CHAPTER 1
HIV drug resistance introduction

It is important to have a basic understanding of how and why HIV develops resistance to antiretroviral medication. This chapter briefly discusses the most important background information, namely:

- The extent of the problem of HIV drug resistance in the world in general and in South Africa in particular.
- The mechanisms of development of HIV drug resistance.
- The risk factors for the development of HIV drug resistance.
- How to interpret a genotype result.
- How to make logical regimen changes in the presence of drug resistance.
- How HIV drug resistance can be prevented.

1.1. Epidemiology of HIV drug resistance

Combination antiretroviral treatment (ART) has proved to be very effective treatment for people infected with HIV. It inhibits viral replication and therefore halts the progression of infection to AIDS and allows for partial restoration of the immune system. If viral replication occurs in the presence of these drugs, however, mutations can occur in the viral proteins targeted by the ART and this can lead to the development of drug resistance[1]. Resistance can develop to any of the drug classes currently in use: nucleoside/nucleotide reverse-transcriptase inhibitors (NRTI/NtRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors (EIs) and integrase inhibitors (INSTIs).

It is important to understand why HIV is particularly prone to develop resistance. One reason is the high level of virus production and turnover. In untreated patients, it has been estimated that there are 10^7 to 10^8 infected cells in the lymphoid tissue. This enormous viral population is furthermore very diverse since the process of reverse transcription of viral RNA to DNA is extremely error-prone. This is due to the absence of any enzymatic proofreading activity, which means that the virus has no mechanism with which to check that the viral copies are similar to the original. This only occurs with RNA viruses and never with DNA viruses. This means that for every viral genome transcribed, an average of one mistake (or mutation) occurs, creating a complex mixture of viral quasispecies in each individual, that each differ by one or more mutations. Some of these mutations are irrelevant, but some confer a survival advantage on the virus, especially if the mutation makes the virus less susceptible to a specific antiretroviral drug. If the patient is then treated with that specific drug, these resistant quasispecies will selectively overtake the other quasispecies and so become the dominant viral population in the patient[1].

This is part of the reason why we treat patients with triple drug therapy or highly active antiretroviral therapy (HAART), consisting of at least two different drug classes. Even if some quasispecies harbour resistance to a drug, it is highly unlikely that they will be resistant to all three of the drugs in the regimen and the entire viral population should therefore be suppressed with HAART. Drug resistance will then most likely only emerge in the presence of HAART if the virus is allowed to replicate in the presence of drugs, as in the case of sub-optimal adherence.

This is presented graphically in FIGURE 1.1:

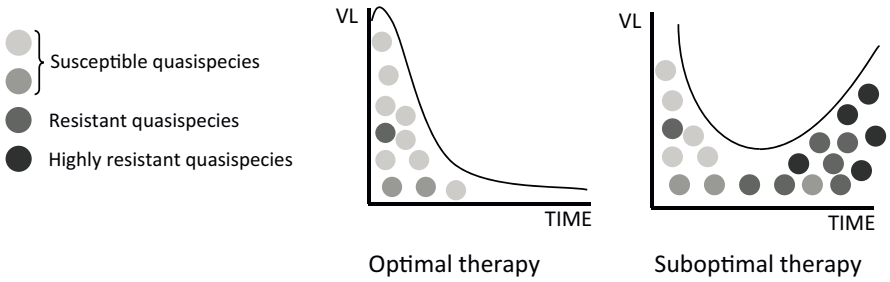

Figure 1.1 *Selection of resistant quasispecies by suboptimal antiretroviral therapy*

Types of drug resistance

There are two major types of HIV drug resistance: primary (or transmitted) resistance and secondary (or acquired) resistance.

Primary or transmitted drug resistance (TDR)

Patients are sometimes primarily infected with a resistant virus. The most common reason is that a patient is infected by a partner (or a mother) who has developed drug resistance secondary to ART.

Secondary or acquired drug resistance

This is the most common type of drug resistance and occurs when HIV continues to replicate in the presence of ART. In order for this to happen, the level of the drug should be too low to block viral replication, but high enough to exert a positive selection pressure on the virus.

Overview of the global figures of transmitted drug-resistant HIV strains

The reported prevalence of transmitted drug-resistant HIV-1 varies widely depending on the location, risk group and sampling time after newly acquired infection. A large increase in overall primary resistance, from 13.2% for the period 1995–1998 to 24.1% for the period 2003–2004, was reported in New York and the rate of transmitted multidrug resistance increased from 2.6% to 9.8% over the same period (TABLE 1.1)[2].

Table 1.1 *Frequency of HIV-1 drug resistance mutations according to drug classes: 1995 to 2004, New York, U.S. Figures in parentheses represent percentage of newly infected individuals in each category. *P values are 2-sided and measured by the exact test for trend. Adapted from reference 2*

	1995-1998	1999-2000	2001-2002	2003-2004	P-value*
N	76	71	102	112	
Any resistance	10(13.2)	14(19.7)	17(16.7)	27(24.1)	0.11
Any NRTI	9(11.8)	11(15.5)	9(8.8)	18(16.1)	0.67
Any NNRTI	2(2.6)	4(5.6)	8(7.8)	15(13.4)	0.007
Any PI	1(1.3)	4(5.6)	5(4.9)	8(7.1)	0.10
Resistance to 2 or more classes	2(2.6)	4(5.6)	4(5.6)	11(9.8)	0.07
	0	1(1.4)	1(1.0)	3(2.7)	0.17

Data from a UK group showed similarly high rates of primary resistance in 2003: 19.2% for any drug, 12.4% for NRTIs, 8.1% for NNRTIs and 6.6% for PIs. High-level resistance was found in 9.3%[3]. A 10-year transmission surveillance study (1996–2005) conducted by the Swiss HIV Cohort Study, however, showed considerably lower rates: 7.7% for any drug, 5.5% for NRTIs, 1.9% for NNRTIs and 2.7% for PIs. Dual- or triple-drug class resistance was observed in only 2% of patients[4].

The World Health Organization (WHO) classifies transmitted drug resistance into three categories: low prevalence (<5%), moderate prevalence (5-15%) and high prevalence (>15%)[5]. When the prevalence is below 5%, the national ART programme should function optimally. When moderate prevalence is detected, the WHO advises public health action, such as (1) examining specific ART programme practices and drug quality measures for specific drugs or drug classes for which prevalence is >5%, (2) increasing support to ART programmes to minimize the emergence of drug resistance in treatment and (3) prevention programmes to minimise the transmission of HIV from persons receiving ART. At high rates of drug resistance, the WHO advises strong public health action, such as increased surveillance and a change in first-line ART regimens.

The high rates of drug resistance in the United States and some European countries partly come from a legacy of monotherapy for ART. In 1987, zidovudine (AZT) was introduced as the first treatment for HIV and it was given as a single drug. Since AZT alone was unable to completely suppress viral replication in the plasma, most patients developed resistance to AZT within a few years and this resistant strain was then transmitted to their partners. It was not until 1996 that new knowledge and drug classes led to the decision to treat HIV with a combination of three drugs or HAART. The advent of HAART saw the dream of virological suppression become a reality for the first time and thus made the emergence of drug resistance less likely.

Due to the high prevalence of drug resistance in some areas in the United States, the International AIDS Society-USA (IAS-USA) advises that patients have resistance testing when they are first diagnosed with HIV and again at the time of initiation of treatment in order to document the resistance pattern that they present with and allow individualization of the first-line regimen[6].

Overview of transmitted drug-resistant HIV strains in Africa

ART was introduced in Africa after 1996 and thus national programmes started with triple-drug regimens. As a consequence, reported levels of drug resistance have been relatively low to date. It should, however, also be added that routine surveillance has not been widely performed on the continent and that the first reports of transmitted resistance have only been published recently.

The first published study of this nature on the continent, outside of South Africa, was performed in Lusaka, Zambia, between 2007 and 2008 and showed an overall baseline prevalence of resistance of 5.7% and a transmitted drug resistance prevalence of 5.2% (FIGURE 1.2)[7].

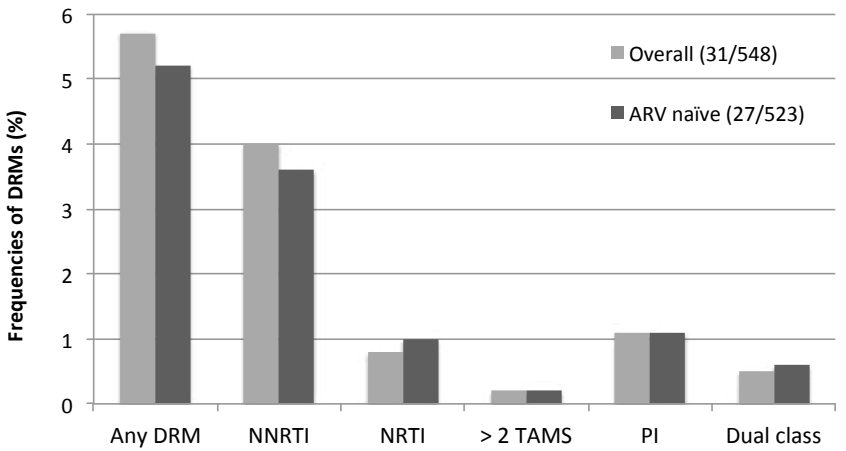

Figure 1.2 *Frequencies of drug resistance-associated mutations (DRMs) in Lusaka, Zambia, between 2007 and 2008. Frequencies are presented separately for antiretroviral-naïve (adapted from reference 7). TAMs= thymidine analogue mutations*

A number of isolated studies have been performed in South Africa and have been put together with data from Hlabisa sub-district in KwaZulu-Natal to reflect a trend over the last ten years (FIGURE 1.3)[8]. It seems as if the level of transmitted drug resistance in South Africa has remained below 5% and this bodes well for the national ART programme.

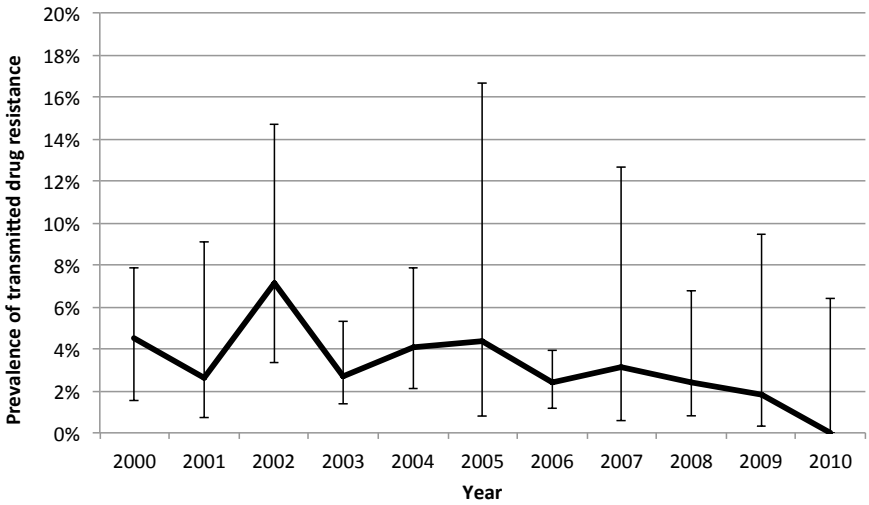

Figure 1.3 *Trend in the prevalence of transmitted drug resistance between 2000 and 2010, in South Africa (adapted from reference 8).*

There are, however, various programmatic problems in Africa that might fuel the development of transmitted drug resistance, such as drug stock-outs and suboptimal regimens. The use of single-dose nevirapine (sdNVP) to prevent mother-to-child transmission (PMTCT) of HIV-1 also deserves special mention. sdNVP selects for nevirapine-resistant HIV-1 in 40%–60% of mothers and 40%-50% of infected babies. Co-administration of other antiretroviral drugs with nevirapine for PMTCT may reduce the risk of drug-resistant infection in adults and children and this has now been incorporated into the WHO and South African guidelines.

It is also important to consider that increasing ART coverage will strain the capacity of an overburdened public health system even further, resulting in compromised quality of care that might fuel the development of resistance. In addition, persistently high HIV incidence due to ineffective prevention strategies, makes an increase in transmitted drug resistance inevitable.

Summary of acquired drug-resistant HIV strains in South Africa

Many local studies have described the patterns of acquired HIV drug resistance in adult patients failing first-line therapy. The most common mutations are NNRTI mutations, followed closely by the lamivudine mutation, M184V. The low number of K65R mutations can be attributed to the unavailability of TDF in the public sector at the time that these studies were done. Fortunately, the mutations that confer complete resistance to the entire NRTI class (Q151M and 69 insertions) occurred only rarely. TABLE 1.2 summarizes the frequencies of resistance mutations detected in different South African studies of patients failing first-line therapy[9-17].

TABLE 1.2 *Resistance mutations in adult patients failing first-line antiretroviral therapy in South Africa (data from references 9-17)*

Author	Location	N	Criteria	M184V (%)	NNRTI (%)	TAM (%)	K65R (%)	PI (%)
Orrell	Cape Town	110	1x VL >1000	78%	88%	23%	9%	1%
Marconi	Durban	115	1x VL >1000	64%	78%	32%	3%	0
Hoffman	Johannesburg	68	1x VL >1000	37%	62%	6%	-	2%
Wallis	Johannesburg	226	2x VL >1000 or 2x VL >5000	72%	77%	31%	4%	0
El-Khatib	Soweto	94	1x VL >400	62%	81%	16%	1%	2%
Sigaloff	Johannesburg	43	2x VL >5000	74%	86%	54%	7%	-
van Zyl	Western Cape	167	1x VL>400	61%	82%	12%	4%	0
Manasa	Africa Centre (rural)	240	1x VL >1000	86%	93%	38%	4%	0
Barth	Limpopo (rural)	21	1x VL >1000	52%	86%	0	0	0

There are still limited data on second-line failure in South Africa. Studies are difficult to compare since some list all PI mutations, whereas others only report on major mutations. For the most part, these studies do not present prevalence but rather the proportion of patients who developed protease inhibitor mutations in a specific patient group. Currently the presence of PI mutations is quite rare in adults failing a second-line PI-based regimen: Wallis *et al*. reported 7% major mutations in patients failing therapy[18], and Rossouw *et al*. reported 5.9%[19]. It should be noted that the number of patients in each group was small. In children, PI mutations occur much more frequently, mostly secondary to ritonavir monotherapy and suboptimal dosing of lopinavir, especially in the presence of concomitant TB treatment. TABLE 1.3 summarises the resistance mutations detected in paediatric patients failing PI-based ART in South Africa[19-22].

TABLE 1.3 *Resistance mutations in paediatric patients failing protease inhibitor-based antiretroviral treatment in South Africa (data from references 19-22)*

Author	Location	N	Criteria	M184V (%)	NNRTI (%)	TAM (%)	K65R (%)	PI (%)
Taylor	Johannesburg	41	1x VL >1000	71%	10%	N/A	N/A	36%
Wallis	Johannesburg	41	1x VL >5000	82%	98%	N/A	N/A	44%
Van Zyl	Cape Town	39	1x VL >4000	83%	N/A	26%	2.5%	43%
Rossouw	Pretoria	49	1x VL >1000	74%	43%	22%	0%	33%

1.2. Risk factors for development of HIV drug resistance

The development of drug resistance is a complex phenomenon and has been associated with various risk factors. These risk factors can be divided into those pertaining to the virus, the host or the treatment regimen. Each of these will now be briefly discussed.

The virus

The extremely high rate of viral replication and lack of proofreading ability makes HIV particularly prone to the development of drug resistance. There is no evidence to show that certain subtypes are more prone to the development of resistance to HAART, although some studies have shown this in mono- or dual therapy for PMTCT. It has been argued that since the plasma viral load of subtype C virus is generally higher than other subtypes, this subtype may be more prone to resistance, although more data are needed. Some subtypes do, however, have unique polymorphisms that might facilitate the development of certain mutational patterns. One example is the K65R mutation, which develops more frequently and more rapidly in subtype C compared to subtype B, due to preferential pausing of reverse transcription at position 65 as a result of differences in the template sequence[23].

The host

Most risk factors pertaining to the host can for the most part be ascribed to adherence issues. Adherence refers to the extent to which a patient follows a prescribed treatment regimen. In HIV treatment, adherence levels of above 90% are needed in order to prevent the emergence of drug resistance. There are a few studies relating specifically to adherence to ART but much of the data are extrapolated from research on other chronic diseases.

Factors affecting adherence[24]

1. Demographic characteristics
   There are no consistent data showing that any of the demographic characteristics such as age, gender, socio-economic status or race are associated with poor adherence.

2. Psychosocial/ behavioural characteristics
   The presence of psychiatric illness, especially major depression and alcoholism, has been associated with lower levels of adherence. Negative attitudes about medication or illness, particularly the denial of the necessity of treatment, may also interfere with adherence. Although some studies have found that poor social relationships, often reflected by lack of involvement of family and friends, social isolation, and living alone, can be risk factors, other studies have had conflicting results. Chaotic lifestyles, such as those found in intravenous drug users, can also predispose to non-adherence.

3. Health care administration and delivery characteristics
   Patient knowledge. It is well recognised that lack of knowledge, on the patient's part, about the diagnosis, the expected course of the illness, the correct dose of the medication and the fact that chronic medication has to be taken continuously, are associated with lower levels of adherence. Interestingly, one study found that patients who learned the names of their medications were more adherent than those who did not. The communication between the healthcare practitioner and patient is vitally important in this regard. The healthcare worker can assist the patient in coming up with a strategy to incorporate the individual drug regimen into a daily schedule. Several strategies have been suggested: timed pill dispensers, alarm clocks and engaging a treatment supporter to act as a reminder. Extrinsic barriers to treatment adherence include cost, lack of transportation, lack of child care, severe illness, place and distance of treatment centre and lack of a primary care physician.

4. Medication characteristics
   Medication characteristics have been found to greatly influence adherence. A complex regimen with a high pill burden and frequent dosing intervals is known to have the potential to cause non-adherence. Complex regimens are difficult to incorporate into daily routines and combination tablets, longer half-life drugs (e.g. a single daily dose), or long-acting controlled-release forms may become important strategies in improving adherence.
   It is also well known that the side-effect profile is important and major side effects, such as gastrointestinal upset and peripheral neuropathy, can lead to decreased adherence and treatment cessation. At times, however, just the fear of side effects is enough to impair adherence.

Other host characteristics that can impact on the development of resistance are relatively rare and can be characterized as follows:

1. Absorption – reduced absorption of drugs due to gastrointestinal abnormalities such as chronic vomiting or diarrhoea, protein-losing enteropathy or bowel resection surgery. Drugs that can interfere with absorption, such as proton-pump inhibitors that change the intestinal pH, may also be to blame.
2. Poor activation – this may be due to host genetics
3. Rapid clearance of drug – this can be due to specific host genetics

The treatment

There are basically three treatment factors that can aid the development of resistance to combination ART.

1. Poor potency – such as NVP monotherapy
2. Wrong dose – sub-therapeutic doses can lead to the rapid accumulation of resistance.
3. Drug-drug interactions – most ARVs have an enormous potential to interact with other medication, especially the NNRTIs and the PIs. Information about these interactions can be sourced from the Medicines Information Centre at UCT at 0800212506 or 0214066829, or the drug interaction website: www.hiv-druginteractions.org.

There are two concepts that are very important in understanding the vulnerability of individual drugs to resistance: the genetic barrier to resistance and the zone of potential replication.

1. The genetic barrier to resistance: this can be understood as the number of mutations required to produce high-level resistance to a specific drug. This varies between and within the different drug classes. This is demonstrated in TABLE 1.4 that reflects the general estimation of the genetic barrier of the approved drug classes. For instance, a single mutation is needed to develop resistance to lamivudine and all the NNRTIs, whereas multiple mutations are needed to develop resistance to thymidine analogues (stavudine, zidovudine) and the boosted PIs.

TABLE 1. 4 *Genetic barrier to resistance of different ARV drug classes*

Drug Class	Genetic Barrier
Unboosted PI	1
NNRTI	1
NRTI – non-thymidine analogues	1
NRTI – thymydine analogues	3
Fusion Inhibitor	1
Boosted PI	3–8

2. The zone of potential replication: the space between the IC_{50} (the drug concentration where 50% of viral replication is suppressed) and IC_{90} (the drug concentration where 90% of viral replication is suppressed) is called the zone of potential replication. This is the zone where viral replication can occur. There is a large difference in the time the drugs spend in this zone after dosing and it is mostly a function of their half-lives. For instance, boosted lopinavir (LPVr) has a relatively short half-life so, when a patient stops taking this drug, the levels rapidly drop through the zone of potential replication, leaving very little opportunity for viral replication. The longer half-lives of nevirapine and efavirenz mean that the drugs spend more time in the zone of potential replication so there is more time for active viral replication to take place in the presence of the drugs. The latter situation represents the perfect set up for resistance to occur.

   When a patient stops all three ARVs at the same time, the drugs with longer half-lives will spend more time in the zone of potential replication than the drugs with shorter half-lives (FIGURE 1.4). This means that the patient is essentially on monotherapy with the longer half-life drug for a period of time and this can lead to the development of resistance to that specific drug.

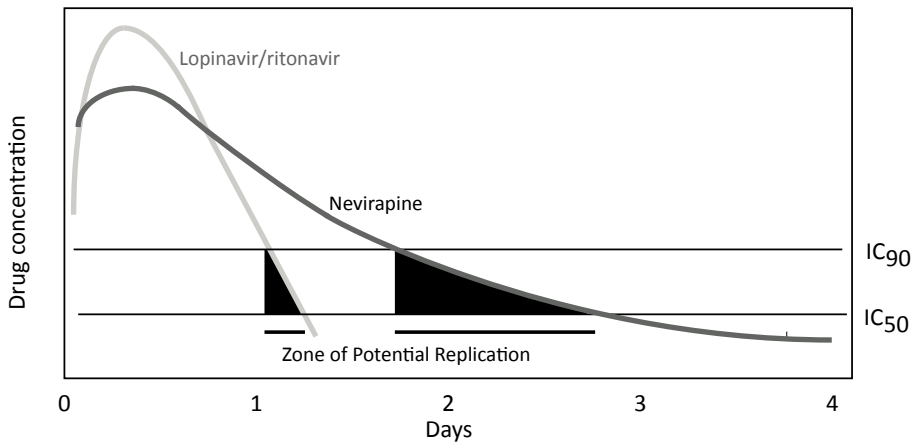

Figure 1.4 *Zone of potential replication (LPVr and NVP are included as examples)*

1.3. Mechanisms of development of HIV drug resistance

Basic nomenclature of resistance

HIV has an RNA genome and RNA codes for all the proteins the virus needs to function. Each codon consists of three nucleotides and encodes one particular amino acid. Changes in the codon – a mutation – may cause encoding of a different amino acid and this is a mechanism that the virus uses to develop resistance and to escape from the action of the antiretroviral treatment. For example, FIGURE 1.5 demonstrates three codons that code for the amino acids lysine (Lys), aspartic acid (Asp) and serine (Ser). If a mutation occurs in the second codon and the G is replaced with an A, that codon no longer codes for aspartic acid but rather for asparagine (Asn) and this new amino acid may enable the virus to escape the action of an ARV drug.

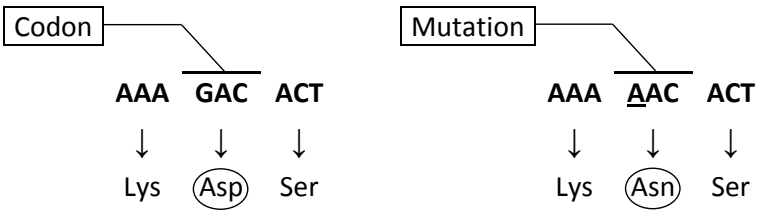

Figure 1.5 *Example of single nucleotide change leading to change in amino acid*

'Wild type' virus is a virus without any resistance mutations. There is a standard manner in which resistance mutations are depicted in the scientific literature. The codon position of the amino acid is given with the amino acid of the 'wild type' virus before the codon position and the mutant amino acid after the codon position, as depicted in FIGURE 1.6. M184V is the signature resistance mutation of lamivudine, where at codon position 184 in the viral genome, methionine (M) has been replaced by valine (V).

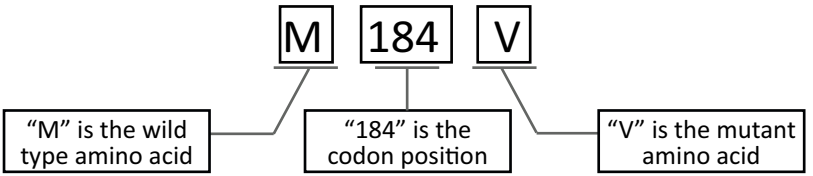

Figure 1.6 *Nomenclature for the signature lamivudine mutation, M184V*

Mechanisms of drug resistance

HIV drug resistance develops via one of two major pathways: selective pressure or transmission of drug resistant virus. As depicted in FIGURE 1.7, there are multiple causes for selective drug pressure and more than one factor might contribute at the same time to the emergence of resistance.

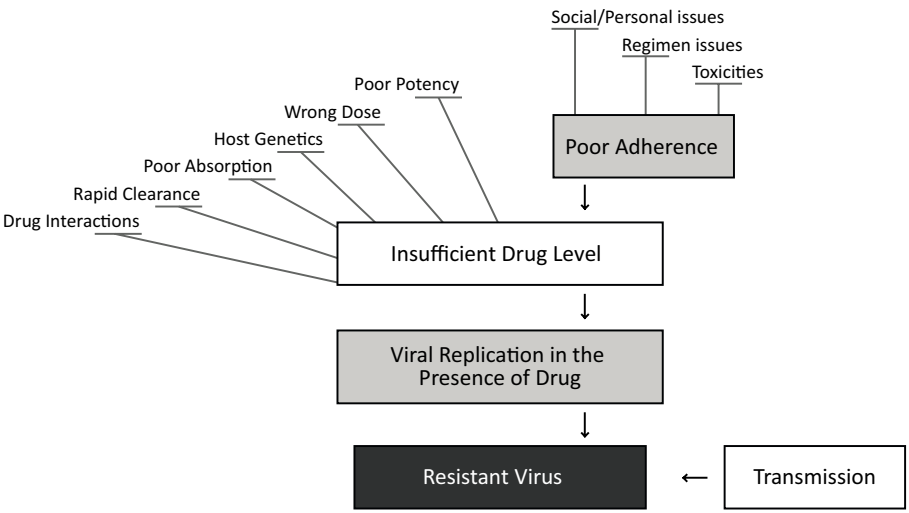

Figure 1.7 *Pathways for the development of drug resistance*

Various mechanisms for the development of drug resistance have been identified and these mechanisms differ between classes of drugs but also within a specific drug class.

Resistance to NRTIs and NtRTIs

The nucleoside and nucleotide analogues inhibit reverse transcriptase by incorporating into the newly developed chain of viral DNA. Because these drugs do not have a 3'hydroxyl group, no additional nucleotides can attach to them and the DNA chain is thus terminated. There are two mechanisms by which resistance develops: the incorporation of the analogue into DNA is impaired or the analogue is removed from the DNA chain.

The first mechanism, impairment of analogue incorporation, is active in the following mutations: M184V, K65R and the Q151M complex of mutations. M184V is the signature lamivudine mutation and confers high-level resistance to lamivudine. It develops within weeks in patients on 3TC monotherapy and is usually the first mutation to emerge in combination therapy. The K65R mutation is the classic tenofovir mutation, but also occurs when a patient fails abacavir

or stavudine-based ART. It confers resistance to most NRTIs and NtRTIs with the exception of zidovudine. The Q151M complex of mutations usually develops in patients failing on stavudine or didanosine. This mutation always starts with the Q151M substitution, which is followed by secondary mutations that increase resistance. Once this complex has developed, it will confer high-level resistance to most NRTIs, except lamivudine and tenofovir. This is fortunately rare and occurs mainly in patients failing ART for a very long time.

The second mechanism, removal of the analogue from the DNA chain, is associated with a group of mutations named the thymidine analogue mutations or TAMs. These mutations usually occur after failure of treatment with the thymidine analogues, such as zidovudine and stavudine. TAMs can, however, cause resistance to all NRTIs and NtRTIs. TAMs develop gradually and in variable order. TAMs occur on six codons: M41L, D67N, K70R, L210W, T215Y/F and K219Q/E. TAMs usually segregate into two pathways, TAM pathway 1: 41L, 210W and 215Y and TAM pathway 2: 67N, 70R, 215F and 219Q. The former is associated with higher-level resistance to tenofovir. Interestingly, the M184V mutation can slow the development of TAMs and may slightly increase the activity of some NRTIs – especially zidovudine – in spite of the presence of TAMs[25]. The most common NRTI mutations and their effects are depicted in TABLE 1.5.

TABLE 1.5 *Everything you need to know about nucleoside analogue resistance (adapted from reference 25)*

Mutation	Selected by	Effects on other NRTIs
M184V	3TC, FTC	- Loss of susceptibility to 3TC, FTC - ↓ susceptibility to ABC, ddI (clinically insignificant) - Delayed TAMs and ↑ susceptibility to AZT, d4T, TDF
TAMs	AZT, d4T	- ↓ susceptibility to all NRTIs based on number of TAMs - Greatest loss of susceptibility with 41/210/215 pathway
Q151M, T69ins	AZT/ddI, ddI/d4T	- Resistance to all NRTIs - T69ins: TDF resistance
K65R	TDF, ABC, ddI	- Variable ↓ susceptibility to TDF, ABC, ddI (and 3TC, FTC) - ↑ susceptibility to AZT
L74V	ABC, ddI	- ↓ susceptibility to ABC, ddI - ↑ susceptibility to AZT, TDF
E44D; V118I	AZT, d4T	- increases NRTI resistance (with 41/210/215 pathway)

Resistance to NNRTIs

NNRTIs block viral synthesis by binding tightly to the catalytic domain of reverse transcriptase. It affects the flexibility of the enzyme and blocks its ability to synthesize DNA (see FIGURE 1.8). Resistance mutations reduce the affinity of the drug to the enzyme. Resistance usually develops rapidly and the resistance patterns depend on the specific NNRTI in the drug regimen. NVP usually selects for Y181C, Y188C, K103N, G190A and V106A. Efavirenz preferentially selects

for K103N but Y188L is also seen.

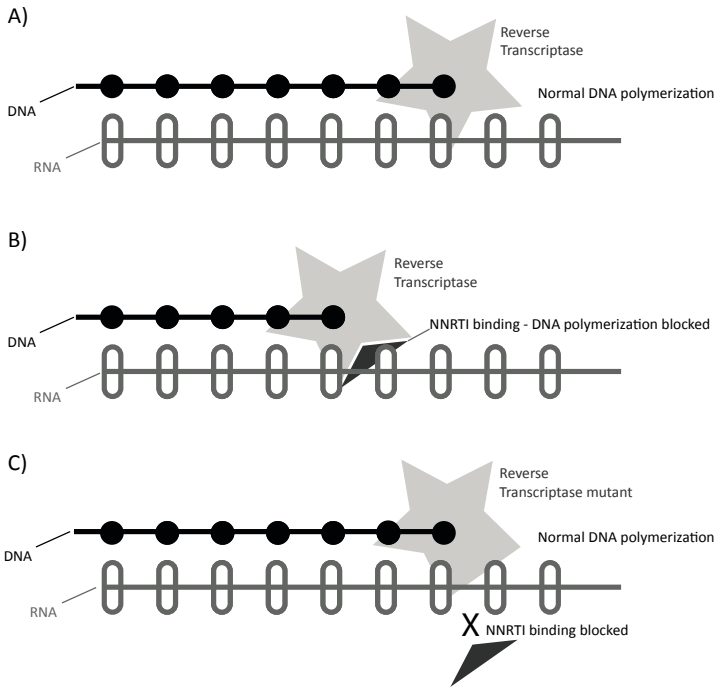

Figure 1.8 *Mechanism of development of resistance to the NNRTI drug class. A) Drug sensitive virus without NNRTI produces normal DNA polymerization; B) Drug sensitive virus with NNRTI blocks DNA polymerization C) Drug-resistant virus with reverse transcriptase mutant blocks NNRTI binding and results in normal DNA polymerization.*

Resistance to protease inhibitors(PIs)

The function of viral protease is to cleave large polyprotein precursors at specific sites, which then release the structural proteins and enzymes necessary for assembly of infectious virions. If protease is inhibited by ART, viral particles are still produced but they are immature and remain uninfectious. Protease inhibitors have a strong affinity for the active site of HIV protease and inhibit the catalytic activity of the enzyme.

Resistance to PIs develops because of amino acid substitutions that occur either inside the substrate-binding domain of protease or at distant sites. These amino acid changes modify the number and nature of the points of contact between the drugs and the enzymes, thereby reducing the affinity of the drugs to the enzyme. Although some PIs only select for specific mutations, considerable overlap exists and there is thus significant cross-resistance within the drug class.

The major PI resistance mutations to lopinavir are V32I, I47V/A and V82A/F/T/S. The first two mutations on their own can confer high-level resistance to lopinavir. There are many minor mutations and the accumulation of 6 or more of these is associated with reduced virological response and the accumulation of 7 or 8 mutations confers complete resistance.

Resistance to other drug classes

HIV resistance has been described to all available drug classes. It is, however, beyond the scope of this book to discuss resistance to the entry inhibitors and integrase inhibitors.

Interpretation of mutations

Fortunately, we do not have to remember all these mutations by heart. The International AIDS Society (IAS-USA) compiles a consensus list of mutations every year and releases this on their website: https://www.iasusa.org/content/hiv-drug-resistance-mutations. FIGURE 1.9 and FIGURE 1.10 reflect the list from November 2011. This list can be downloaded from the IAS website and pocket guides can be ordered from the organization.

There are also a number of websites available that assist with the interpretation of mutation patterns, the most well-developed being the Stanford University HIV Drug Resistance Database. A SATuRN mirror site exists in South Africa and can be accessed on http://www.bioafrica.net/hivdb/ and http://hivdb.stanford.edu/. This website has a wealth of information and also has a function called the HIVdb program that does genotype resistance interpretation.

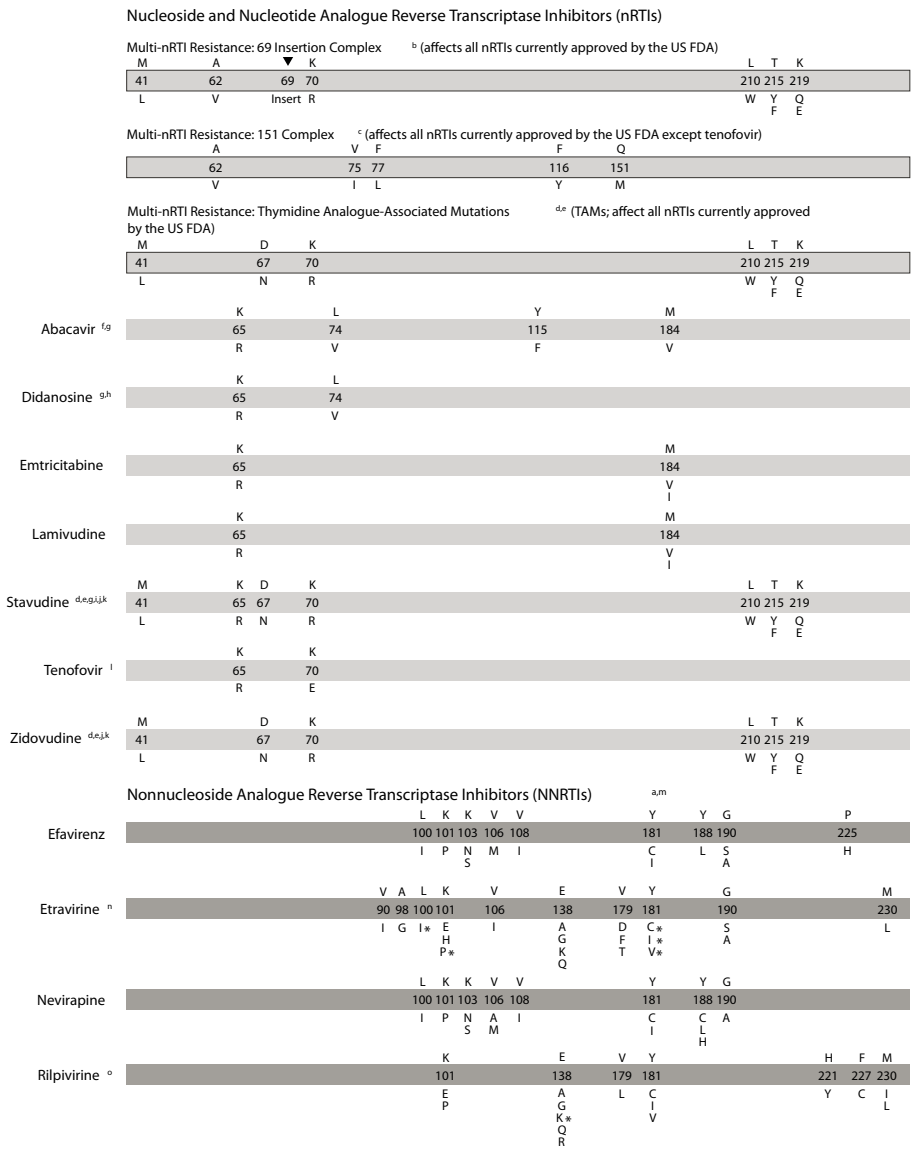

Figure 1.9 *IAS-USA mutation list. Mutations in the reverse transcriptase gene associated with resistance to reverse transcriptase inhibitors*

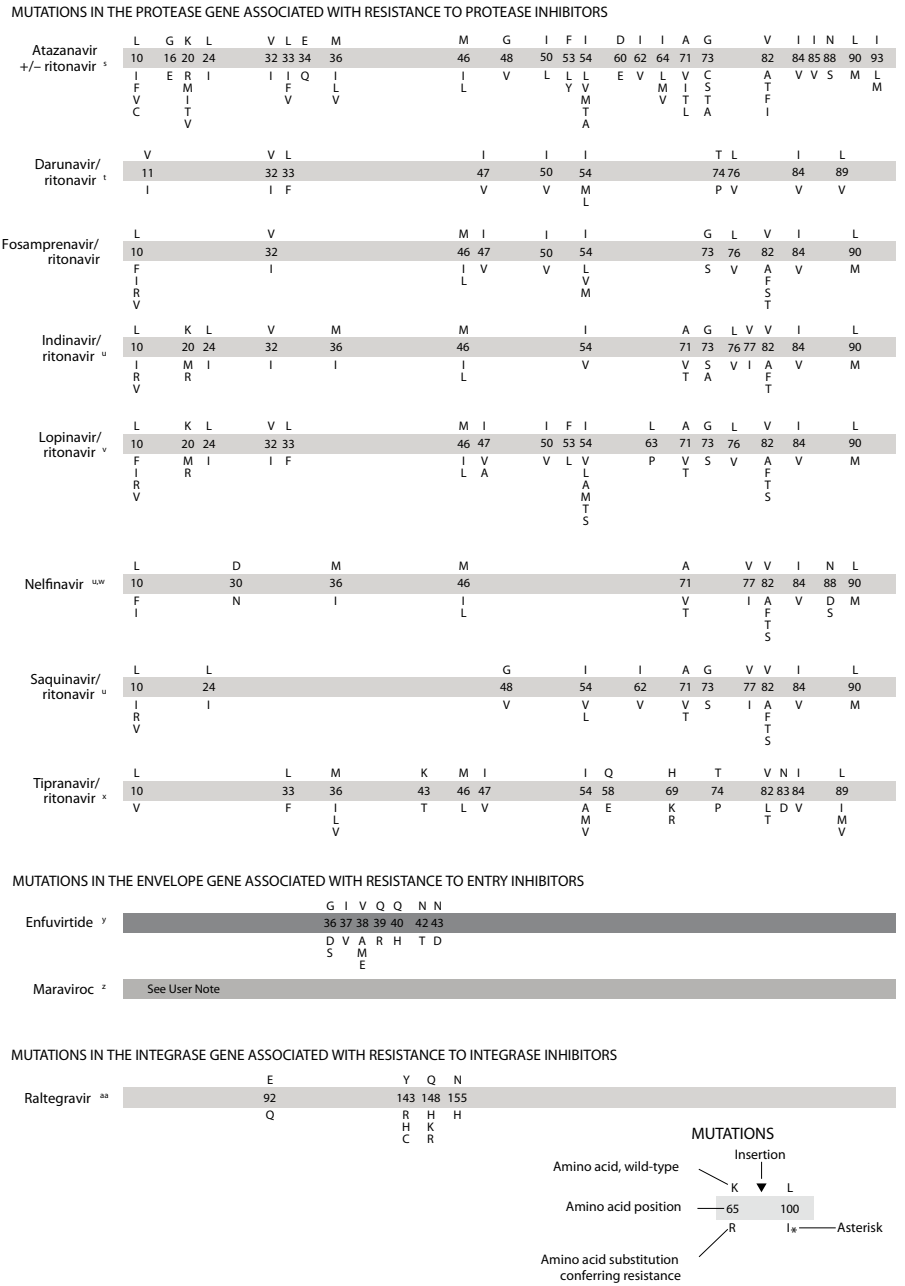

Figure 1.10 *IAS-USA mutation list. Mutations in the protease gene associated with resistance to protease inhibitors*

1.4. Types of resistance testing

There are two ways to test for HIV drug resistance. The first method is phenotypic testing, which is the standard way of testing for antimicrobial drug resistance. This is done by cloning the virus and then incubating it at different strengths of the antiretroviral medication in tissue-culture systems. Phenotypic testing has some advantages, such as the potential for easier interpretation. Since it is a quantitative measure indicating the degree of resistance, it is able to assess the interactions between mutations. It is, however, very expensive and requires a high-safety laboratory for cloning. It is thus only a research tool at present.

The main form of resistance testing is genotypic testing. Genotypic testing is based on PCR technology and detects the presence of mutations in a virus population by identifying codon changes that are different from the standard or 'wild-type' genetic sequences of HIV. These codon changes are also called point mutations and many of these have been linked to the phenotypic expression of drug resistance.

A typical genotypic resistance report from the SATuRN RegaDB Clinical and Resistance Database using the Stanford HIVDB 6.0.5 algorithm will look as follows:

TABLE 1.6 *Antiretroviral HIV drug resistance interpretation report.*

Sample ID / Sample Date: RES001 - 20/04/2011
Antiretroviral experience: [d4T, 3TC, NVP]
Subtype: HIV-1 Subtype C
Resistance interpretations: HIVDB 6.0.5

Drug	Mutations	Description	Level	GSS
zidovudine	184V	Susceptible	1	1.0
zalcitabine	N/A	N/A	N/A	N/A
didanosine	184V	Susceptible	1	1.0
lamivudine	184V	High-level resistance	5	0.0
stavudine	184V	Susceptible	1	1.0
abacavir	184V	Potential low-level resistance	2	1.0
emtricitabine	184V	High-level resistance	5	0.0
tenofovir	184V	Susceptible	1	1.0
nevirapine	103R 106M 179D	High-level resistance	5	0.0
delavirdine	103R 106M 179D	High-level resistance	5	0.0
efavirenz	103R 106M 179D	High-level resistance	5	0.0
etravirine	106M 179D	Low-level resistance	3	0.5
saquinavir	N/A	N/A	N/A	N/A
saquinavir/r		Susceptible	1	1.0
ritonavir	N/A	N/A	N/A	N/A
indinavir	N/A	N/A	N/A	N/A
indinavir/r		Susceptible	1	1.0
nelfinavir		Susceptible	1	1.0
fosamprenavir	N/A	N/A	N/A	N/A
		Susceptible	1	1.0
lopinavir/r		Susceptible	1	1.0
atazanavir	N/A	N/A	N/A	N/A
atazanavir/r		Susceptible	1	1.0
tipranavir/r		Susceptible	1	1.0
darunavir/r		Susceptible	1	1.0

The genotypic susceptibility score (GSS) is automatically calculated for each antiretroviral drug by the Stanford HIVDB algorithm. A score of 1 means complete susceptibility and a score of 0 complete resistance. The level of resistance is another measure of the extent of resistance to an individual drug, where 1 means full susceptibility and 5 means high-level resistance.

Limitations of resistance testing

It is very important to understand the limitations of standard genotypic resistance testing. There are four major limitations that will be briefly discussed.

1. It cannot detect 'minority' populations. Minority populations are viral populations that occur at a level of less than 20% of the total population. Resistance testing can thus only detect the dominant population of virus in the plasma. This dominant population does not always reflect the diversity of viral quasispecies in patients failing treatment. It is believed that the minority populations may serve as a reservoir for the generation of novel resistant viral strains that might ultimately take over from the dominant population. Although so-called ultra-deep sequencing for minority populations is possible, interpretation of these results is complex and this is only used for research purposes at present.

2. It cannot detect 'archives' or 'reservoirs'. When patients with drug-resistant HIV are treated with alternative drugs for a period of time, the mutations associated with resistance to the initial regimen may no longer be present in the virus obtained from the plasma. These mutations do not go away, however, but are archived within the cells. If therapy with the initial drugs is resumed, these archived resistant strains can re-emerge and cause treatment failure. The same is true for a patient who has completely stopped his ART. The patients should be back on his ART for a minimum of six weeks before a genotype can be requested. Resistance testing thus gives the most reliable results for the drugs the patient is currently taking.

3. It is better at determining which drugs won't work than which drugs will. In light of the previous two limitations, it should be understood that the absence of a mutation on the genotype does not mean that it is not there. Apparent susceptibility can be further compromised by the imprecision of some assays, the short time required for some initially susceptible viruses to develop full cross-resistance to the new agents and confounding variables, such as the pharmacokinetics of individual drugs.

4. It requires a minimum viral load. At present all the tests generally require a plasma viral load of at least 1000 copies/ml in order to ensure adequate viral amplification. Resistance tests are, therefore, not useful in determining the presence of resistance in patients with low-level viraemia.

1.5. Approach to virological treatment failure

There are three basic steps to be followed before treatment substitutions are made.

The first step in assessing treatment failure is confirming the viral load. This should be done after 12 weeks of intensified adherence counselling.

The second step, which can be done while the viral load result is awaited, is assessing the adherence of a patient. This can be done in a variety of ways, but usually consists of an in-depth interview with a patient where a detailed adherence history is taken. It is also possible to look at pharmacy records of collection of medication and missed appointments. In addition, it is possible to do therapeutic drug monitoring, but this is rather expensive and can be difficult to interpret. An intensified adherence strategy should be followed in which patients are provided with adherence strategies and tools, if possible.

The third step is determining the reason for treatment failure. The cause of failure should first be addressed before a switch to second-line treatment is made. If this is not done, the patient is as likely to fail the second-line regimen.

Failing NNRTI-based therapy (adult first-line)

This section will deal with recommended treatment switches when resistance testing is not available. When resistance genotypes are available they should, of course, be used to inform treatment changes.

Thymidine-analogue based regimens

It can be very complicated to suggest treatment changes for patients failing on thymidine-analogue regimens since long-term failure may induce TAMs that can limit all subsequent treatment regimens. If a patient has failed for less than 1 year, it can be assumed, however, that the K65R mutation has not yet developed on stavudine and that there will be limited TAMs, so a switch to a tenofovir-based regimen should be adequate. This regimen should then consist of a combination of TDF, 3TC or FTC, and a PI – usually boosted lopinavir (LPVr) or atazanavir (ATV/r). If TDF cannot be used, an alternative of zidovudine or abacavir should be considered. If a patient has failed for longer than one year, a resistance genotype is indicated, if at all possible.

TDF-based regimens

TDF usually selects for the K65R mutation. This mutation causes reduced susceptibility to abacavir, didanosine, lamivudine and emtricitabine. However, it increases susceptibility to zidovudine and a second-line regimen consisting of AZT, 3TC or FTC and a boosted PI should be adequate. TDF can, however, occasionally induce the development of TAMs, which could reduce susceptibility to stavudine and zidovudine.

Failing PI-based therapy (adult second-line)

The majority of patients who fail second-line protease-inhibitor-based treatment do not have any PI mutations. Second-line treatment failure is usually a continuation of poor adherence in the first regimen. When step-up adherence is performed in second-line failure, a large number of patients will re-suppress their viral load. Having said this, there are patients who do develop PI mutations, especially young children and patients with extensive previous ART experience. Constructing a regimen in second-line failure that does not respond to intensified adherence support is very complicated and should preferably be done in conjunction with a resistance genotype.

A standard third-line regimen has not yet been included in the South African HIV treatment guidelines. Third-line regimens are, however, frequently used in the private sector, and are usually based on the resistance genotype. Such a regimen often consists of a combination of entry inhibitors such as maraviroc, new-generation NNRTIs such as etravirine, integrase inhibitors such as raltegravir and new-generation PIs such as darunavir that have a different mutation pattern to the other PIs.

1.6. Adherence resources

Liu *et al*. demonstrated that three commonly used adherence tools – electronic monitoring devices (such as MEMS), pill counts and patient interview – all have limitations[26]. They advised a comprehensive, combination approach. This is however mostly unattainable in the developing world due to financial and human resource constraints. Patient interview seems to be the most feasible in a developing world setting and Knobel *et al*. developed a simplified medication adherence questionnaire (SMAQ), consisting of 6 questions (TABLE 1.7). It showed sensitivity of 72%, specificity of 91% and a likelihood ratio of 7.94 in identifying non-adherent patients[27]. A meta-analysis of self-reported adherence showed that, although not ideal, it could distinguish between clinically meaningful patterns of medication-taking behaviour[28].

TABLE 1.7 *A simplified medication adherence questionnaire (SMAQ) (taken from reference 27)*

SMAQ:
(1) Do you ever forget to take your medicine?
(2) Are you careless at times about taking your medicine?
(3) If at times you feel worse, do you stop taking your medicine?
(4) Thinking about the last week. How often have you not taken your medicine?
(5) Did you not take any of your medicine over the last weekend?
(6) Over the past 3 months, how many days have you not taken any medicine at all?

For further discussion on adherence tools, go to:

- Machtinger EL & Bangsberg R. Adherence to HIV Antiretroviral Therapy. HIV InSite Knowledge Base Chapter. Available from: http://hivinsite.ucsf.edu/InSite?page=kb-03-02-09#S1X
- Chesney MA, Ickovics JR, Chambers DB, Gifford AL, Neidig J, Zwickl B, *et al*. Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG adherence instruments. Patient Care Committee & Adherence Working Group of the Outcomes Committee of the Adult AIDS Clinical Trials Group (AACTG). AIDS Care 2000; 12: 255-66

For a discussion on strategies to improve adherence, go to:

- Bain-Brickley D, Butler LM, Kennedy GE, Rutherford GW. Interventions to improve adherence to antiretroviral therapy in children with HIV infection. Cochrane Database of Systematic Reviews 2011, Issue 12. Art. No.: CD009513. DOI: 10.1002/14651858.CD009513.
- Rueda S, Park-Wyllie LY, Bayoumi A, Tynan AM, Antoniou T, Rourke S, *et al*. Patient support and education for promoting adherence to highly active antiretroviral therapy for HIV/AIDS. Cochrane Database of Systematic Reviews 2006, Issue 3. Art. No.: CD001442. DOI: 10.1002/14651858.CD001442.pub2.
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- Bärnighausen T, Chaiyachati K, Chimbindi N, Peoples A, Haberer J, Newell ML. Interventions to increase antiretroviral adherence in sub-Saharan Africa: a systematic review of evaluation studies. Lancet Infect Dis 2011; 11: 942-951

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