conforming the quasispecies down to approximately 1% frequency[4-6].

Whereas much attention has been placed on the clinical impact of MDRVs in patients initiating antiretroviral therapy (ART), their possible influence on the effectiveness of ART following prevention of mother-to-child transmission (pMTCT) strategies in resource-limited settings remains largely unexplored.

Clinical significance of minority variants

Studies have shown that MDRVs may be found in approximately 14% of ART-naive, chronically HIV-1-infected subjects harboring a wild-type virus by population sequencing[3].

Minority drug-resistant variants may be generated spontaneously or be transmitted[7]. Such variants tend to persist for some time after discontinuation of ART[8,9] and may quickly reappear after they fade if antiretroviral selective pressure is reinitiated. Studies have shown, for example, that minority K103N variants may reappear during treatment interruption, and often persist after interruption of suppressive ART[10]. In one study, minority thymidine analogue mutations (TAM) were found in patients exposed to zidovudine (AZT) up to 10 months after ART cessation[11].

There are an increasing number of studies investigating the clinical impact of minority variants. Minority drug-resistant variants are independent predictors of virological failure to nonnucleoside reverse transcriptase inhibitor (NNRTI)-containing antiretroviral therapy[12]. A systematic review and pooled analysis of 10 studies using NNRTI-based regimens found that the presence of minority variants increased the risk of virological failure by 2.5 to 3 times, even at adherence levels of 95% or more[13]. These data confirm previous studies showing that pre-existing minority Y181C mutants more than double the risk of virological failure in adherent patients on efavirenz (EFV)-based therapy, either as first-line ART[14] or after NNRTI exposure[15].

Although HIV drug resistance poses a clinical and public health problem in pMTCT programs[16-21], limited information is available on the relevance of minority variants in pMTCT.

Detection of minority variants

The field of next-generation sequencing is very dynamic, with rapid improvements on present technologies and the introduction of novel technologies. One good example is the intense but short lifespan of 454 sequencing, which will discontinue reagent production in 2016. Although most next-generation sequencing science on HIV has been performed with 454 sequencing, newer platforms like Illumina (MiSeq) and Ion Torrent PGM™ are as sensitive as 454 and provide equivalent results. Moreover, new sequencing technologies provide more output while being faster, cheaper, and easier to manipulate and automate (Table 1).

HIV drug resistance in prevention of mother-to-child transmission strategies

Strategies for pMTCT for resource-limited settings have evolved in the past years according to scientific advances, public health needs, and affordability. Some of these include single-dose nevirapine (sd NVP) administered intrapartum[22], AZT monotherapy according to the ACTG 076 protocol[23], short course AZT and lamivudine (3TC)[24,25], AZT, 3TC ,and sd NVP[26] amongst others. Tables 2 and 3 summarize ARV drug resistance after pMTCT strategies in developing and developed countries, respectively. The current World Health Organisation (WHO) guidelines include three strategies, each with their own advantages and disadvantages. Prophylaxis for pMTCT is given to women with a $CD4^+$ lymphocyte count ≥ 350 cells/μl.

WHO Option A includes antepartum AZT from 14 weeks gestation, intrapartum sd NVP with first dose of AZT plus

Table 1. Techniques and platforms for detection of HIV minority drug-resistant variants

	Principle	Sensitivity	Output (Mb)	Read length
Standard cloning	Analysis of individual colony forming units containing gene of interest	Approximately 10%[4]	Not applicable	Not applicable
Sanger sequencing (viral population sequencing)	Dideoxy-terminator sequencing	15-20%	Not applicable	950 bases
AS-PCR	Real-time PCR amplification of mutants in relation to wild-type	0.003-0.4%[4]	Not applicable	950 bases
454 sequencing (GS FLX and Junior Platforms, Roche)	Amplification of single stranded DNA copies. Sequencing-by-synthesis in water/oil emulsion[78]	0.5-1%[4]	35 Mb (Junior)	400-750 bases[79]
MiSeq, HiScan™SQ system (Illumina)	Sequencing-by-synthesis using solid phase bridge amplification of genomic DNA[78]	0.5-1%	15 Gb (MiSeq) 135-150 Gb (HiScan)	2 × 300 base pairs 2 × 100 base pairs[80]
Ion Torrent PGM™ (Life Technologies)	Converts chemically encoded information (A, C, T, G) into digital (0,1) using semiconductor sequencing technology	0.2-0.1%	600 Mb-2 Gb (Ion 318™ Chip V2)	200-400 bases[81]
PacBio RS II (Pacific Biosciences)	Single-Molecule, Real-Time (SMRT®) technology enabling DNA synthesis by DNA polymerase in real time	< 0.1%	275-375 Mb (data per SMRT® cell)	3,000-5,000 bases per run[82]

AS-PCR: allele-specific polymerase chain reaction.

3TC, and postpartum AZT plus 3TC for seven days. Option B includes triple ART starting at 14 weeks gestation and continued throughout pregnancy and childbirth until one week after cessation of breastfeeding. Option B+ is the initiation of ART in pregnant women at diagnosis and continued lifelong regardless of $CD4^+$ lymphocyte count[27].

The risk for development of resistance is variable for each option, depending on the level of adherence, correct ART administration during labor and postpartum period (e.g. in Option A where 3TC/AZT should be given for seven days), correct staggered approach when stopping antiretrovirals (ARV), and other factors like constant ARV supply[28]. There is much controversy presently regarding the implementation of Option B+ in resource-limited settings.

The benefits of Option B+ include a simplification of the pMTCT regimen and programme requirements, protection in future pregnancies[27], superior maternal health benefit compared to Options A and B, and possibly lower risk of resistance prevented by ART interruptions and simplified ART schedules[28]. However, poor adherence, interruptions in ARV supply, and programmatic and economic issues pose a challenge. Still, these challenges need to be weighed against the long-term cost effectiveness of Option B+. The cost effectiveness of using Option B+ in four countries, including South Africa, Kenya, Zambia, and Vietnam, was investigated. Option B+ is more cost effective than Option A and B and averts more HIV infections in children than does Option A and B[29].

The South African pMTCT guidelines were revised in 2008, 2010, and 2013. The 2010 guidelines expanded

Table 2. Antiretroviral drug resistance after prevention of mother-to-child transmission strategies in developing countries

pMTCT strategy	Drug-resistant mutation	Method of sequencing
sd NVP	25% NVP resistance[33]	Sanger
sd NVP with or without ante/intrapartum ARVs	37.5% NVP (pooled estimate)[83]	Sanger
sd NVP with AZT and 3TC postpartum	4.5% NVP (pooled estimate)[83]	Sanger
sd NVP with or without ante/intrapartum ARVs	62.4% NVP (pooled estimate)[83]	Ultrasensitive sequencing
sd NVP + AZT/3TC postpartum (4 days)	11.7% NVP resistance[84]	Sanger
sd NVP with AZT/3TC (7 days)	7.3% NVP resistance[84]	Sanger
Short-course AZT from 34 weeks with sd NVP	75% NVP resistance[85]	AS-PCR
Short-course ART with AZT + 3TC + NVP from 34	18% NVP resistance[85]	AS-PCR
Antenatal AZT, intrapartum sd NVP, postpartum AZT + 3TC (similar to WHO Option A)	22% AZT resistance[43] 18% NVP resistance	AS-PCR AS-PCR

pMTCT: prevention of mother-to-child transmission; sd: single dose; NVP: nevirapine; ARV: antiretroviral; AZT: zidovudine; 3TC: lamivudine; AS-PCR: allele-specific polymerase chain reaction.

the provision of ART prophylaxis to women not eligible for triple therapy. Pregnant women not eligible for lifelong ART received antenatal AZT from 14 weeks gestation, intrapartum sd NVP and three-hourly AZT, and postpartum a single dose of tenofovir/emtricitabine (TDF/FTC)[88]. A fixed-dose combination for pMTCT prophylaxis with TDF/FTC and EFV was introduced in South Africa in 2013. South African pMTCT guidelines between 2008 and 2013 are summarized in table 4. It is well known that sd NVP used in the pMTCT setting selects for mutations (e.g. K103N and Y181C) that confer NNRTI resistance[18,30-32]. Nevirapine resistance was detected in 25% of Ugandan women 6-8 weeks after ingestion of sd NVP[33]. Mutations of NNRTI, in particular Y181C, were detected in 62% of infants aged less than six months who were exposed to sd NVP[16].

Strategies to reduce NNRTI resistance conferred by sd NVP include the addition of ARVs during pregnancy and the addition of ARVs after exposure to sd NVP in order to cover the NVP 'tail". During pregnancy, AZT monotherapy has been used from 34 weeks[34], 28 weeks[35], and 14 weeks[27].

After exposure to sd NVP, several ARV strategies have been investigated for their potential to reduce resistance. These include a single dose of TDF and FTC at delivery[17], short-course Combivir (AZT/3TC)[21], and AZT and didanosine (DDI)[36,37], amongst others. Interestingly, even drugs other than ARVs, like carbamazepine, have been assessed for their potential to reduce NVP resistance[38].

Several studies have assessed AZT resistance in women exposed to AZT monotherapy, with most reporting no or minimal resistance[34,39-41].

Resistance to AZT was not detected in women exposed to short-course AZT as part of pMTCT in the Ivory Coast. A majority of the women received short-course AZT plus 3TC and sd NVP. Others received short-course AZT and sd NVP, short-course AZT plus 3TC, and sd NVP alone[39].

Eastman, et al. assessed AZT resistance in women who participated in the Pediatric AIDS Clinical Trial Group (PACTG) 076 protocol at study entry and at delivery. Both high-level resistance (detection of T215Y/F) and low-level resistance (detection of K70R) were assessed. No high-level resistance was detected at study entry or at delivery, whilst detection of low-level resistance was seen in 1/61 (1.6%) at entry and 2/47 (4.3%) at delivery. The low levels of resistance detected may be explained by the short duration of exposure to AZT, high median CD4 counts, and low median viral load[40].

Similarly, a study in Cote d'Ivoire also found no resistance in samples from women receiving AZT monotherapy from 36 weeks gestation[42]. In Cape Town, South Africa, women in the pMTCT program who received AZT monotherapy from 34 weeks gestation and sd NVP at delivery were assessed for resistance. The AZT resistance was found to be low. The study also confirmed that the addition of AZT reduces NVP resistance[34].

Factors that favor the development of AZT resistance include a longer duration of exposure[28,43,44] and lower $CD4^+$ lymphocyte count[45,46].

It is important to note that in many of the studies reporting low levels of resistance, standard population sequencing was applied and further evaluation for the presence of MDRVs using more sensitive assays are

Table 3. Antiretroviral drug resistance after prevention of mother-to-child transmission strategies in developed countries

pMTCT strategy	Drug-resistant mutation	Method of sequencing
AZT monotherapy (ACTG 076)	No high level AZT resistance Minimal low-level resistance (4.3% at delivery)[40]	Differential hybridization, oligoligation, or direct sequencing
Pregnancy-limited ART (similar to WHO Option B)	28.7% 3TC (M184V/I) resistance[49] 51.6% 3TC (M184 V/I) resistance[49] 25% NNRTI resistance (K103N)[49] 37.5% NNRTI resistance (K103N)[49] 1.1% PI resistance[49] 1.1% PI resistance[49]	Sanger AS-PCR Sanger AS-PCR Sanger AS-PCR
PLAT (AZT + 3TC + nelfinavir)	23.5% nelfinavir resistance[87]	Sanger

pMTCT: prevention of mother-to-child transmission; AZT: zidovudine; 3TC: lamivudine; AS-PCR: allele-specific polymerase chain reaction; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor; PLAT: pregnancy-limited antiretroviral therapy.

required to fully estimate the prevalence of AZT resistance after exposure to AZT monotherapy.

Minority drug-resistant mutations in prevention of mother-to-child transmission

The OCTANE (Optimal Combination Therapy After Nevirapine Exposure)-1 study, which compared TDF/FTC and NVP to TDF/FTC and ritonavir- boosted lopinavir in patients previously exposed to NVP, found that patients in the NVP arm had significantly higher rates of virological failure. Nevirapine resistance by population sequencing was strongly associated with the primary endpoint (time to virological failure or death)[47]. However, two-thirds of endpoints occurred in patients with no detectable NVP resistance by population sequencing.

Following the OCTANE-1 study, Boltz, et al. postulated that minority NVP-resistant mutations may have contributed to the virological failures where resistance was not detected by population sequencing[6].

Indeed, it was found that in the women with prior exposure to sd NVP, minority NVP-resistant mutations were associated with an increased risk of virological failure when initiated on NVP-containing regimens[6]. This finding is consistent with another study, which showed that minority NNRTI drug-resistant variants may impact future clinical response with NNRTI-containing regimens. Women who received AZT from 34 weeks gestation and sd NVP, and who were subsequently initiated on NVP-containing regimens and failed treatment, were assessed for minority NNRTI drug-resistant variants. No resistance was seen by population sequencing prior to ART initiation. Although the numbers are small, minority NVP-resistant mutations were found in 6/7 (86%) of pre-ART samples of patients who failed treatment[5].

After exposure to sd NVP, women with minority K103N drug-resistant mutations were also found to have inadequate virological response[48].

The use of dual therapy was associated with higher rates of minority M184V/I mutation amongst women who received pregnancy-limited ART. Using AS-PCR, minority M184V/I drug-resistant variants were seen in 95% of patients who received dual pregnancy-limited ART. The frequency of this mutation was higher with increased duration of exposure to AZT[49].

Hauser, et al. assessed the emergence of resistance (including MDRVs) in women exposed to pMTCT prophylaxis in Tanzania. Prophylaxis comprised AZT monotherapy during pregnancy, sd NVP at onset of labor, and AZT plus 3TC during labor and one week postpartum. Zidovudine-resistant mutations were detected in 22% of women, including the detection of minority K70R, T215Y, and T215F mutations. Although K70R confers low-level resistance to AZT, T215Y and T215F result in high-level resistance and were detected by AS-PCR in 8% of women. The investigators of the study go on to say that these results are in conflict with the WHO statement that "the available evidence suggests that the time-limited use of AZT monotherapy during pregnancy for prophylaxis (for approximately six months, or less) should not be associated with a significant risk of developing AZT resistance". Other TAMs, including M41L, L210W, D67N, and K219E/Q, were not assessed in this study and the impact of these drug-resistant mutations on future treatment regimens is not defined[43].

Studies on MDRVs in pMTCT are summarized in table 5.

Table 4. South African prevention of mother-to-child transmission guidelines between 2008 and 2013

	2008[35]	2010[88]	2013[89]
Gestation at initiation	28 weeks	14 weeks	Any
Regimen during pregnancy	AZT monotherapy	AZT monotherapy	FDC: FTC/TDF/EFV
Regimen during labor	sd NVP at onset of labor Continue with AZT 3-hourly until delivery	sd NVP at onset of labor Continue with AZT 3-hourly until delivery	Continue FTC/TDF/EFV
Post delivery	Stop all ARVs	Start dose of Truvada (TDF + FTC)	Continue FTC/TDF/EFV
Postpartum period	No continuation during postpartum period	No continuation during postpartum period	Continue FDC for one week after cessation of breastfeeding
Infant regimen	sd NVP and AZT (for 7 or 28 days)	NVP for 6 weeks or for duration of breastfeeding	Eligible to start HAART
Cd4 cutoff for initiation of HAART	≤ 200 cells/μl	≤ 350 cells/μl	≤ 350 cells/μl

AZT: zidovudine; FDC: fixed-dose combination; FTC: emtricitabine; TDF: tenofovir; EFV: efavirenz; sd NVP: single dose nevirapine; ARV: antiretroviral.

Clinical impact of minority variants for specific antiretroviral classes

Minority drug-resistant mutations, in particular those conferring resistance to NNRTIs which are extensively used in the pMTCT context, may have a negative impact on future ARV regimens. Studies relating to this for each ARV class are discussed using the current South African first (TDF/FTC/EFV) and second line (AZT/3TC/LPV/r) regimens as an example.

Nonnucleoside reverse transcriptase inhibitors

Studies have predominantly focused on the impact of minority NVP-resistant mutations after use of sd NVP for pMTCT. These minority NVP-resistant mutations are associated with an increased risk of virological failure when patients are initiated on NVP-containing ARV regimens[6,5].

Whilst the presence of minority NNRTI-resistant variants was associated with virological failure in women exposed to sd NVP[6], it was not associated with virological failure in women not exposed to sd NVP[50], suggesting that exposure to sd NVP may also play a key role. This raises particular concerns in sub-Saharan Africa and other resource-constrained areas where sd NVP is extensively used in the pMTCT context. In South Africa, intrapartum sd NVP with AZT during pregnancy was implemented in 2004. Only in 2013 did the guidelines include the use of HAART during pregnancy (WHO Option B). Thus it remains to be seen whether the large numbers of women exposed to sd NVP who have initiated or will initiate ART develop virological failure to first-line NNRTI-containing ART (i.e. TDF/FTC/EFV).

As much as exposure to sd NVP may be an important contributor to ART failure, the presence of minority NNRTI-resistant variants detected in ART-naive patients has also been associated with a poor clinical outcome when patients are initiated on NNRTIs. Paredes, et al. investigated the impact of pre-existing minority NNRTI drug-resistant mutations on first-line EFV-based therapy. The risk of virological failure tripled in adherent patients in whom pre-existing minority Y181C variants were detected[14].

Johnson, et al. detected MDRVs (including Y181C and K103N) in 17% of ART-naive patients in whom no resistance was detected by standard population sequencing. Further assessment of the impact of these MDRVs was conducted in a separate case-control study where patients initiated an EFV-based regimen. Minority drug-resistant variants were detected in 7% of patients who failed treatment compared to 0.9% who had treatment success[51]. The clinical impact of minority Y181C mutations on first-line regimens, e.g. TDF/FTC/EFV, in sub-Saharan Africa requires further studies.

Table 5. Studies of minority variants in prevention of mother-to-child transmission using allele-specific polymerase chain reaction

Study	Antiretrovirals investigated	Findings
Boltz, et al.	NNRTIs (NVP) (based on OCTANE A5208 study)	In women previously exposed to sd NVP, minority NVP-resistant mutations are associated with an increased risk of virological failure when initiated on NVP-containing regimens
Rowley, et al.	NNRTI (NVP)	High level of pre-ART (and post-sd NVP exposure) detection of minority NVP-resistant mutations in women failing NNRTI regimens
Coovadia, et al.	NNRTI (NVP)	Persistence of K103N as minority variants was predictive of poor durability of virological response in patients subsequently exposed to NNRTI-containing regimens
Hauser, et al.	NRTI/NNRTI	High rates of minority AZT-resistant mutations in women receiving AZT monotherapy during pregnancy, sd NVP at delivery and AZT/3TC one week postpartum
Paredes, et al.	NRTI/NNRTI/PI	Higher rates of resistance detected in dual therapy compared to triple therapy (95% compared to 51.6% for M184V/I)

NNRTI: nonnucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor NVP: nevirapine; sd: single-dose; ART: antiretroviral therapy; AZT: zidovudine; 3TC: lamivudine; PI: protease inhibitor.

Nucleoside reverse transcriptase inhibitors

Thymidine analogue mutations

There is a risk of development of AZT resistance with the use of AZT monotherapy[43,52]. High-level resistance to AZT requires the accumulation of several TAMs, which take time to develop[28,53]. However, exposure to AZT monotherapy in multiple pregnancies may increase the risk for development of TAMs[28].

Firstly, let's consider the impact of TAMs that may arise from the use of AZT monotherapy for pMTCT on first-line therapy. There are two patterns of TAMs that have been described. The TAM-1 pathway includes M41L, L210W, and T215Y, and TAM-2 includes D67N, K70R, and K219Q/E[54]. The TAM-1 pathway will result in high-level resistance to AZT as well as significant cross resistance to TDF. Thus, the presence of three or more TAMs (that include M41L or L210W) may result in resistance to TDF[55], compromising the use of a first-line regimen that includes tenofovir, e.g. TDF/FTC/EFV.

Secondly, where AZT is used as part of the second-line regimen, e.g. AZT/3TC/LPV/r, exposure to AZT monotherapy for pMTCT in women who subsequently develop TAMs could potentially compromise the second-line regimen.

Zidovudine mutations may persist for long periods of time[56,57]. It is possible that AZT-resistant mutations may be archived in long-lived cells, resulting in reduced efficacy of AZT-containing regimens[52]. Hence, exposure to AZT-containing ART regimens or to subsequent prophylactic AZT will select for mutations that may compromise future treatment. Prolonged exposure to AZT was associated with high levels of AZT resistance[43] and, in the context of dual pregnancy-limited ART, with selection of M184V[49].

Lamivudine-M184V/I

Higher rates of M184V mutation, which confers high-level resistance to 3TC, are seen in patients exposed to dual verses triple pregnancy-limited ART[49]. Due to significant cross resistance to FTC seen with MI84V, first-line therapy with FTC (TDF/FTC/EFV) may also be compromised from M184V arising in the context of dual therapy for pMTCT.

Where 3TC is used intrapartum and seven days postpartum in combination with AZT (WHO Option A), the potential for development of M184V/I does exist[28]. Resistance to 3TC was seen in 8% of women at very low levels (< 1%) using AS-PCR[43]. Although resistance to 3TC use in WHO Option A is low, high rates of resistance as dual therapy (i.e. AZT/3TC) during pregnancy, may compromise 3TC- or FTC-containing regimens. However, M184V mutants are lost very quickly once 3TC is withdrawn[28,43].

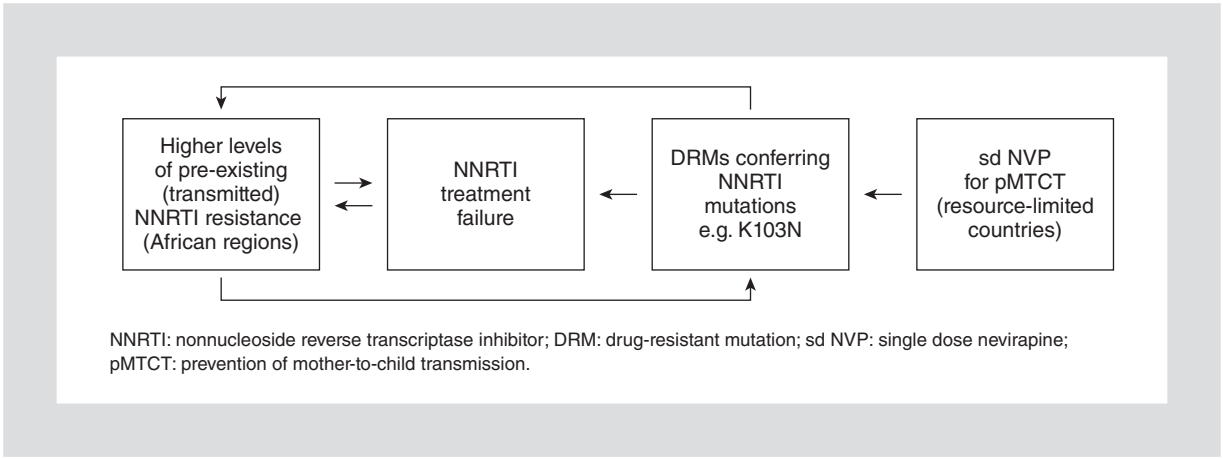

Figure 1. *Contributors to nonnucleoside reverse transcriptase inhibitor treatment failure in resource-limited countries (population level).*

Nucleotide reverse transcriptase inhibitors

Tenofovir

The K65R mutation is selected at higher levels in subtype C infections than in other subtypes[58]. High rates (69.7%) of K65R mutation were found in one South African study of patients failing TDF-based first-line ART[59], although these rates were not confirmed by a similar South African study[60].

Minority K65R drug-resistant variants were found at higher levels in subtype C than B and AE[61].

The prevalence of minority K65R drug-resistant variants in ART-naive patients in South Africa with Subtype C is 4%[61] compared to 2.7% in Subtype B[3]. A case of treatment failure due to minority K65R drug-resistant mutation[62] highlights the clinical impact of pre-existing minority K65R variants when patients commence TDF-containing regimens.

The clinical impact of a TDF-containing first-line regimen in sub-Saharan Africa with possibly a higher prevalence of K65R MDRVs remains to be seen.

The inclusion of a stat dose of TDF/FTC after delivery as part of pMTCT has been used in some countries including South Africa (Table 4). Although we know that this intervention reduces NVP resistance, it is unknown whether this might select for higher levels of K65R MDRVs in the context of the pMTCT regimen where it is included, especially in subtype C virus.

Primary antiretroviral drug resistance

Another important consideration is the increased reports of primary resistance to NNRTIs. The WHO HIV drug resistance report of 2012 indicates that in the African region, the prevalence of transmitted resistance has increased significantly. The major contributor is the increased levels of mutations conferring resistance to NNRTIs. In 2003 the prevalence of NNRTI resistance in Africa was 1%, rising to 6.4% in 2010[96]. The most common mutation detected was K103NS. Indeed, in countries like South Africa and India there seems to be an increase in primary NNRTI resistance detected in the last decade[91,92]. Gupta, et al. conducted a meta-analysis of transmitted resistance in resource-limited areas and noted an increase in the prevalence of transmitted drug resistance in sub-Saharan Africa fuelled by the increase in NNRTI-associated drug resistance in East and Southern Africa[90].

Higher levels of pre-existing resistance to NNRTIs, particularly in Africa where sd NVP is extensively used, may potentiate a cycle of NNRTI resistance at a population level, leading to possible treatment failure (Fig. 1). Whilst conventional sequencing is able to detect transmitted resistance, using ultra-deep sequencing, higher levels of transmitted resistance (30.5%) were detected in ART-naive patients, with about half of those being present in < 20% of the viral population[63].

Prevalence of primary drug resistance, i.e. in ART-naive patients, which include those of transmitted resistance, are summarized in table 6, focusing on resource-limited countries.

Future perspectives

One of the major issues for both developed and developing countries is the implementation of next-generation sequencing in clinical practice. Although there are an

Table 6. Primary drug resistance (i.e. in antiretroviral-naive patients) using Sanger and next generation sequencing in resource-limited countries

Geographical location	Primary mutations	Method of sequencing
Resource-limited countries (transmitted resistance)	Substantial increase of NNRTI resistance in East Africa (36% per year) and Southern Africa (23%) per year[90]	Sanger
South Africa	Overall prevalence 7.4% NRTI-M184V, K219E/R, K65R NNRTI-K103N, V106M, Y181C[91]	Sanger
India	Overall prevalence of 2.6% NRTI: T69D, D67N NNRTI: L100I, K101E, K103N, Y181C Significant increase in NNRTI drug-resistant mutations over time[92]	Sanger
Asia	Overall prevalence 4.6% NRTI: M184I/V, T215D/E/F/I/S/Y NNRTI: Y181C PI: M461 K70R (recently infected)[93]	Sanger
Thailand	Overall prevalence 4% NNRTI: K103N, Y181C[94]	Sanger
Africa (OCTANE-2 trial)	NVP-resistant variants 18%[50]	AS-PCR
South Africa	NNRTI: K103N 15%[48]	AS-PCR
Malawi	Overall prevalence 11% K65R (1-20% of variant prevalence) G190A, Y181 C (> 20% variant prevalence)[95]	454 sequencing
Africa, Asia, Europe, North and South America. CASTLE study	Overall prevalence 30.5% NRTI: TAMs, M184V, K65R NNRTI: K103N, Y181C/I, G190A/E[63]	454 sequencing

NRTI: nucleoside reverse transcriptase inhibitor NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor; NVP: nevirapine; AS-PCR: allele-specific polymerase chain reaction; TAM: thymidine analogue mutation.

increasing number of studies showing the added benefits of using next-generation sequencing from a clinical[13-15,51,64] as well as a correlation and feasibility perspective[11,65-70], the clinical utility of such expensive techniques for resistance testing, especially in resource-limited countries, will need to be proven beyond doubt if clinicians are to utilize these tests in the future. Indeed, in many resource-limited countries, even conventional resistance testing is not yet part of the treatment guidelines and the cost-effectiveness[71] and logistical challenges of implementation[72] are still being realized. The analysis of minority variant detection in addition to population sequencing did not add any additional clinical benefit in a large retrospective trial using AS-PCR for the detection of K103N and Y181C[73]. Although the use of next generation sequencing provides massive amounts of data and may actually be more cost-effective, the start-up costs of various platforms are a huge limitation for resource-limited settings. Recently, using multiplexed amplicon-based next generation sequencing for HIV drug resistance surveillance proved cost-effective in low- and middle-income countries[74]. The implementation will no doubt require robust technical support and training and in resource-limited countries such efforts may not be justified, especially for smaller laboratories. Besides the technical constraints for individual assays, one of the major obstacles is the sophisticated bio-informatics support required to obtain meaningful clinical information

complicated by the reported "error rates"[75] of sequencing very low frequency variants, usually below 1%. Finally, these next generation sequencing assays will require the necessary Food and Drug Administration (FDA) approvals[97].

However, even given the limitations, the dynamic field of next generation sequencing coupled with the high turnover of studies evidently showing its cost effectiveness and clinical utility means it might replace conventional sequencing at least in developed countries.

Another issue is whether there is a difference in the frequency and type of MDRVs across HIV subtypes. Gonzalez, et al. assessed minority variants in HIV subtype C ART-naive patients. Minority NRTI, NNRTI, and protease inhibitor drug-resistant mutations were detected in these patients[76]. In Thailand where subtype CRF01_AE is common, low levels of Y181C and M184V MDRVs were found in a group of patients including recently infected and first-line NNRTI failures[77].

It is well known that single ARVs do not suppress HIV viral replication as effectively as HAART and may lead to resistance. However, is the same true for MDRVs? Are MDRVs more likely in the context of mono or dual therapy?

Conclusion

In general, ARV drug resistance in pMTCT is a concern and has been the focus of research. However, MDRVs in this setting are also proving to be a significant concern as more studies relating to this particular field are published.

Probably one of the most important questions relating to minority variants remains their clinical significance, especially in the era of dynamic improvements in sequencing, scaling up of ARV programmes, higher levels of transmitted NNRTI resistance, and continued use of sd NVP and mono and dual therapy for pMTCT. The clinical impact of this warrants further studies especially for resource-limited countries, in particular in sub-Saharan Africa with its longstanding use of sd NVP and high HIV burden.

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Conflict of interest

No conflict of interest.

References

1. Charpentier C, Laureillard D, Piketty C, et al. High frequency of integrase Q148R minority variants in HIV-infected patients naive of integrase inhibitors. AIDS. 2010;24:867-73.
2. Gianella S, Richman DD. Minority variants of drug-resistant HIV. J Infect Dis. 2010;202:657-66.
3. Metzner K, Rauch P, Braun P, et al. Prevalence of key resistance mutations K65R, K103N, and M184V as minority HIV-1 variants in chronically HIV-1 infected, treatment-naive patients. J Clin Virol. 2011;50:156-61.
4. Paredes R, Clotet B. Clinical management of HIV-1 resistance. Antiviral Res. 2010;85:245-65.
5. Rowley C, Boutwell C, Lee E, et al. Ultrasensitive detection of minor drug-resistant variants for HIV after nevirapine exposure using allele-specific PCR: clinical significance. AIDS Research Hum Retroviruses. 2010;26:293-300.
6. Boltz V, Zheng Y, Lockman S, et al. Role of low-frequency HIV-1 variants in failure of nevirapine-containing antiviral therapy in women previously exposed to single-dose nevirapine. Proc Nat Acad Sci USA. 2011;108:9202-7.
7. Metzner K, Scherrer A, Preiswerk B, et al. Origin of minority drug-resistant HIV-1 variants in primary HIV-1 infection. J Infect Dis. 2013;208:1102-12.
8. Charpentier C, Dwyer D, Mammano F, Lecossier D, Clavel F, Hance A. Role of minority populations of human immunodeficiency virus type 1 in the evolution of viral resistance to protease inhibitors. J Virol. 2004;78:4234-47.
9. Dykes C, Najjar J, Bosch R, et al. Detection of drug-resistant minority variants of HIV-1 during virologic failure of indinavir, lamivudine, and zidovudine. J Infect Dis. 2004;189:1091-6.
10. Metzner K, Leemann C, Di Giallonardo F, et al. Reappearance of minority K103N HIV-1 variants after interruption of ART initiated during primary HIV-1 infection. PloS One. 2011;6:e21734.
11. Avidor B, Girshengorn S, Matus N, et al. Evaluation of a benchtop HIV ultradeep pyrosequencing drug resistance assay in the clinical laboratory. J Clin Microbiol. 2013;51:880-6.
12. Li J, Paredes R, Ribaudo H, et al. Relationship between Minority NNRTI resistance mutations, adherence, and the risk of virologic failure. AIDS. 2012;26:185-92.
13. Li JZ, Paredes R, Ribaudo H, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA. 2011;305:1327-35.
14. Paredes R, Lalama C, Ribaudo H, et al. Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure. J Infect Dis. 2010;201:662-71.
15. Halvas E, Wiegand A, Boltz V, et al. Low frequency nonnucleoside reverse-transcriptase inhibitor-resistant variants contribute to failure of efavirenz-containing regimens in treatment- experienced patients. J Infect Dis. 2010;201:672-80.
16. Hunt G, Coovadia A, Abrams E, et al. HIV-1 drug resistance at antiretroviral treatment initiation in children previously exposed to single-dose nevirapine. AIDS. 2011;25:1461-9.
17. Chi B, Sinkala M, Mbewe F, et al. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. Lancet. 2007;370:1698-705.
18. Flys T, Mwatha A, Guay L, et al. Detection of K103N in Ugandan women after repeated exposure to single dose nevirapine. AIDS. 2007;21:2077-82.
19. Johnson J, Li J, Morris L, et al. Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. J Infect Dis. 2005;192:16-23.
20. Lallemant M, Jourdain G, Le Coeur S, et al. Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. N Engl J Med. 2004;351:217-28.
21. Palmer S, Boltz V, Chow J, et al. Short-course Combivir after single-dose nevirapine reduces but does not eliminate the emergence of nevirapine resistance in women. Antivir Ther. 2012;17:327-36.
22. Guay L, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet. 1999;354:795-802.
23. ACTG 076 and reduction of perinatal transmission. Update Natl Minor AIDS Counc. 1997:4-7, 9.
24. Mandelbrot L, Landreau-Mascaro A, Rekacewicz C, et al. Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. JAMA. 2001;285:2083-93.
25. Chaisilwattana P, Chokephaibulkit K, Chalermchockcharoenkit A, et al. Short-course therapy with zidovudine plus lamivudine for prevention of mother-to-child transmission of human immunodeficiency virus type 1 in Thailand. Clin Infect Dis. 2002;35:1405-13.
26. Dabis F, Bequet L, Ekouevi D, et al. Field efficacy of zidovudine, lamivudine and single-dose nevirapine to prevent peripartum HIV transmission. AIDS. 2005;19:309-18.
27. WHO. Use of Antiretroviral Drugs for treating Pregnancy women and preventing HIV infection in Infants. 2012. Available at: http://www.who.int/hiv/pub/mtct/programmatic_update2012/en/

28. Paredes R, Marconi V, Lockman S, Abrams E, Kuhn L. Impact of antiretroviral drugs in pregnant women and their children in Africa: HIV resistance and treatment outcomes. J Infect Dis. 2013;207(Suppl 2):S93-100.
29. Gopalappa C, Stover J, Shaffer N, Mahy M. The costs and benefits of Option B+ for the prevention of mother-to-child transmission of HIV. AIDS. 2014;28(Suppl 1):S5-14.
30. Eshleman S, Mracna M, Guay L, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS. 2001;15:1951-7.
31. Loubser S, Balfe P, Sherman G, Hammer S, Kuhn L, Morris L. Decay of K103N mutants in cellular DNA and plasma RNA after single-dose nevirapine to reduce mother-to-child HIV transmission. AIDS. 2006;20:995-1002.
32. Kuhn L, Sinkala M, Kankasa M, Kasonde P, Thea D, Aldrovandi G. Nevirapine resistance viral mutations after repeat use of nevirapine for prevention of perinatal HIV transmission. J Acquir Immune Defic Syndr. 2006;42:260-2.
33. Eshleman S, Guay L, Mwatha A, et al. Comparison of nevirapine (NVP) resistance in Ugandan women 7 days vs. 6-8 weeks after single-dose nvp prophylaxis: HIVNET 012. AIDS Res Hum Retroviruses. 2004;20:595-9.
34. van Zyl G, Claassen M, Engelbrecht S, et al. Zidovudine with nevirapine for the prevention of HIV mother-to-child transmission reduces nevirapine resistance in mothers from the Western Cape, South Africa. J Med Virol. 2008;80:942-6.
35. Policy and Guidelines for the Implementation of the PMTCT Programme. South Africa. National Department of Health. 2008. Available at: http://southafrica.usembassy.gov/root/pdfs/2008-pmtct.pdf
36. Lallemant M, Ngo-Giang-Huong N, Jourdain G, et al. Efficacy and safety of 1-month postpartum zidovudine-didanosine to prevent HIV-resistance mutations after intrapartum single-dose nevirapine. Clin Infect Dis. 2010;50:898-908.
37. Van Dyke R, Ngo-Giang-Huong N, Shapiro D, et al. A Comparison of 3 Regimens to Prevent Nevirapine Resistance Mutations in HIV-Infected Pregnant Women Receiving a Single Intrapartum Dose of Nevirapine. Clin Infect Dis. 2012;54:285-93.
38. Muro E, Fillekes Q, Kisanga E, et al. Intrapartum single-dose carbamazepine reduces nevirapine levels faster and may decrease resistance after a single dose of nevirapine for perinatal HIV prevention. J Acquir Immune Defic Syndr. 2012;59:266-73.
39. Coffie P, Ekouevi D, Chaix M, et al. Maternal 12-month response to antiretroviral therapy following prevention of mother-to-child transmission of HIV type 1, Ivory Coast, 2003-2006. Clin Infect Dis. 2008;46:611-21.
40. Eastman P, Shapiro D, Coombs R, et al. Maternal viral genotypic zidovudine resistance and infrequent failure of zidovudine therapy to prevent perinatal transmission of human immunodeficiency virus type 1 in pediatric AIDS Clinical Trials Group Protocol 076. J Infect Dis. 1998;177:557-64.
41. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med. 2004;351:229-40.
42. Ekpini R, Nkengasong J, Sibailly T, et al. Changes in plasma HIV-1-RNA viral load and CD4 cell counts, and lack of zidovudine resistance among pregnant women receiving short-course zidovudine. AIDS. 2002;16:625-30.
43. Hauser A, Sewangi J, Mbezi P, et al. Emergence of Minor Drug-Resistant HIV-1 Variants after Triple Antiretroviral Prophylaxis for Prevention of Vertical HIV-1 Transmission. PloS One. 2012;7:e32055.
44. Frenkel L, McKernan J, Dinh P, et al. HIV type 1 zidovudine (ZDV) resistance in blood and uterine cervical secretions of pregnant women. AIDS Res Hum retroviruses. 2006;22:870-3.
45. Richman D, Grimes J, Lagakos S. Effect of stage of disease and drug dose on zidovudine susceptibilities of isolates of human immunodeficiency virus. J Acquir Immune Defic Syndr. 1990;3:743-6.
46. Land S, McGavin C, Lucas R, Birch C. Incidence of zidovudine-resistant human immunodeficiency virus isolated from patients before, during, and after therapy. J Infect Dis. 1992;166:1139-42.
47. Lockman S, Hughes M, McIntyre J, et al. Antiretroviral therapies in women after single-dose nevirapine exposure. N Engl J Med. 2010;363:1499-509.
48. Coovadia A, Hunt G, Abrams E, et al. Persistent minority K103N mutations among women exposed to single-dose nevirapine and virologic response to nonnucleoside reverse-transcriptase inhibitor-based therapy. Clin Infect Dis. 2009;48:462-72.
49. Paredes R, Cheng I, Kuritzkes D, Tuomala R. Postpartum antiretroviral drug resistance in HIV-1-infected women receiving pregnancy-limited antiretroviral therapy. AIDS. 2010;24:45-53.
50. Boltz V, Bao Y, Lockman S, et al. Low-frequency nevirapine (NVP)-resistant HIV-1 variants are not associated with failure of antiretroviral therapy in women without prior exposure to single-dose NVP. J Infect Dis. 2014;209:703-10.
51. Johnson J, Li J, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med. 2008;5:e158.
52. Ton Q, Frenkel L. HIV drug resistance in mothers and infants following use of antiretrovirals to prevent mother-to-child transmission. Curr HIV Res. 2013;11:126-36.
53. Anderson PL, Rower JE. Zidovudine and Lamivudine for HIV Infection. Clin Med Rev Ther. 2010;2:a2004.
54. Acosta-Hoyos A, Scott WA. The Role of Nucleotide Excision by Reverse Transcriptase in HIV Drug Resistance. Viruses. 2010;2:372-94.
55. Miller M, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis. 2004;189:837-46.
56. Smith M, Koerber K, Pagano J. Long-term persistence of AZT-resistance mutations in the plasma HIV-1 of patients removed from AZT therapy. Leukemia. 1994;8(Suppl 1):S179-82.
57. Albert J, Wahlberg J, Lundeberg J, et al. Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in post-treatment sera. J Virol. 1992;66:5627-30.
58. Theys K, Vercauteren J, Snoeck J, et al. HIV-1 subtype is an independent predictor of reverse transcriptase mutation K65R in HIV-1 patients treated with combination antiretroviral therapy including tenofovir. Antimicrob Agents Chemother. 2013;57:1053-6.
59. Sunpath H, Wu B, Gordon M, et al. High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen. AIDS. 2012;26:1679-84.
60. Hoffmann C, Ledwaba J, Li J, et al. Resistance to tenofovir-based regimens during treatment failure of subtype C HIV-1 in South Africa. Antivir Therapy. 2013;18:915-20.
61. Li J, Lipscomb J, Wei X, et al. Detection of low-level K65R variants in nucleoside reverse transcriptase inhibitor-naive chronic and acute HIV-1 subtype C infections. J Infect Dis. 2011;203:798-802.
62. Bansal V, Metzner K, Niederost B, et al. Minority K65R variants and early failure of antiretroviral therapy in HIV-1-infected Eritrean immigrant. Emerg Infect Dis. 2011;17:1966-8.
63. Lataillade M, Chiarella J, Yang R, et al. Prevalence and clinical significance of HIV drug resistance mutations by ultra-deep sequencing in antiretroviral-naive subjects in the CASTLE study. PloS One. 2010;5:e10952.
64. Simen B, Simons J, Hullsiek K, et al. Low-abundance drug-resistant viral variants in chronically HIV-infected, antiretroviral treatment-naive patients significantly impact treatment outcomes. J Infect Dis. 2009;199:693-701.
65. Fleury H, Bellecave P, Recordon-Pinson P, et al. Detection of Low-Frequency HIV-1 RT Drug Resistance Mutations by Ultra-Deep Sequencing in Naive HIV-1 Infected individuals. AIDS Res Hum Retroviruses. 2014;30:170-3.
66. Nicot F, Saliou A, Raymond S, et al. Minority variants associated with resistance to HIV-1 nonnucleoside reverse transcriptase inhibitors during primary infection. J Clin Virol. 2012;55:107-13.
67. Ji H, Li Y, Graham M, et al. Next-generation sequencing of dried blood spot specimens: a novel approach to HIV drug-resistance surveillance. Antivir Ther. 2011;16:871-8.
68. Mild M, Hedskog C, Jernberg J, Albert J. Performance of ultra-deep pyrosequencing in analysis of HIV-1 pol gene variation. PloS One. 2011;6:e22741.
69. Mohamed S, Penaranda G, Gonzalez D, et al. Comparison of ultra-deep versus Sanger sequencing detection of minority mutations on the HIV-1 drug resistance interpretations after virological failure. AIDS. 2014; 28:1315-24.
70. Hunt G, Morris L, Moorthy A, et al. Concordance between allele-specific PCR and ultra-deep pyrosequencing for the detection of HIV-1 non-nucleoside reverse transcriptase inhibitor resistance mutations. J Virol Methods. 2014;207C:182-7.
71. Rosen S, Long L, Sanne I, Stevens W, Fox M. The net cost of incorporating resistance testing into HIV/AIDS treatment in South Africa: a Markov model with primary data. J Int AIDS Soc. 2011;14:24.
72. Lessells R, Stott K, Manasa J, et al. Implementing antiretroviral resistance testing in a primary health care HIV treatment programme in rural KwaZulu-Natal, South Africa: early experiences, achievements and challenges. BMC Health Serv Res. 2014;14:116.
73. Metzner K, Scherrer A, Von Wyl V, et al. Limited clinical benefit of minority K103N and Y181C-variant detection in addition to routine genotypic resistance testing in antiretroviral therapy-naive patients. AIDS. 2014. [Epub ahead of print].
74. Ekici H, Rao S, Sonnerborg A, Ramprasad V, Gupta R, Neogi U. Cost-efficient HIV-1 drug resistance surveillance using multiplexed high-throughput amplicon sequencing: implications for use in low- and middle-income countries. J Antimicrob Chemother. 2014. [Epub ahead of print].
75. Zagordi O, Klein R, Daumer M, Beerenwinkel N. Error correction of next-generation sequencing data and reliable estimation of HIV quasispecies. Nucleic Acids Res. 2010;38:7400-9.
76. Gonzalez S, Tully D, Gondwe C, Wood C. Low-abundance resistant mutations in HIV-1 subtype C antiretroviral therapy-naive individuals as revealed by pyrosequencing. Curr HIV Res. 2013;11:43-9.
77. Le Nguyen H, Pitakpolrat P, Sirivichayakul S, Delaugerre C, Ruxrungtham K. Minority HIV-1 resistant variants in recent infection and in patients who failed first-line antiretroviral therapy with no detectable resistance-associated mutations in Thailand. J Med Virol. 2012;84:713-20.
78. Mardis E. Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet. 2008;9:387-402.
79. Loman N, Misra R, Dallman T, et al. Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol. 2012;30:434-9.
80. Illumina. http://www.illumina.com/systems.ilmn.
81. Ion-Torrent. http://www.lifetechnologies.com/za/en/home/brands/ion-torrent.html.
82. PACBIO. http://files.pacb.com/pdf/PacBio_RS_II_Brochure.pdf.

83. Arrive E, Newell M, Ekouevi D, et al. Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Internat J Epidemiol. 2007;36:1009-21.
84. McIntyre J, Hopley M, Moodley D, et al. Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: a randomized clinical trial. PLoS Med. 2009; 6:e1000172.
85. Lehman D, Chung M, Mabuka J, et al. Lower risk of resistance after short-course HAART compared with zidovudine/single-dose nevirapine used for prevention of HIV-1 mother-to-child transmission. J Acquir Immune Defic Syndr. 2009;51522-9.
86. Eshleman S, Guay L, Mwatha A, et al. Characterization of nevirapine resistance mutations in women with subtype A vs. D HIV-1 6-8 weeks after single-dose nevirapine (HIVNET 012). J Acquir Immune Defic Syndr. 2004;35:126-30.
87. Kakehasi F, Tupinambas U, Cleto S, et al. Persistence of genotypic resistance to nelfinavir among women exposed to prophylactic antiretroviral therapy during pregnancy. AIDS Res Hum Retroviruses. 2007; 23:1515-20.
88. Clinical Guidelines : PMTCT (Prevention of Mother-to-child transmission). South Africa: National Department of Health. 2010. Available at: http://www.fidssa.co.za/images/PMTCT_Guidelines.pdf
89. Updates on Revised Antiretroviral Treatment Guidelines 2013. South Africa: National Department of Health. 2013 (27 March). Available at: http://www.sahivsoc.org/upload/documents/FDC%20Training%20Manual%2014%20March%202013(1).pdf
90. Gupta R, Jordan M, Sultan B, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet. 2012;380:1250-8.
91. Parikh U, Kiepiela P, Ganesh S, et al. Prevalence of HIV-1 drug resistance among women screening for HIV prevention trials in KwaZulu-Natal, South Africa (MTN-009). PloS One. 2013;8:e59787.
92. Neogi U, Gupta S, Palchaudhuri R, et al. Limited evolution but increasing trends of primary non-nucleoside reverse transcriptase inhibitor resistance mutations in therapy-naive HIV-1-infected individuals in India. Antivir Ther. 2014. [Epub ahead of print].
93. Kiertiburanakul S, Chaiwarith R, Sirivichayakul S, et al. Comparisons of Primary HIV-1 Drug Resistance between Recent and Chronic HIV-1 Infection within a Sub-Regional Cohort of Asian Patients. PloS One. 2013;8:e62057.
94. Sungkanuparph S, Pasomsub E, Chantratita W. Surveillance of transmitted HIV drug resistance in antiretroviral-naive patients aged less than 25 years, in Bangkok, Thailand. J Int Assoc Provid AIDS Care. 2014; 13:12-4.
95. Bansode V, McCormack G, Crampin A, et al. Characterizing the emergence and persistence of drug resistant mutations in HIV-1 subtype C infections using 454 ultra deep pyrosequencing. BMC Infect Dis. 2013;13:52.
96. WHO Drug Resistance Report 2012. http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf.
97. Gibson RM, Schmotzer CL, Quinones-Mateu ME. Next-Generation Sequencing to Help Monitor Patients Infected with HIV: Ready for Clinical Use? Current infectious disease reports. 2014;16(4):401.