

Introduction to HIV drug resistance

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Extensive analysis of the HIV-1 lifecycle has resulted in the identification of several drug targets, namely, 1) viral entry (entry inhibitors [EI] such as Fusion Inhibitors and CCR5 coreceptor antagonists); 2) viral replication (Nucleoside Reverse Transcriptase Inhibitors [NRTIs] and non-NRTIs [NNRTIs]); 3) viral integration (Integrase inhibitors) and 4) viral processing (Protease Inhibitors [PIs]).

Factors that impact on antiretroviral treatment outcome are: compliance, drug-drug interactions, environmental factors such as antiretroviral drug supply, antiretroviral drug toxicity and an array of either viral or host genetic factors.

Virus Drug

Patient

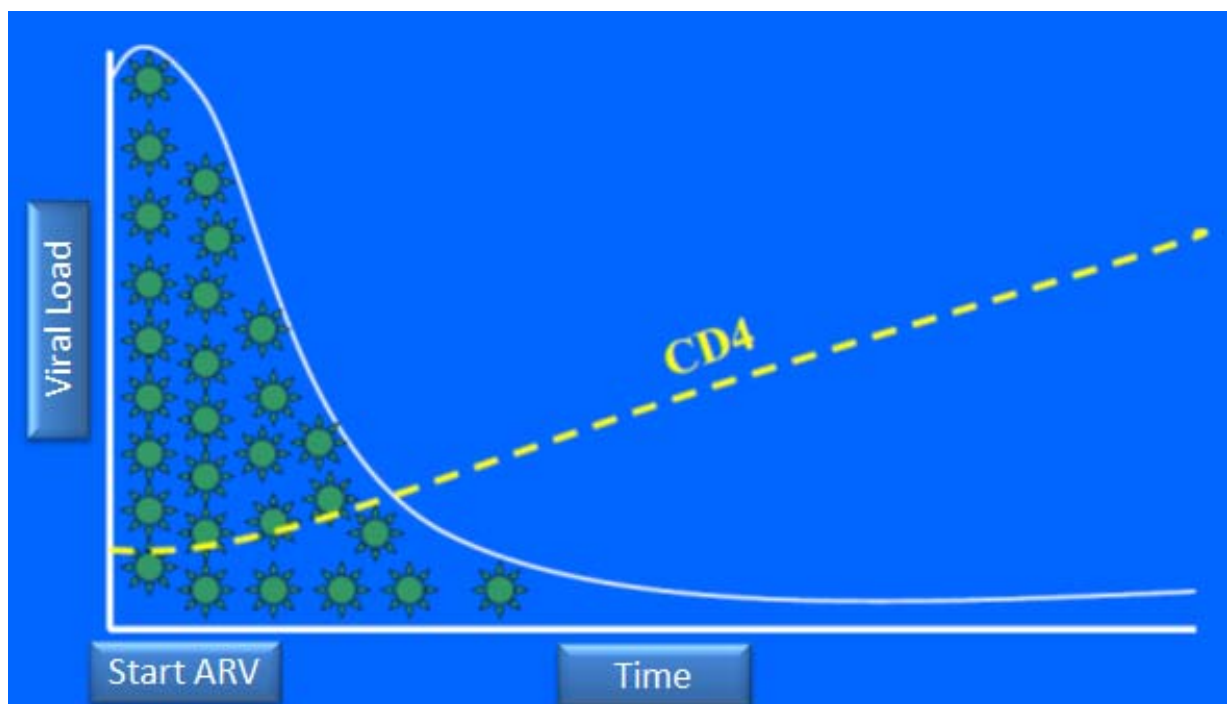
- The **level of compliance** can differ and depends on complicated dosage requirements, adverse side effects (toxicity) from antiretrovirals or non-adherence. All these factors can lead to virological failure. Non-compliance can be monitored by clinical algorithms such as pill-count or treatment diaries, or in the laboratory by measuring the viral load to detect continued virological suppression.

- **Drug-drug interactions** can lead to insufficient antiretroviral drug bioavailability to adequately suppress viral replication. For example, drug interactions with concomitant therapy, in particular anti-tuberculosis drugs can alter the circulating concentration of antiretroviral drugs. **Therapeutic drug monitoring is available at Lancet to assess if adequate drug concentrations are being achieved.**
- **Environmental factors** such as antiretroviral drug supply, alcohol consumption and recreational drug usage linked to non-adherence may impact on treatment outcome. Other factors that may contribute to altered drug concentrations in the local South African environment include the use of traditional remedies such as St. John's Wort and milk thistle.

The above factors can result in the antiretrovirals achieving either low or high concentrations. When ARVs are present in low concentrations in the blood, this can result in continued viral replication, whereas high drug concentrations can lead to ARV toxicity in subjects, both of these scenarios can result in antiretroviral treatment failure.

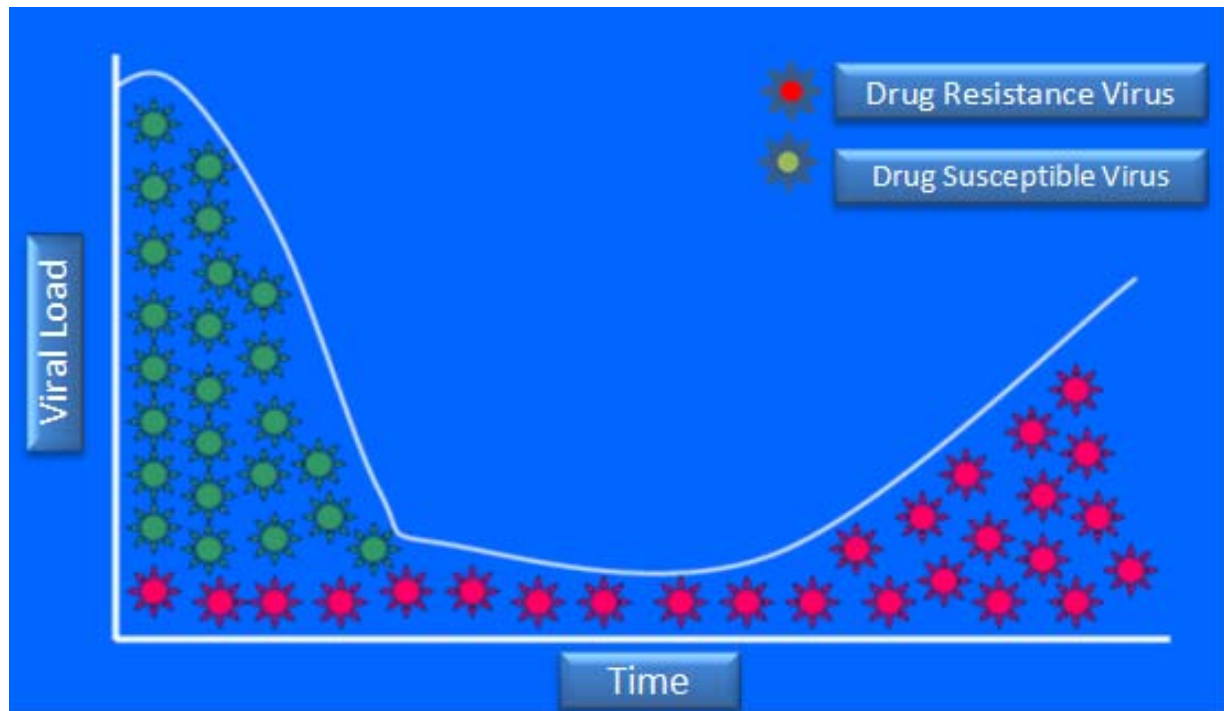
Viral dynamics once antiretroviral therapy has started:

Once antiretroviral therapy is started the HIV viraemia normally decreases rapidly and the immune system starts to recover (monitored by CD4 cell count).

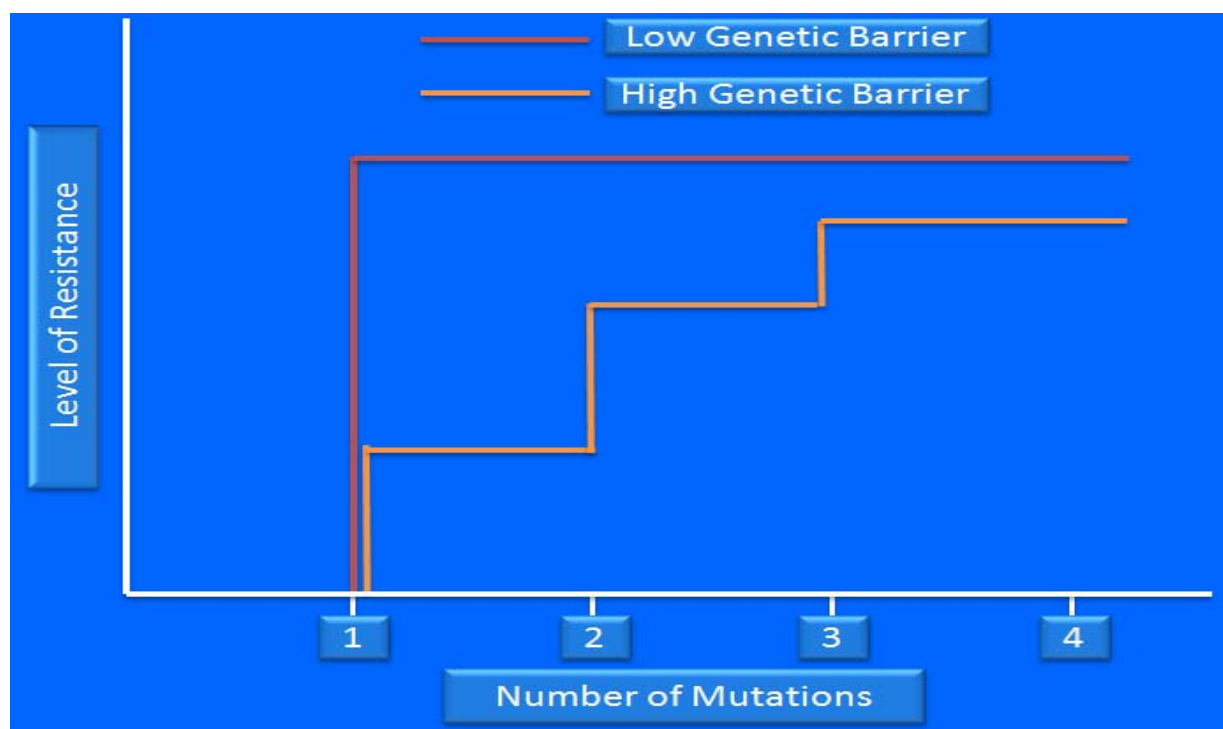


Development of HIV-1 Drug Resistance:

This is a direct result of the poor proof-reading capability of the HIV-1 reverse transcriptase enzyme during replication, which results in the introduction of either polymorphisms or mutations which reduce the efficacy of the antiretroviral drugs. These mutations developed at or around the gene targeted by the antiretroviral drugs. Furthermore, these mutations are either present prior to ARV initiation or spontaneously develop during ARV treatment. The development of mutations coupled with the rapid replication rate of HIV-1 results in a proliferation of virus, which is resistant to the antiretroviral drugs the subject is taking.

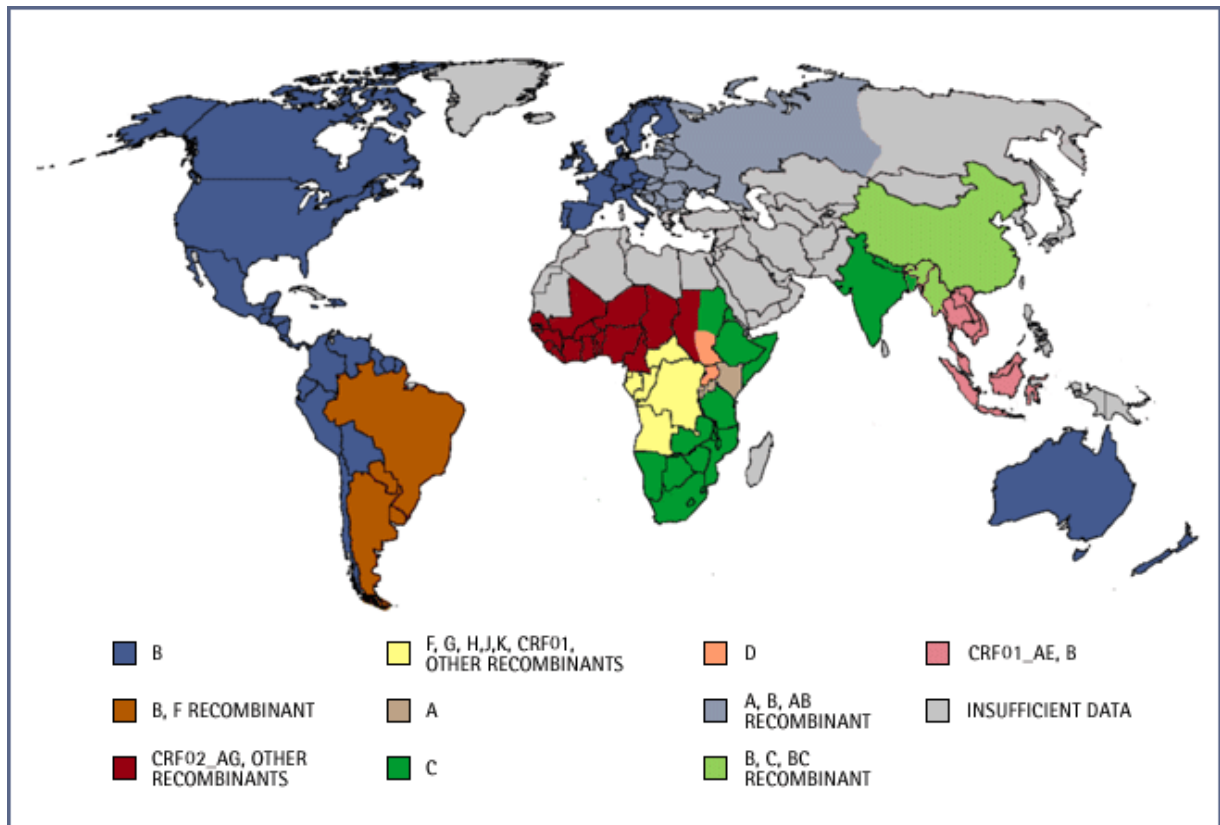


The mutations that develop can either be specific for one antiviral drug or lead to reduced susceptibility to a number of drugs (cross-resistance). For each mutation, the level (fold) of resistance which develops varies per mutation. **Low Genetic Barrier:** A single mutation may result in a completely resistant virus (e.g. 3TC or NVP). **High Genetic Barrier:** Resistance only develops when there is an accumulation of mutations, and as the number of mutations increase, so does the level of resistance (e.g. AZT or PIs).



Viral subtypes

The frequency and patterns of mutations that develop differ according to viral subtype. HIV-1 subtype C infected patients in Botswana and Ethiopia showed that subtype C viruses from Botswana developed Nelfinavir resistance (D30N) via a different mutation pathway than those from Ethiopia (L90M). A comparison of two clinical trials using single dose NVP to prevent mother to child transmission, namely the HIVNET 012 and NVAZ, indicated that the prevalence of the K103N drug resistance mutation in the RT of HIV-1 subtype C (69.2%) was significantly higher than that of subtype A (19.4%) or subtype D (36.1%). Furthermore, NVP resistance was more frequent in subtype C infected infants from Malawi (87%) versus subtype A or D infected infants from Uganda (46%).



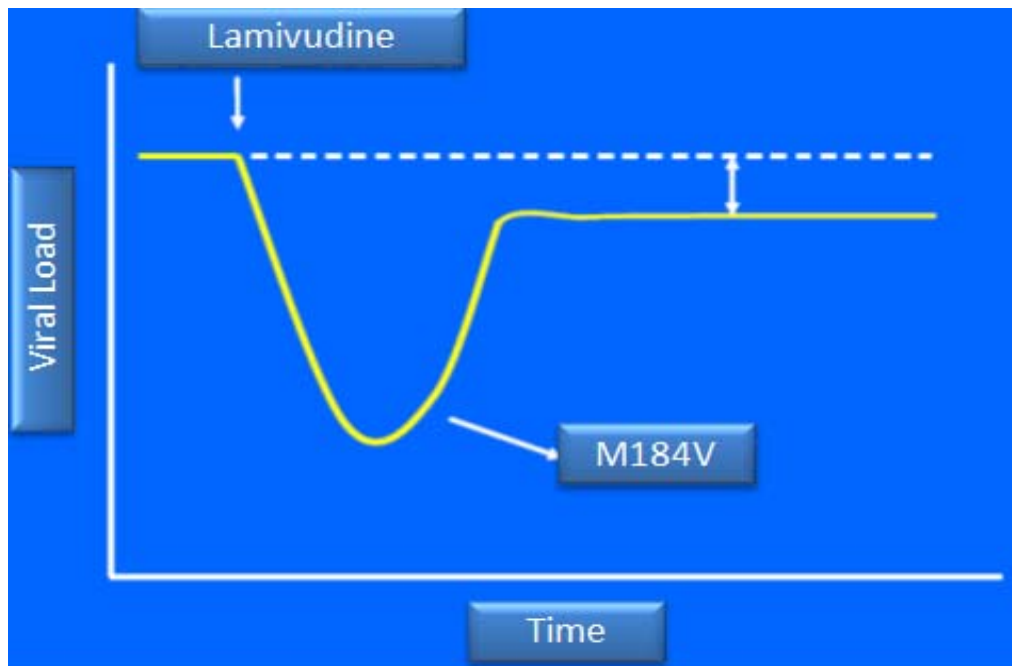
Distribution of HIV-1 subtypes, <http://www.pbs.org/wgbh/pages/frontline/aids/atlas/clade.html>

Mutations associated with Nucleoside Reverse Transcriptase Inhibitors (NRTIs):

Abacavir	K 65 R	L 74 V	Y 115 F	M 184 V
Didanosine	K 65 R	L 74 V		
Emtricitabine	K 65 R			M 184 V I
Lamivudine	K 65 R			M 184 V I
Stavudine	M 41 L	K 65 R	D 67 N	K 70 R
Tenofovir	K 65 R	K 70 E		
Zidovudine	M 41 L	D 67 N	K 70 R	L 210 W
				T 215 Y
				K 219 Q E

(Adapted from: Johnson V, et al., J I Topics in HIV Medicine 2010; 18; 5: 156-163)

M184V/I: The most common mutation that occurs with **lamivudine (3TC)** and **emtricitabine (FTC)** is the M184V/I mutation. Resistance to 3TC emerges rapidly as this is a low genetic barrier requiring the development of only one mutation (M184V/I). M184V/I have a limited cross-resistance pattern and decrease the replicative fitness capacity of the viruses that harbor these mutations. The M184V/I mutations partially restores susceptibility to AZT, d4T and tenofovir and delays the emergence of thymidine analogue mutations (TAMs) as a result of the antagonistic relationship of these mutations.



(Adapted from: Schuurman R, et al., J Infect Dis 1995; 171: 1411-9)

Thymidine Analogue Mutations: This group of mutations are associated with **stavudine (d4T)** and **zidovudine (AZT)** usage and give rise to a high level of resistance. Three or more TAMs are required to confer a reduced level of susceptibility to NRTIs, so the longer a subject is left on a failing regimen the more TAMs accumulate. There are two TAM pathways:

- TAM pathway 1: M41L, L210W, T215Y
- TAM pathway 2: D67N, K70R, T215F and T219Q/E

TAM pathway 1 has been associated with higher resistance to AZT, a higher level of cross resistance to NRTIs and there is less effect of the M184V/I mutations partially restoring susceptibility to AZT.

There is no known reason determining which pathway the virus will choose to go down.

K65R: This mutation is associated with **tenofovir (TDF)** usage, but has recently been shown to occur in higher frequencies in the reverse transcriptase of HIV-1 subtype C subjects administered d4T compared to HIV-1 subtype B. The K65R mutation results in cross-resistance to most NRTIs, except AZT. This mutation has an antagonistic effect with TAMs and therefore TDF is often used once TAMs are present in a subject or vice versa.

Mutations associated with Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs):

Efavirenz	L K K V V					Y Y G			P	
	100	101	103	106	108	181	188	190	225	
	I	P	N	M	I	C	L	S	H	
						I		A		
Etravirine	V A L K	V				E	V	Y	G	M
	90 98 100 101	106				138	179	181	190	230
	I G	I*	E		I	A	D	C*	S	L
			H			G	F	I*	A	
			P*			K	T	V*		
Nevirapine	L K K V V					Y Y G				
	100	101	103	106	108	181	188	190		
	I	P	N	A	I	C	C	A		
				M		I	L	H		

(Adapted from: Johnson V, et al., J Topics in HIV Medicine 2010; 18; 5: 156-163)

Nevirapine (NVP): Has a low genetic barrier for resistance, and the resistance mutations that occur generally result in cross-resistance to efavirenz (EFV). The most common mutations that occur with NVP are either **Y181C** or **K103N** and both result in a high replicative capacity (therefore are present for an extended amount of time after the ARV drug pressure has been removed).

Efavirenz (EFV): Like NVP has a low genetic barrier for resistance, and the mutations that occur with EFV cause resistance to NVP. The **V106M** mutation which occurs in HIV-1 subtype C develops with EFV pressure. Furthermore, **V108I** and **P225H** only confer resistance to EFV in combination with other NNRTI-associated mutations

Etravirine (ETR): This is a second-generation NNRTI. ETR's increased potency has been linked to the increased flexibility of the molecule, thereby preserving its use after the development of K103N. However, a high level of decreased susceptibility is observed in the presence of **Y181C**. The resistance for ETR is determined using the weighted scoring system.

Mutations associated with Protease Inhibitors (PIs):

In the protease region of non-subtype B, several naturally occurring polymorphisms are observed (classified as minor mutations in HIV-1 subtype B), for example M36I and L63P. These may impact on the efficiency of PIs. The clinical relevance of this for HIV-1 subtype C infected subjects remains unclear, and further investigation is required. These polymorphisms may result in a predisposed resistance to the antiretrovirals or result in novel mutations.

Darunavir/ritonavir	V	V L		I	I	I	T L		I	L
	11	32 33		47	50	54	74 76	84	89	
	I	I F		V	V	M L	P V	V	V	
Lopinavir/ritonavir	L	K L	V L	M I	I F I	L	A G L	V	I	L
	10	20 24	32 33	46 47	50 53 54	63	71 73 76	82	84	90
	F I R V	M I	I F	I V L A	V L V L A M T S	P	V T S V	A F T S	V	M
Nelfinavir	L	D	M	M			A	V V	I N	L
	10	30	36	46			71	77 82	84 88	90
	F I	N	I	I L			V T	I A F T S	V D S	M

(Adapted from: Johnson V, et al., J Topics in HIV Medicine 2010; 18; 5: 156-163)

Resistance to protease inhibitors is uncommon in subjects failing a boosted-PI for the first-time. This is the result of a high genetic barrier that is achieved with a boosted protease inhibitor and often the failure is linked to poor tolerability of the regimen. However, major protease mutations observed are **M46L/I**, **V82A** and **L90M** and result in reduced susceptibility to commonly used **lopinavir/ritonavir (Kaletra or Aluvia)**. In children the higher frequency of PI mutations observed has been linked to the use of ritonavir only prior to starting a boosted-PI regimen.

Darunavir/ritonavir is a second generation PI and is effective in viruses which contain **M46L/I**, **V82A** and **L90M** mutations, making it a viable option for use after PI failure when protease mutations have been observed.

Mutations associated with Integrase Inhibitors:

	E	Y	Q	N
Raltegravir	92	143	148	155
	Q	R	H	H
		H	K	
		C	R	

(Adapted from: Johnson V, et al., J Topics in HIV Medicine 2010; 18; 5: 156-163)

Raltegravir: has a low genetic barrier and resistance is defined by three distinct genetic pathways. Each pathway has a major mutation, Q148H/K/R or N155H or Y143R/H/C, and a few minor mutations. The most common pathway is the Q148H mutation with G140S and is associated with the highest level of resistance.

Diagnostic Assays to monitor for the development of HIV-1 drug resistance

The development of HIV-1 drug resistance can be determined using either a phenotypic or a genotypic assay.

Phenotypic assays:

- Based on growing the HIV-1 primary virus isolates from the patient in the presence of different levels of ARVs.
- The amount of viral growth is quantified.
- This quantification allows for the level of susceptibility of the virus to each ARV to be determined.
- They are extremely expensive, time consuming, require highly skilled staff.

Genotypic Assays:

- These assays sequence protease, reverse transcriptase and integrase, and compare the sequence to a consensus sequence for differences (polymorphisms or mutations).

The most commonly used assay is the population based genotyping assay. These assays are accurate in assessing resistance to current regimens. The absence of resistance to previously used antiretroviral drug does not rule out reservoirs of resistant virus (present in less than 25% of the viral population) that might emerge after re-initiation of that drug. If resistance to a given drug has ever been detected, that drug should probably not be used again, even if current test results suggest viral susceptibility.

Factors to consider when requesting an HIV Drug Resistance Test:

- Only possible if a detectable viral load (currently VL >1000cp/ml).
- Testing should be performed while the patient is on therapy.
- Treatment history and other clinical factors should be considered.
- Other reasons for therapy failure should be considered.
- Genotypic testing does not pick up “minority species” therefore information is given on predominant strain only.

What are the benefits of an HIV Drug Resistance Test:

- Shows which drugs not to use.
- Save costs associated with switching drugs.
- Avoid toxicity of inactive drugs.
- May avoid further development of resistance.
- Shown to improve short term response in several prospective studies.

Lancet Laboratories offer HIV-1 drug resistance testing using a published and extensively used **In-house Assay**. This assay starts with an automated viral RNA extraction followed by RT-PCR and sequencing of the **entire protease and partial reverse transcriptase** (RT1-300) regions. Unlike the ViroSeq assay (which was initially designed for HIV-1 subtype B and therefore a high “no amplification” rate is observed in HIV-1 subtype C), the in-house assay has been designed for HIV-1 subtype C, and has also been evaluated in subtype A, D, AG and several circulating recombinants.

Apart from the above mentioned assay, Lancet is also able to offer HIV sequencing of:

- **Only the reverse transcriptase region (RT 1-250);**
- **Integrase sequencing;**
- **Full-length sequencing of the reverse transcriptase region** (several studies are starting to emerge linking mutations developing in the RNase H and connection domains of polymerase to antiretroviral resistance).

Additional Services

- **Monthly Report of Resistance Mutations:**
 - o These reports will describe the frequency of the mutations observed by drug class (Protease Inhibitors; Nucleoside and Non-nucleoside Reverse Transcriptase Inhibitors; Integrase Inhibitors)
 - o Number of samples with no drug resistance mutations observed.
 - o If antiretroviral drugs used by each patient are supplied, an analysis by drug will be added.
- **Long Term storage of “FASTA files”:**
 - o This will enable historic resistance data to be re-evaluated and assessed for drug resistance that may have been present, but was not reported previously as a result of the development of second generation antiretrovirals.

- Accumulated drug resistance reports:
 - o Antiretroviral susceptibility will be determined on 1) the single resistance test and 2) accumulated data using both the current and historic resistance tests on the patient, when available. This will allow clinicians to have increased information and understanding of the patient's resistance patterns, when mutations may have faded.

How to prepare samples for testing?

- Collect 2 x 6ml EDTA tubes.
- Invert tubes 10-15 times to mix.
- Send EDTA tubes at room temperature to:

**Specialty Molecular Division
Molecular Pathology
Lancet Corner
Lancet Laboratories
Richmond
Johannesburg**

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