



# Principles of HIV drug resistance

## for clinical management in South Africa

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## Introduction

The rapid scale-up of antiretroviral therapy (ART) during the past decade has led to dramatic reductions in HIV-related morbidity and mortality. Efforts are now focused on maintaining virological suppression of patients on first line ART, detecting treatment failure and switching to second-line regimens where necessary.

A major threat to sustaining the positive impacts of ART is the increasing issue of drug resistance. Drug resistance may be primary (transmitted), whereby a person is infected by a strain of HIV that is not fully susceptible to antiretroviral medications (ARVs), or secondary (acquired), whereby a person develops resistance to ARVs over time. In South Africa, the level of primary resistance has been below 5% for the last decade<sup>1</sup> and so this article will focus on the more pressing problem of secondary resistance.

In Southern Africa, routine viral-load monitoring is recommended to identify treatment failure but it is often not done with sufficient frequency, nor reacted to appropriately. There can be a reluctance to switch patients to second-line therapy, in spite of clear guidelines. This is in part because of a lack of certainty regarding the reason for treatment failure – whether it is due to poor patient adherence, the development of drug resistance, or a combination of these issues. Nonetheless, patients who continue taking a failing ART regimen are at risk of developing resistance to

those medications. This article reviews the South African guidelines for viral load monitoring and regimen switch and introduces basic concepts of drug resistance for nurses and health care workers. The information presented here is useful to practitioners throughout Southern Africa as most HIV epidemics in the region are dominated by the same HIV subtype (HIV-1 subtype C), and drug resistance develops by similar mechanisms.

## Guidelines on VL monitoring

The goal of ART is to keep the viral load as low as possible for as long as possible<sup>2</sup>. HIV viral load tests are reported as the number of HIV copies in a milliliter (copies/ml) of blood. If the viral load measurement is above detection (> 50 copies/ml), this indicates that HIV is reproducing as evidenced by its presence in the blood, and that disease will likely progress faster than if the viral load is not detectable. Consistent suppression of viral load levels is associated with reduced morbidity and mortality and a lower probability of sexual transmission of HIV<sup>3</sup>.

The South African national ART program provides viral load monitoring free of charge to patients on ART. Two viral loads are measured in the first year of treatment, at 6 and 12 months, and the test is repeated every 12 months thereafter. In response to a detectable viral load, adherence should be carefully assessed and the test repeated, as described in table 1.

## Barriers to correct viral load monitoring

Many barriers to viral load testing in South Africa are due to Health System challenges which are a combination of financial, logistical and human resource issues<sup>4</sup>. Viral load is a costly and complex test. The price for viral load testing is about 4 to 5 times that of CD4 testing. Currently available viral load platforms are laboratory-based and require significant infrastructure compared with CD4 point-of-care technologies<sup>5</sup>. Currently available viral load technologies require delicate instruments, a reliable cold chain and a secure electricity supply – luxuries which are not available in many areas of Southern Africa. Physical and human resources at laboratory and clinic level further impede efficient processing and reporting of results<sup>4</sup>. There is evidence that continued ART scale up may exacerbate the health system crisis in South Africa<sup>6</sup>. These various factors present obstacles to consistent and timely viral load monitoring. However, as the most sensitive indicator of treatment success or failure, viral load monitoring is a vital component of care for patients taking ART.

## Why viral load monitoring is important: resistance to antiretroviral drugs

Treatment of HIV-infected people with antiretroviral treatment (ART) is very effective. It works by preventing HIV from making copies of itself, allowing cells of the immune system to survive and fight infections. These effects are

**Table 1: South African National Guidelines 2010, 3:19**

Viral load (VL)	Action to be taken
<400 copies/ml	Routine adherence support 6 monthly viral load monitoring and then at 12 months annually
400-1000 copies/ml	Assess adherence carefully Repeat viral load after 6 months
>1000 copies/ml	Intensive adherence assessment and counseling Repeat viral load in 3 months, check hepatitis B status if not done already. If <1000, return to routine 6 monthly monitoring If >1000 and adherence issues addressed, switch to second line therapy

reflected in a falling viral load and a rising CD4 count. If a person is taking ART and the viral load is still detectable in the blood, this indicates that the virus is still making copies of itself despite presence of the antiretroviral drugs. HIV has a very high rate of replication, coupled with a lack of quality control checks when this replication occurs. That is, HIV that is not controlled by antiretroviral medications (ARVs) produces billions of copies of itself every day, and none of these copies are double-checked to ensure that they are the same as the original. Under these conditions, the structural make-up of the virus is altered, and this is known as the development of 'mutations'. Some of these mutations do not impact how well the virus responds to ARVs; however some make the virus less susceptible (or more resistant) to one or more antiretroviral drugs. In general, resistance to a specific ARV only occurs if that ARV is present in the patient. This is why adherence to ART is essential – adequate levels of the drugs must be present to ensure that the virus does not get a chance to replicate. Drug resistance is strongly predictive of virological failure after highly-active ART<sup>7</sup>. Moreover, resistance to drugs in first-line ART regimens increases the probability of virological failure to subsequent regimens<sup>8,9</sup>.

### **Virological failure and switching patients to second line ART**

The South African National Department of Health (NDoH) defines virologic failure as VL > 1000 copies on two occasions, despite intensive adherence counseling. In many Southern African countries, viral load monitoring is not available and in these circumstances, immunological (fall of CD4 count to baseline, or 50% fall from peak value on treatment, or persistent CD4 level below 100 cells/mm<sup>3</sup>) or clinical (new or recurrent World Health Organization stage 4 condition) criteria are used to detect treatment failure<sup>10</sup>.

Recommended second line regimens are based on the likelihood that

patients with treatment failure will have developed resistance to their first-line ARVs. In the case of the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), a patient who is resistant to efavirenz (EFV) is likely to be resistant to nevirapine (NVP), and vice-versa. For this reason, second-line regimens contain Protease Inhibitors (PIs) rather than NNRTIs. Empirical treatment switches should be made as follows:

- Patients failing on a d4T or AZT based first line regimen – switch to TDF+3TC/FTC+LPV/r
- Patients failing on a TDF based first-line regimen – switch to AZT+3TC/FTC+LPV/r

First-line drug resistance and treatment switch is explained in more detail later in this article. Patients failing any second-line regimen require specialist referral<sup>11</sup>

### **Patient-specific risk factors for the development of virological failure and HIV drug resistance**

Virologic outcomes improve with increased levels of adherence to first-line (NNRTI-based) ART<sup>12</sup>. Factors that impact patient adherence are often complex, and a number of factors have been linked to poor adherence in Sub-Saharan Africa<sup>13-15</sup>.

- Disease and treatment factors such as experiencing side effects, and the maintenance of adherence even when a person feels well
- Social factors such as having disclosed to a trusted family member or friend and having social support
- Individual factors such as alcohol use, being away from home, fear of stigma, preferential use of traditional medicines, non-acceptance of one's own HIV positive status
- Health care characteristics such as provider/patient relationship, waiting times, access to health care facility
- Additional issues affect children and adolescents taking ART, where

the importance of full disclosure of HIV status by caregivers, as well as strong parental relationships are associated with good adherence.

Aside from adherence issues, drugs may be poorly absorbed in the gastrointestinal tract, for example due to chronic vomiting or diarrhea or protein-losing enteropathy.

Drug-drug interactions are also a common issue affecting patients on ART, and these can lead to drug toxicity, poorer adherence, or decreased efficacy of either the ARVs or the coadministered medication<sup>16</sup>. Potential interactions are commonly presented when a patient is started on treatment for tuberculosis: rifampicin reduces the concentration of PIs and, to a lesser extent, NNRTIs. In addition, patients taking TB drugs as well as ARVs are exposed to increased risk of toxicity such as liver damage and peripheral neuropathy<sup>17</sup>. Prescribing errors are a further concern, and particular care must be taken with children, for whom drug doses must be calculated at each visit to ensure accuracy.

### **Nomenclature of resistance mutations**

HIV RNA is a code for the proteins that the virus requires in order to function. RNA is made up of a sequence of codons. Each group of three codons makes an amino acid. Amino acids are the basic units that make up proteins and it is at the amino-acid level that resistance mutations are described. Because ARVs target certain HIV proteins, mutations in these proteins mean that the drugs no longer work, or work less effectively. This is drug resistance. When a mutation occurs, it is described according to the position of the affected amino acid. The intended amino acid is named before the position, and the amino acid resulting from the mutation is named after. For example, M184V is a one of the most common drug resistance mutations. It happens in the reverse transcriptase (RT) protein and it is associ-

**Table 2. Introduction on HIV-1 drug resistance mutations to first line ART in South Africa and its effects on other ARVs (adapted from the HIV & TB Drug Resistance Clinical Cases Book. Russouw, Lessells & de Oliveira, ISBN 978-1-920014-91-9)**

Mutation	Selected by	Effects on other ARV
K103N, V108M, Y181C	EFV, NVP	- The presence of one of more mutations result in loss of susceptibility to EFV and NVP - Drug resistance mutations commonly transmitted from mother to child due to sdNVP
M184V	3TC, FTC	- Loss of susceptibility to 3TC, FTC - Increased susceptibility to AZT, d4T, TDF
TAMs: M41L, D67N, K70R, L210W T215Y or F, K219Q or E	AZT, d4T	- Decreased susceptibility to all NRTIs based on number of TAMs - Three or more TAMs are usually related to high-level resistance
Q151M, T69ins	AZT/ddI, ddI/d4T	- Resistance to all NRTIs - T69ins: TDF resistance
K65R	TDF, ABC, ddI	- Variable decrease in susceptibility to TDF, ABC, ddI (and 3TC, FTC) - Increased susceptibility to AZT
L74V	ABC, ddI	- Decreased susceptibility to ABC, ddI - Increased susceptibility to AZT, TDF

ated with lamivudine (3TC) resistance. In this mutation, 'M' refers to the 'wild-type' amino acid, methionine; 184 means that the affected amino acid is at position 184 in the genetic code of HIV's RT protein; and 'V' refers to the amino acid resulting from the mutation in the RNA, valine. Because the amino acid at this position has been altered, the protein produced is different to that which was intended, and the virus is now resistant to lamivudine.

Even more worrying is the fact that these mutations can cause resistance to more than one ARV. In the example of the lamivudine mutation M184V, this single amino acid switch alone makes HIV resistant to lamivudine and emtricitabine, as well as potentially resistant to abacavir and didanosine<sup>18</sup>.

#### **Resistance to first-line therapy: NRTI and NNRTI resistance**

RT is a type of protein known as an enzyme. It is essential for HIV to make new copies of itself. Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Tran-

scriptase Inhibitors (NNRTIs) are designed to prevent reverse transcriptase from performing this function. These are the ARVs used in first line therapy in South Africa. Mutations that make HIV resistant to these classes of drugs are summarized in table 2. Particularly concerning are Thymidine Analogue Mutations (TAMs), which are a group of mutations that can cause resistance to all of the NRTIs.

#### **Resistance to second-line therapy: PI resistance**

Protease is another enzyme that is needed for the virus to become infectious. Protease Inhibitors (PIs) block protease so that HIV cannot infect a new cell. One mutation is usually not enough to make HIV resistant to PIs but if there are a multiple mutations, these drugs will become less effective than they should be<sup>19</sup>. Unlike with the NRTIs and NNRTIs, PI mutations do not tend to affect the entire family of drugs, so even if a person is resistant to one PI they may still be susceptible to another PI. Resistance to second-line therapy is not simple to manage; for this reason

South African guidelines suggest that once a patient is resistance to PIs, he or she should see a specialist physician.

#### **HIV drug resistance in South Africa**

A number of studies have described the patterns of HIV drug resistance in patients failing first-line therapy in South Africa. Patients with virological failure on ART who have demonstrable resistance mutations have been shown to range between 73 and 88 percent<sup>20-25</sup>. The most common mutations found are the M184V mutation, already described, and mutations that present resistance to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) – namely efavirenz and nevirapine. This is clearly a huge concern with respect to our first-line treatment options here in South Africa. Patients who continue their first-line treatment despite raised viral loads are likely to accumulate numerous drug-resistant mutations as time progresses<sup>23,25,26</sup>. A recent study in rural KwaZulu-Natal identified that 1 in 6 patients failing

**Table 3: Example of an HIV-1 genotypic resistance test report.**

Drug	Mutations	Description	Level	GSS
Zidovudine	41L 65R 184V	Susceptible	1	1.0
Didanosine	41L 65R 74I 184V	High-level resistance	5	0.0
Lamivudine	41L 65R 184V	High-level resistance	5	0.0
Stavudine	41L 65R 184V	Low-level resistance	3	0.5
Abacavir	41L 65R 74I 115F 184V	High-level resistance	5	0.0
Emtricitabine	41L 65R 184V	High-level resistance	5	0.0
Tenofovir	41L 65R 115F 184V	Intermediate resistance	4	0.5
Nevirapine	106M 190A	High-level resistance	5	0.0
Delavirdine	106M	High-level resistance	5	0.0
Efavirenz	106M 190A	High-level resistance	5	0.0
Etravirine	106M 190A	Low-level resistance	3	0.5
saquinavir/r		Susceptible	1	1.0
indinavir/r		Susceptible	1	1.0
Nelfinavir		Susceptible	1	1.0
fosamprenavir/r		Susceptible	1	1.0
lopinavir/r		Susceptible	1	1.0
atazanavir/r		Susceptible	1	1.0
tipranavir/r		Susceptible	1	1.0
darunavir/r		Susceptible	1	1.0

first line ARVs developed high-level resistance that compromises second line therapy<sup>27</sup>. This was due to patients failing treatment for an average of 27 months (i.e. 27 months with detectable viral load) without being switched to second line. These findings highlight the need to detect and react to raised viral loads as soon as they occur.

### Testing for resistance

There are two types of resistance tests; phenotypic testing and genotypic testing. The former is relatively simple to interpret and can assess the interactions between different mutations. Genotypic testing uses polymerase chain reaction (PCR) technology to find the changes in HIV's genetic sequences that we have discussed. A genotypic resistance report will describe all of the resistance mutations and their impact on the level of resistance to each drug from 1 (no resistance) to 5 (complete

resistance), as well as the genotypic sensitivity score (GSS): either 0 (drug has no activity), 0.5 (drug has partial activity), or 1 (drug has full activity). The perfect regimen has a GSS score of 3, meaning that all drugs are fully active (Table 3).

The first-line regimen of the patient described in table 3 was TDF/3TC/EFV. This regimen has a cumulative GSS of 0.5, because only TDF is partially active. The standard second-line regimen for this patient, as per South African guidelines, should be AZT/3TC/LPV/r, which has a less-than-perfect cumulative GSS of 2.0. However, given that this patient has the M184V mutation (described in table 2), he/she should do well on the standard second-line treatment as this mutation increases susceptibility to AZT. HIV-1 genotypic resistance testing is very useful both in terms of clinical management of

patients, and as a research tool. In the clinical management of patients it allows clinicians to see whether the drugs the patient is taking are active against that patient's HIV, and whether different drugs may be more appropriate.

In addition, given that there is currently no accepted questionnaire-based adherence assessment tool<sup>28</sup>, resistance testing can serve as a useful proxy indicator for adherence, as follows:

- High viral load and resistance to patient's drugs shown on test result: Patient may or may not be taking their drugs properly at the present time, but because they have resistance they will not be able to suppress their viral load with their current regimen.
- High viral load and NO resistance shown on test result: either...
  - a) Patient has resistance but the

level of resistance is too low for the test to detect (less than 20%). This could happen if the patient was not taking their drugs at all around the time of the test, but had been taking them previously and so developed resistance. In this scenario, re-initiation of the same regimen may not work as the resistant HIV will re-emerge once adherence to the same drugs improves. This is why we sometimes repeat the resistance test after 6 months if the viral load is still high despite intensive adherence support and if the patient reports good adherence. Alternatively...

- b) Patient is not taking the drugs at all and genuinely has no resistance. If this is the case, the same regimen should work for this patient if he or she is able to adhere correctly. Patients failing antiretroviral therapy in the absence of drug resistance are particularly difficult to manage and often have serious adherence problems. They are in great danger of disease progression with the development of AIDS and require intensive adherence support and care.

## Conclusion

Drug resistance testing is not currently available in the public sector in most provinces of South Africa. However, with the recent publication of guidelines by the SA HIV Clinicians Society, it is hoped that drug resistance testing will become more widely available in the near future. In the meanwhile, attention must be focused on frequent, proactive monitoring for treatment failure. Research is underway to design simple, inexpensive viral load tests (e.g. fingerprick) and it is hoped that they will be available throughout Southern Africa and beyond in the near future<sup>29</sup>. Patients with treatment failure should be supported with intensified adherence support, in conjunction with the correct application of criteria for regimen switching. This will

reduce the amount of time that patients spend on failing regimens and limit the development of complex resistance patterns, encouraging a durable treatment response. **R**

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