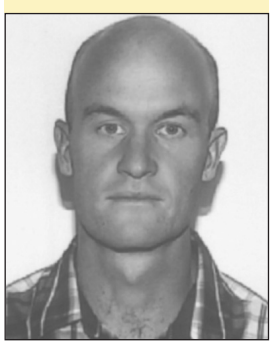
ANTIRETROVIRAL RESISTANCE

Highly active antiretroviral therapy (HAART) reduces opportunistic infections, improves quality of life and prolongs survival in HIV-infected individuals. The major threat to this is the development of resistance to sequential antiretroviral drugs, which compromises therapeutic options.



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If patients develop multidrug-resistant HIV they risk immunological and clinical progression that can only be slowed but no longer reversed by HAART, essentially returning their disease status to the 'pre-HAART' era.

Much can be done to avoid the development of resistance: efficacious choices and rational sequencing of regimens on the part of clinicians, thorough adherence counselling and impeccable adherence on the part of patients. This article focuses on these issues with particular reference to the drug regimens in the National Department of Health Antiretroviral Treatment Guidelines.[1]

MECHANISMS OF RESISTANCE

Antiretrovirals act by inhibiting the activity of viral proteins that are essential for the replication of HIV:

- NRTIs and NNRTIs inhibit the enzyme reverse transcriptase (RT)
- Protease inhibitors inhibit the enzyme protease
- Fusion inhibitors block the surface protein GP 41.

Resistance develops when one or more mutations occur in the genes encoding the amino acid sequence of these proteins. Typically these are single-base substitutions. The resultant alteration in the amino acid sequence means a reduction in the affinity of the drug for the protein and thus reduced drug inhibition of viral replication. As an example, the M184V mutation is selected for in the RT gene by 3TC and results in high-level resistance to 3TC. It is a single-base substitution in the RT gene that results in a replacement of methionine by valine at position 184 in the RT amino acid sequence.

Resistance may be low level (with partial reduction in the susceptibility of the virus to the drug) or high level (with the virus fully resistant to the drug).

HIV is highly prone to developing resistant mutations when it is allowed to replicate in the presence of antiretroviral drugs. The reasons for this are:

- The high rate of HIV production and turnover. Between 1 and 10 billion new HIV particles are formed each day in an untreated HIV-infected individual.
- Reverse transcriptase (which performs a critical step in transcribing viral RNA into DNA as part of the replication process) is infamously error prone with no proofreading capability. This results in an average of 1 mutation occurring in each of the 1 - 10 billion viral genomes transcribed each day.

The result is that prior to the introduction of antiretrovirals a very heterogenous pool of viruses exists, with all possible mutations at all alleles in the genome existing across this diverse population of viruses. Individual resistance mutations exist in discrete viral genomes, however.

These small numbers of drug-resistant mutants are selected out when viral replication persists in the presence of antiretrovirals, because it is only in this context that the otherwise less fit mutant is fitter than the wild type and outgrows it (Fig. 1).

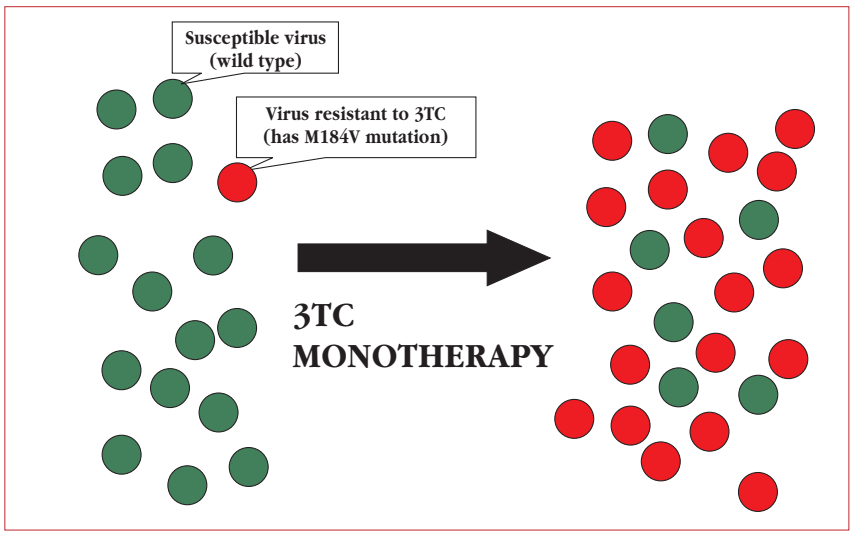

Fig. 1. This shows the hypothetical situation of 3TC used as monotherapy. A few viruses containing the M184V mutation precede the introduction of 3TC. This mutation confers high level resistance to 3TC. Thus, within days to weeks of starting 3TC the wild type (green) is suppressed and the resistant mutant (red) is selected out and comes to dominate the viral population.

HAART is far more effective than mono- or dual therapy at preventing the evolution of resistance because:

- The only effective means of avoiding the selection of resistance mutants is by preventing replication. Multiple drugs suppress viral replication more efficiently.
- Although all single resistance mutations are present in different viruses, it is highly improbable that a single viral genome containing resistance mutations to all 3 drugs will be present before the introduction of HAART (unless the patient was infected by a person with multidrug-resistant HIV). Thus while single-drug therapy will select out for mutants that are resistant to that drug, no virus resistant to all 3 drugs exists to be selected out by HAART. If, however, replication continues in the presence of HAART this mutant will evolve because ongoing random mutations continue to occur (see Figs. 2a and 2b).

Once mutations have been selected out they cannot be erased. They remain archived in memory T cells as proviral DNA. The resistant mutant viruses may be overtaken by wild type in the circulating viral population if the selection pressure of the drug(s) is removed but will re-emerge as soon as replication occurs in the context of the drug(s) to which they are resistant.[2]

ROLE OF ADHERENCE

By far the most common reason for ongoing viral replication while a patient is on HAART is inadequate adherence to therapy.

The level of adherence that is most likely to select for resistant viruses is between 70% and 90%. At this level, drug levels are inadequate to suppress replication, but enough to apply potent selection pressure for the emergence of resistant mutants.

Other reasons are drug absorption problems, vomiting of medication, drug interactions which reduce antiretroviral levels or the prescription of a non-suppressive regimen (e.g. dual NRTI therapy).

Resistance is most often the consequence rather than the cause of initial treatment failure,[2] yet once resistant mutations develop it becomes impossible to suppress viral replication and this begets the development of further resistance mutations (Fig. 3, Table I).

EMERGENCE OF RESISTANCE

A viral load (VL) of < 50 copies/ml is required to prevent the emergence of resistant mutants. Even patients who suppress to between 50 and 400 copies/ml are far more likely to rebound than those who suppress to < 50 copies/ml.[4] However, isolated 'blips' (a single isolated rise in viral load (VL) to between 50 and 1 000 copies/ml) have not been shown to result in the emergence of resistance.[5]

One study that looked at the VL at which new resistance mutations occurred showed that the first new RT mutation (usually M184V) occurs at a median VL of 500 and the first protease mutation at a median VL of 200.[6]

Viral resistance manifests as a VL that fails to suppress or rebounds after initial suppression, despite optimal adherence. In clinical practice it can be difficult to differentiate adherence problems from resistance, but in the long run this becomes less important as poor adherence will beget resistance. The exception is patients who are very poorly adherent and fail to create enough selection pressure to select for resistance. This is suggested by a failure to achieve any significant reduction of VL (> 1 log) when therapy is initiated. This may also occur in patients infected by a multidrug-resistant virus (primary resistance), but this is unlikely to be a common occurrence in South Africa at present, given that widespread access to antiretroviral therapy is a recent phenomenon.

PRINCIPLES OF CHANGING THERAPY FOR RESISTANCE

Because of the entity of VL 'blips' and potential technical errors, resistance should never be assumed or therapy ever changed based on a single VL reading. The Department of Health guidelines take this into account, advising that the switch from 1st to 2nd line only be made if the VL is documented to rebound over a 3 - 6-month period with the latter

Table I. **Important resistance mutations**[2]

RT SEQUENCE

- **M184V**
  Selected for by 3TC
  Causes high-level resistance to 3TC
  Reduces viral fitness by 1/3 (i.e. 'crippling')
  Increases susceptibility to AZT and D4T in context of TAMs

- **Thymidine analogue mutations (TAMs)**
  M41L, D67N, K70R, L210W, T215Y/F, K219Q/E
  Selected for by AZT and D4T and confer resistance to these drugs
  Can cause NRTI class resistance if ≥ 4 accumulate (resistance to all NRTIs except 3TC)
  M41L, L210W in particular are associated with tenofovir resistance

- **L74V**
  Selected for by ddI and abacavir and causes resistance to these 2
  Causes hypersusceptibility to AZT and tenofovir

- **K65R**
  Selected for by ddI, abacavir and tenofovir
  Resistance to most NRTIs
  Causes hypersusceptibility to AZT

- **Multinucleoside resistance mutations**
  Q151M and T69ins
  Selected for by AZT+ddI or D4T+ddI
  Result in NRTI class resistance

- **NNRTI mutations**
  K103N, Y181C, Y188C and others
  Selected for by EFZ and NVP
  Most cause high-evel class resistance as a single mutation

PROTEASE SEQUENCE

- Major protease inhibitor mutations
  46, 82, 84, 90
  Combinations of these mutations associated with PI class resistance

- **Drug-specific PI mutations**
  Certain mutations confer drug-specific resistance
  e.g. D30N confers nelfinavir resistance

value > 5 000 copies/ml. Another important reason not to change immediately is that when viral rebound manifests it may be reversible by improved adherence. Obviously once there is persistent rebound in patients taking therapy some degree of resistance inevitably develops and subsequent suppression becomes very unlikely.

The development of resistance to drugs in a regimen is sequential, with resistance to drugs with a low genetic barrier emerging before resistance to drugs with a high genetic barrier. Certain mutations, typically M184V, are also more likely to occur with early failure.

Thus, when a regimen fails it can be assumed that resistance has developed to those drugs with a low genetic barrier, and these and related drugs with the same resistance pattern cannot be used in later regimens. The aim is to change to another regimen before the accumulation of further mutations that threaten those drugs with a high genetic barrier and result in the avoidable development of cross resistance.

DEPARTMENT OF HEALTH REGIMENS: IMPORTANT MUTATIONS

First-line therapy consists of D4T, 3TC and one of the NNRTIs (efavirenz or nevirapine). The mutational pattern that is present when virological failure occurs on this regimen is fairly predictable. The first mutation to occur is usually the M184V. This is followed (and sometimes preceded) by an NNRTI mutation. Both the M184V and the NNRTI mutation occur rapidly in a non-suppressive regimen, and confer high-level resistance to 3TC and the NNRTI class respectively.

Thereafter there is an accumulation of thymidine analogue mutations (or TAMs) that are selected for by D4T. There are 6 possible TAMs. If 1 or 2 TAMs are present there is low to intermediate level resistance to D4T and AZT (which have very similar mutational patterns). However, once 3 or more TAMs accumulate, this resistance becomes high level and there is also the risk of developing cross-resistance to other drugs in the class (e.g. ddI, abacavir) and the nucleotide reverse transcriptase inhibitor, tenofovir (Fig. 4).

TAMS accumulate gradually. One study of patients on AZT/3TC dual therapy showed that after 72 months in those failing therapy in whom genotyping was done, 29% had one TAM and 21% had more than one TAM.[7] It is important to change before sufficient TAMs have accumulated to significantly compromise D4T and AZT and, worse still, cause broad NRTI class resistance, thereby compromising second-line options.

Thus, when first line fails, assume 3TC and NNRTI class resistance. If the regimen has failed for 6 months or less, it is likely that D4T has not selected for sufficient TAMs to have significantly compromised AZT or ddI.

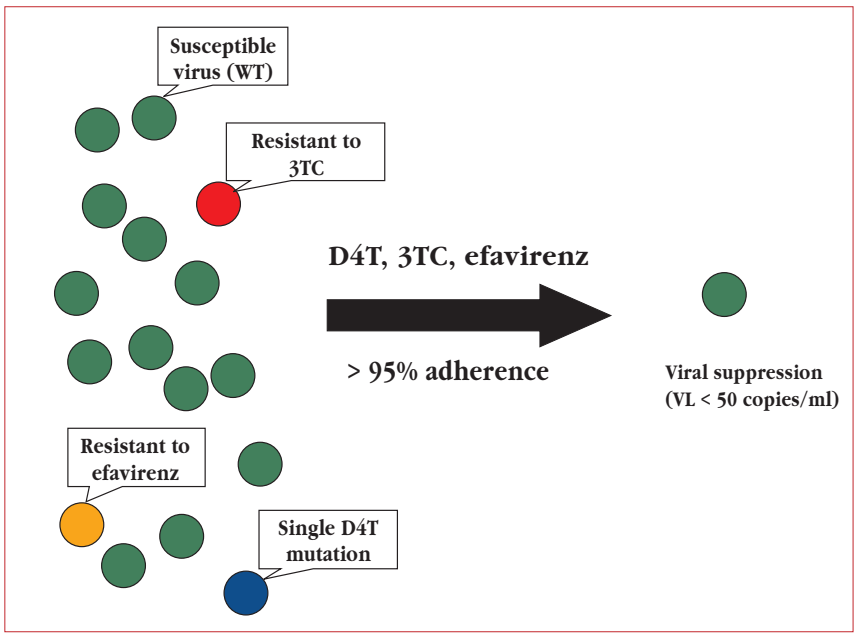

Fig. 2a. Although viruses containing all resistance mutation precede the introduction of HAART these reside in discrete genomes, and no single genome contains resistance mutations to all the drugs. Thus, HAART taken with optimal adherence is able to suppress viral replication. If this suppression is sustained then evolution of resistant mutants is prevented.

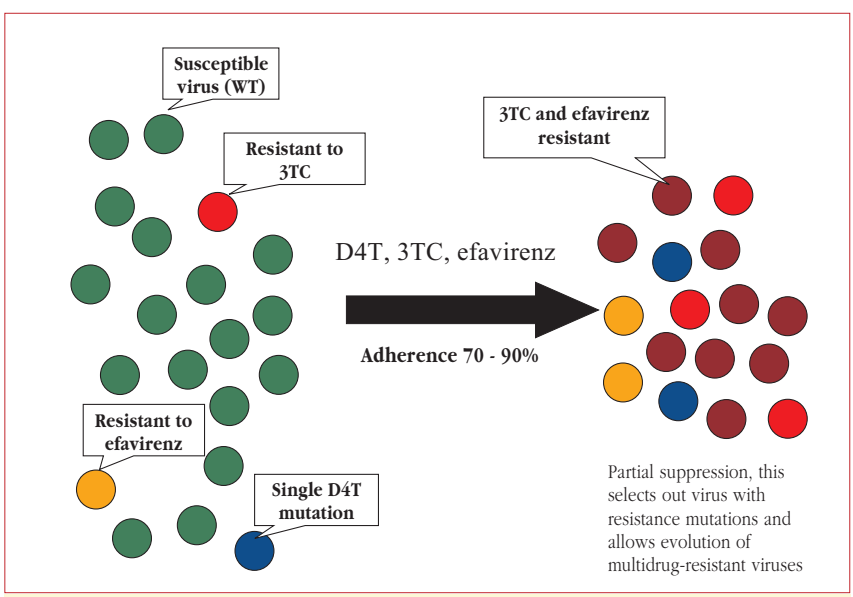

Fig. 2b. However, if replication is allowed to continue in the presence of HAART (usually due to suboptimal adherence) mono-resistant viruses are selected out and then further random mutations in their genome occur that make them two-drug (and later three-drug) resistant.

If the patient was, however, left on a failing first-line regimen for much longer than 6 months, they run the risk of having accumulated sufficient TAMs to compromise the efficacy of AZT and even ddI.

Second-line treatment consists of AZT, ddI and lopinavir/ritonavir (Kaletra).

The rationale for this choice is based on the likely resistance patterns that are mentioned above. 3TC resistance is likely to have developed and thus it is changed to ddI. NNRTI class resistance is likely to have developed, so a class switch to the PI, Kaletra, is made. As significant D4T resistance is unlikely to be present if a timeous switch is made, it could be continued. However, rather than combing D4T and ddI, which together carry a high risk of mitochondrial toxicity, a switch to AZT is made.

When patients fail second-line therapy they develop (further) TAMs that are selected for by AZT. ddI may result in the selection of the L74V mutation, but the presence of AZT tends to drive the mutational pattern down the TAM pathway rather than toward the L74V mutation. Resistance to Kaletra, when it is used as the first protease inhibitor to which a patient is exposed, is extremely unlikely, with a large trial showing no Kaletra mutations after 5 years of follow-up in 100 patients.[8] There are only isolated case reports in the literature of Kaletra resistance when it is used as such.[9,10] This is because Kaletra is potently suppressive and has a very high genetic barrier to resistance with 8 mutations required for high-level resistance. Thus, if patients fail a Kaletra-based regimen (when Kaletra is the first PI to which they are exposed) this is more likely to reflect non-adherence rather than the development of Kaletra resistance. Kaletra is still usually a viable option if patients can be successfully counselled to improve adherence.

RESISTANCE TESTING

There are two methods of detecting resistance to antiretroviral drugs – genotyping (GT) and phenotyping (PT). Genotyping detects resistance mutations in the RT and protease genes of the patient's virus by genetic probes or gene sequencing. Phenotyping involves inserting the RT and protease genes from a patient's virus into a laboratory virus depleted of these genes and measuring its replication in a tissue-culture system in the presence of drugs relative to a standard wild type.

The problems with GT are that it is costly, and clear evidence for its benefit in clinical practice is lacking. A recent meta-analysis of GT trials[11] showed minimal benefit in terms of virological response for GT-guided

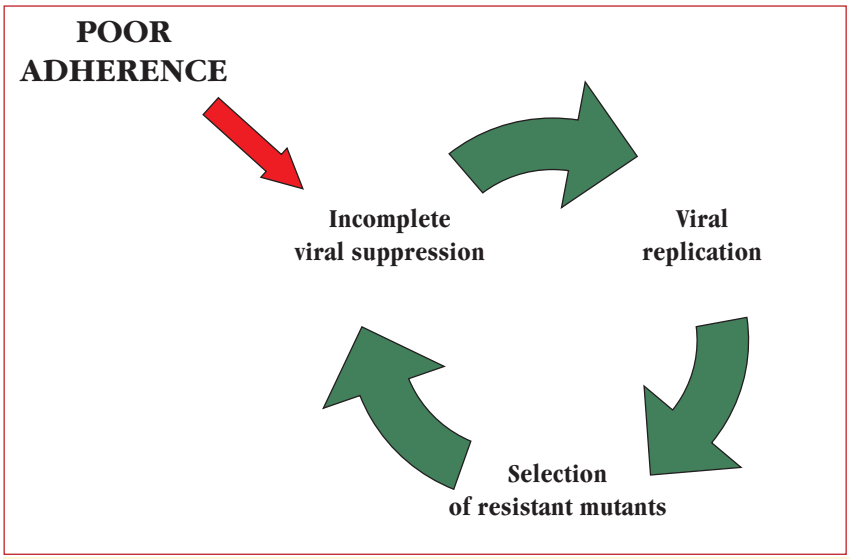

Fig. 3. Resistance is most often the consequence rather than the cause of initial treatment failure. Initial treatment failure (ongoing viral replication) usually results from suboptimal adherence. If there is ongoing replication in the presence of drugs, resistant mutations are selected out. Once resistant mutations accrue it becomes impossible to suppress viral replication and this begets the development of further resistance mutations.

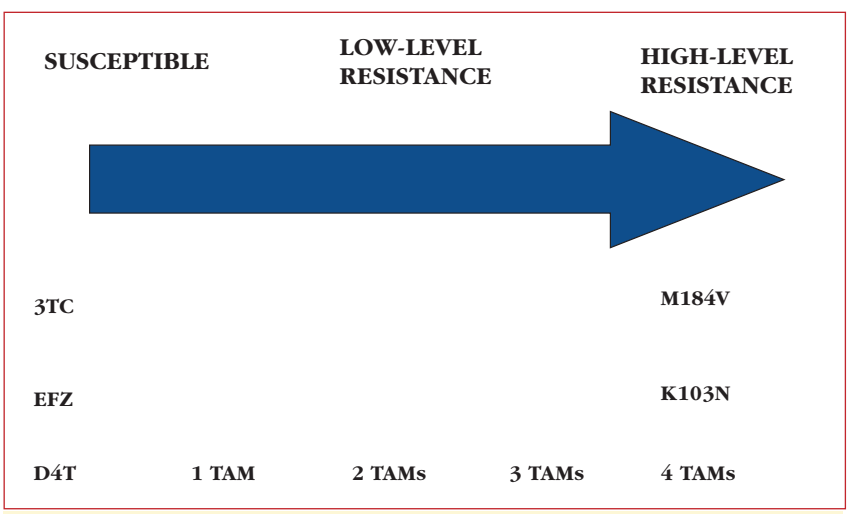

Fig. 4. Evolution of resistance on first-line therapy consisting of 3TC, efavirenz and D4T. Single mutations confer high-level resistance to 3TC and efavirenz respectively, whereas the accumulation of 3 or more thymidine analogue mutations (TAMs) is required for resistance to D4T.

therapy choices as opposed to controls who did not have GT. It is currently unavailable in the State sector.

GT will only detect mutations if the VL is greater than 1 000 copies/ml and the mutant virus constitutes more than 20% of the viral population. Genotyping should thus only be done when the patient is on therapy, otherwise the mutants are overgrown by wild type and the mutations will not be detected. Similarly, even if the patient is on therapy, if they are not on a drug that selects for a given mutation it will not be detected even if it is archived. For instance if a patient is on HAART, but not receiving 3TC then the M184V mutation may be archived and not detectable by GT.

PT is unavailable in SA at present and it is regarded as inferior to GT.

SALVAGE THERAPY

Salvage therapy refers to the construction of an antiretroviral regimen for patients who have failed multiple prior regimens. Finding a virally suppressive regimen is often impossible because of multidrug resistance. Thus the aim is often immunological and clinical stabilisation rather than VL suppression.

The choice of drugs in a salvage situation is best directed by GT. Drug choices may involve those with residual activity (e.g. tenofovir) or those known to 'cripple' the virus effectively (e.g. 3TC, PIs). In a developed world setting, dual boosted PI options (e.g. fosampenavir and lopinavir boosted with ritonavir), fusion inhibitors (e.g. enfuvirtide) and new-generation PIs (e.g. tipranavir) are available as salvage options.

Viral 'crippling' involves using a drug to which the virus is known to be resistant, but which selects for a less fit resistant mutant and suppresses the wild type, thus reducing viral replicative capacity and slowing immunological and clinical decline.[12] The aim here is not viral suppression and this strategy is only appropriate where all available suppressive options have been exhausted.

No salvage therapy options are currently offered in the Department of Health guidelines and if patients fail second line, the options are to continue this for its 'crippling' effect and/or institute palliative therapy when clinical decline occurs.

TWO IMPORTANT PRACTICAL CONCEPTS

Protecting the 'NNRTI tail'

In the context of mother-to-child transmission prevention, 14 - 40% of mothers will demonstrate NNRTI resistance mutations after a single

dose of nevirapine.[13] This is due to the fact that nevirapine has a very long half-life and persists in significant yet sub-therapeutic concentrations in the plasma for days to 2 weeks after a single dose, selecting for mutations. Similarly, if an NNRTI-containing HAART regimen is stopped, because nevirapine and efavirenz have a much longer half-life than the NRTIs, they persist in the plasma effectively as monotherapy for 7 days after the regimen is stopped.[14] This too will select out for mutations. Thus in the context of nevirapine use in MTCT prevention it is advisable to cover this 'NNRTI tail' with AZT/3TC for 5 - 10 days. This has been shown to reduce the emergence of NNRTI mutations.[15] In the context of stopping an NNRTI-containing HAART regimen electively (e.g. patient's request or after use in a pregnant woman with a high CD4 for MTCT prevention) it is advisable to continue the NRTIs for 5 days after stopping the NNRTI to protect the 'NNRTI tail'.[14] However, if the regimen is being stopped for a serious adverse event such as lactic acidosis, then the immediate safety concerns take precedence and all drugs in the regimen should be stopped simultaneously.

Interchangeability of AZT and D4T

It is increasingly understood that AZT and D4T select for, and are compromised by, very similar mutational patterns. Thus it is not necessary to swap these drugs merely for resistance concerns. If a patient has been on AZT/3TC as the nucleoside backbone of their first-line regimen with an NNRTI, rather than changing both NRTIs to the more mitochondrial toxic combination of D4T/ddI in second line, it is more appropriate to persist with AZT and change the 3TC to ddI and the NNRTI to Kaletra.

GLOSSARY OF TERMS

- **Archiving** – A resistance mutation is selected out by drug pressure, but when the drug pressure is removed it is overtaken in the circulating viral pool by the fitter wild type. It however remains 'archived', persisting at low levels in circulating viruses and as proviral DNA in the chromosomes of resting memory T-cells in lymph nodes. If the drug is reintroduced the mutation will re-emerge.
- **Cross resistance** – The development of resistance to a drug to which the patient has not been exposed. It occurs within classes. This occurs because certain drugs or whole classes share similar resistance mutational patterns. A single NNRTI mutation can cause cross class resistance whereas multiple mutations are required in the case of PIs and NRTIs.
- **Genetic barrier to resistance** – This refers to the ease with which resistance develops to a given drug. LOW = a single mutation is required for resistance to develop, thus in a non-suppressive regimen resistance to these drugs develops in days to weeks. HIGH = multiple mutations are required for resistance to develop to these drugs, thus it takes months to years for high-level resistance to develop.
- **Primary resistance** – Resistance mutations that are acquired with the virus at the time of infection. This may be due to the fact that drug exposure and resistance have occurred in the individual from whom infection was acquired. In addition, some viruses are inherently resistant to certain drugs, like HIV 2 has reduced susceptibility to NNRTIs.
- **Viral fitness (replicative capacity)** – The ability of a given virus or viral pool to replicate relative to wild type. A virus with multiple resistance mutations usually has reduced fitness because the mutations, while protecting against the drugs, modify the function of key viral proteins. PI and NRTI mutations reduce viral fitness whereas NNRTI mutations do not.
- **Wild-type virus** – Virus that does not carry resistance mutations.

References available on request.

IN A NUTSHELL

The prevention of resistance is the single most important way in which the long-term efficacy of HAART can be assured. This is best done by optimising adherence.

Adherence rates of 70 - 90% are most likely to select for resistance.

When there is virological failure on an NNRTI-containing regimen assume class resistance to the NNRTIs.

When there is virological failure on a 3TC-containing regimen assume 3TC resistance.

If a first-line regimen containing D4T or AZT has failed for 6 months or less, significant resistance to these drugs is unlikely.

An antiretroviral regimen should be changed if the viral load continues to rebound despite optimising adherence. A change should never be made on the basis of one reading alone.

If a patient is left on a failing regimen then there is a risk of developing resistance to those drugs in the regimen with a high genetic barrier (e.g. AZT) and cross resistance. This compromises later options.