

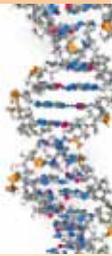
HIV drug resistance:

a guide for treatment advocates



i-Base treatment training manual for advocates

May 2014



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This booklet is one section of the i-Base training manual for advocates, available online (www.i-Base.info). Other sections include: The immune system and CD4 count; Virology, HIV and viral load; Introduction to ARVs; Side effects of ARVs; OIs and co-infections; HIV and pregnancy; Drug users and ARVs, Understanding clinical trials and other learning resources.

This resource is part of a copyright-free project that is available on the i-Base website to download in various formats, or to work online. As with other treatment information produced by i-Base we encourage translations into other languages.

Thanks to an advisory group of community advocates, and to David Dunn and researchers at UK HIV Drug Resistance Database group for comments.

Written and compiled by: Simon Collins for HIV i-Base, Third edition, May 2014.

Thanks to The Monument Trust for their support in funding this publication

HIV drug resistance:

a guide for treatment advocates

- This is an introduction to HIV drug resistance.
- It is written for people who want to understand this aspect of their treatment.
- It was originally developed as a training course for treatment advocates.
- Although the subject sounds technical, this guide is written in mainly non-technical language.

1. Introduction

1.1 Introduction

Resistance can be a daunting subject. It sounds complicated, scientific, technical and difficult.

- Drug resistance is important. It determines whether your treatment will work and whether it will fail. It determines which drugs you can use.
- Most people use HIV treatment for years without developing resistance.
- However, if resistance develops, it stays with you for life. Avoiding resistance makes sure you keep the widest choice of drugs.
- The principles behind resistance are simple. If an organism (i.e. a virus) continues to reproduce in the presence of a drug, resistance will develop.

1.2 Resistance on a personal level

If you are reading this guide for your own health, then there may be more information, including technical information, than you expect or need.

One of the aims of the resource is to collect and explain information that is not usually easy to find in one place. So has more detail than most community guides to drug resistance. It was designed as a course based on reading one section each week. Take time to understand each section.

However, if you like to know more about this geeky stuff, more technical information is included in the appendices. We use non-technical language throughout but when technical terms are important, we explain them. We also include a glossary.

Luckily, most people are able to use HIV combination for many years without developing resistance.

This is an important point.

In the UK, less than 5% of people each year develop resistance, once they have had an undetectable viral load for more than a year. This depends on continuing to take treatment.

If resistance does develop, in many cases this is linked to difficulties with adherence.

The most active thing you can do to avoid resistance is therefore to get into a good routine for taking your meds on time.

1.3 Questions about resistance

This guide started with a list of over 30 questions about resistance from a group of HIV-positive people.

- Which drugs can someone use if they have resistance?
- How are treatment choices made?
- Can resistance be passed from mother to child?
- Can someone develop resistance even with perfect adherence?
- What is “wild-type” virus and what does it do?
- When should you have a resistance test?
- How expensive (or cheap) are resistance tests?
- Are the tests always 100% accurate?
- Is resistance inevitable?
- What should I expect to hear back from my resistance test?
- How does resistance affect me?
- How can I avoid resistance?
- Is resistance permanent?
- What happens if I get resistance?

These are questions that lots of people have. The guide was designed to work through the science behind the answers.

These and other questions are listed in Appendix 1. The answers are in the online version of this guide but they are also answered in the text through the guide.

If you have question after reading this resource, please email the i-Base Q&A service and we will do our best to help.

questions@i-Base.org.uk

1.4 Course outline

Each section in this booklet was written as part of a course. Each section looks at a different aspect of resistance.

Most people do not have a medical background, so we start with basics. Each section describes a different aspect of drug resistance.

- The resource requires active participation.
- Each section involves reading, taking your own notes, and responding to questions. Each section should take about 30-60 minutes. The additional material in appendices does not need to be learned by heart. These are references that will be referred to through the course.
- We asked participants from the original course to ask questions and complete online evaluations. This involved sending at least one email back for each section. We included these questions as part of the training.

This course was developed over several months.

As well as learning, it is meant to be fun.

1.5 Learning objectives

By the end of the training you should have an understanding of:

- Key concepts: genetics, HIV structure and lifecycle.
- Basic mechanisms of how and why resistance to HIV drugs occurs.
- How resistance is measured, when to test and how test results are interpreted.
- The impact of resistance on HIV treatment and treatment options; Treatment strategies for people with drug resistance; How new drugs can overcome resistance.
- Transmission of drug resistance.
- Examples from research into drug resistance.

The training should help advocates advise on resistance research from a community perspective. For example, by working with researchers on local or national research studies.

It should help HIV positive people who want to understand this aspect of treatment in more detail.

1.6 Introductory reading

The following three short sections from the i-Base Introduction to Combination Therapy are included as background reading. This information should help prompt questions that we cover later in the course.

What is resistance?

<http://i-base.info/guides/starting/resistance>

How do I avoid resistance?

<http://i-base.info/guides/starting/avoiding-resistance>

A missed or late dose increases the risk of resistance

<http://i-base.info/guides/starting/missed-dose>

If you are not reading this guide online or do not have internet access, don't worry about this reading. Everything will be covered anyway ;)

1.7 Feedback

The online version of this guide includes a short online survey for each section.

If you are reading this as a booklet in one go, there is a single feedback survey for the whole guide at this link:

<http://www.surveymonkey.com/s/L8ZJM7P>

A paper version is included on page 67 that you can send back by FREEPOST if you prefer this format.

Your feedback is important.

Thank you for your help us in this way.

2: Key concepts: genetics and HIV lifecycle

2.1 Recap from previous section

The introductory reading in section 1 was general information about some of the practical issues about HIV resistance.

2.2 Introduction to section 2

For the first proper section we need to start with basics and learn about a few important concepts.

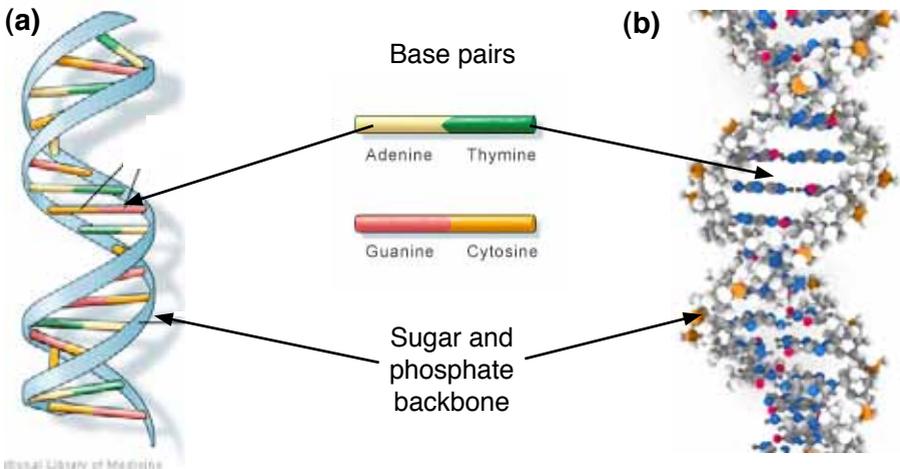
This includes an introduction to genetics, how HIV makes copies of itself when it reproduces or replicates and how it makes tiny mistakes each time.

2.3 Genetics

The structure of things that reproduce, grow and die is usually dependent on genetic material. This is the case for bacteria, viruses, insects, animals, a tomato, a beanstalk or a human.

This is usually a double strand of RNA called DNA (see Figure 1).

Figure 1: Illustrations of DNA: (a) simplified to show bases, and (b) showing molecular structure of the bases and the sugar and phosphate groups that form the backbone ribbon strips



Source: US National Library of Medicine. [<http://ghr.nlm.nih.gov/handbook/basics/dna>]

DNA is like a recipe book for how to make a new organism (tomato/human/virus etc). For humans, DNA is in cells that have a nucleus - skin cells, bone cells, brain cells, liver cells, blood cells and many others.

The genetic structure of HIV is slightly different because it is single-stranded RNA. Before it can replicate inside the nucleus of a human cell, it needs to be transformed into double stranded DNA. To do this, HIV mainly uses CD4 cells. These are a type of blood cells that are part of the immune system.

DNA is made up of a chain of chemicals called nucleotides (or bases). There are only four bases and the order of the bases determine what they do. The chain of bases are held together by a backbone of two strands of sugar and phosphate molecules. This makes the familiar double helix structure in Figure 1.

Human DNA is a chain of 3,000,000,000 bases. The four bases are abbreviated to letters: A (adenine), T (thymine), C (cytosine) and G (guanine).

The code for a human will look very similar (but is much longer):

```
TACCTTGAGGTGTTGAGGTGCCAAAATGGTGACCCCTT
GAGGTGCCAAAATGGTGTTGAGGTGCCAAAATGGTGAC
CATTGAGGT etc (continuing for 3,000,000,000 letters).
```

Because DNA is a double strand, this is actually a double chain of 3,000,000,000 base pairs. The pairs always twin A with T, and C with G.

So the chain looks like:

```
  T A C C T T G A
  | | | | | | |
  A T G G A A C T etc
```

HIV is a similar chain, but much shorter with about 9,700 bases.

This is the recipe for HIV to replicate. If these letters change for any reason, it is like changing the the recipe. The next generation of HIV will then be slightly different.

Changes in each generation is called evolution. Evolution occurs for every living thing - for humans, tomatoes and viruses.

See Appendix 2 for more information about DNA.

2.4: Life cycles and replication

Every living thing, by definition, has a life cycle. This is repeated from generation to generation. At it's most basic, this includes:

1. early development and birth,
2. replication, perhaps many times, and then
3. death.

The life cycle for HIV is very fast. HIV in an active CD4 cell only survives for 1–2 days. Over this time, a cell is infected, the virus replicates and then the cell dies. Infected cells also signal to uninfected cells to die more quickly. In an HIV negative person, CD4 cells live for 3-4 days, so HIV causes all activated CD4 cells to live for a shorter time. However, most of the immune system is resting or asleep. HIV in a resting cell is also resting.

HIV is also very prolific—it replicates a lot! Each infected CD4 cell produces several hundred new infectious particles of HIV (called virions). A virus is called a virion when it is not inside a cell. These virions infect new CD4 cells and the cycle repeats. When not on treatment, millions of CD4 cells become infected every day and at least 100 million new HIV virions are produced each day.

HIV has one of the highest and fastest replication rates of all viruses. It replicates a lot in a very short time.

HIV has to reproduce its genetic code which is in the form of a strand of 9,200 bases. Small mistakes in copying the genetic RNA is like print errors in a recipe. Because HIV does not have a way to proofread, mistakes are common. In every reproduction cycle it makes at least one mistake.

By comparison, human DNA replication usually has very accurate proof-reading. If it detects an error it goes back to correct it. In humans an error occurs only once in every 10-100 million bases. In humans, many changes are not important and the role of much of human DNA is not understood.

Although 90% of DNA was thought to be junk more recent research thinks it may be more important and that we just have not yet understood it.

If a recipe spelled 'sugar' as 'suger' you would probably guess right and still make a good cake. But changing '2 eggs' to '20 eggs' would make a mess.

With HIV, some changes are important and some make no noticeable difference. Sometimes, one change affects the way a drug works.

The lack of proof reading, together with the vast amount of new viruses produced each day, makes it likely that at least one HIV mutation will be produced in every cycle (when not on treatment).

Sometimes dual mutations may occur on the same strand of HIV. Luckily, even with so much virus being produced triple mutations relating to drug resistance rarely occur by chance.

To understand how different mutations affect drug resistance it is useful to use a different diagram for the structure of HIV. (See Appendix 5).

This shows the genetic structure of the single strand of RNA for HIV as nine main genes. In order to picture this structure, the genetic structure of HIV RNA that shows each gene, is shown as a block, some of which overlap.

Each of these main genes plays an important role in making new HIV. You don't need to learn about the function of each gene but it is useful to know that they exist.

By comparison, the chains of nucleotides in human DNA is organised into over 20,000 genes (in 23 pairs of chromosomes).



2.5 HIV replication

The third point in this section involves combining points one and two:

- i) HIV is a chain of 9,200 bases that replicates every 1–2 days.
- ii) Even with a viral load of only 10,000 copies/mL, over 100 million new viruses are produced each day.
- iii) Every reproduction cycle includes at least one mistake: somewhere an A could change to a C; or a G to an A etc; just by accident. HIV does not proofread.**

Before starting treatment (ie before viral load is dramatically reduced) every single base change is likely to be present. Some of these mutations cause drug resistance.

2.6 Section 2: Learning points

- HIV is a virus made up of two single strands of genetic material, called RNA.
- The genetic material in HIV is much shorter than human DNA. It is like comparing a pea to the Titanic.
- The order of the four bases determines everything about the structure of an organism (whether this is a virus, a tomato or a human).
- The lifecycle for HIV is short (only 1-2 days).
- The natural process of replication sometimes involves slight changes to the genetic structure. These are called mutations.
- HIV doesn't have a way to proofread for these mutations. This means that everyday slightly different new versions of HIV are produced.
- Often these new mutations make no difference, but some can stop an HIV drug from working.

3. Drug resistant mutations (with and without drugs): 'selective pressure' and 'survival of the fittest'

3.1 Recap of previous section

The previous section set the stage:

- We have a genetic organism – in this case a virus (HIV).
- After infecting a cell, the virus can replicate many times. HIV produces more than 100 million new virions every 1–2 days.
- But HIV has no proofreading mechanism. It makes at least one mistake (mutation) just by chance in each replication cycle.
- Someone who is not on treatment is likely to have every possible single mutation the HIV genome. We don't have one virus but a pool or soup of thousands of slightly different types of HIV.

3.2 Introduction to section 3

Section 3 looks at how HIV mutations behave when HIV drugs are around.

3.3 Wild-type virus and drug pressure

In someone not on treatment, mutations that develop that can affect how a drug works are made at random.

Mutations generally make HIV less fit at replicating. Wild type HIV is therefore stronger and fitter than drug resistant HIV.

When not on treatment, drug resistant HIV has no advantage over the wild type. It is less fit and so wild type continues to be dominant. The mutation may still be in the pool of viruses but it will stay a minority.

- The main strain will be the fittest virus (ie wild type when not on treatment).
- A mutation that stops a drug working is called 'a mutation associated with drug resistance' or, more commonly, 'a drug resistant mutation'.
- As long as you are not taking the drug associated with this resistance, this mutation will have no relative advantage over wild type virus.

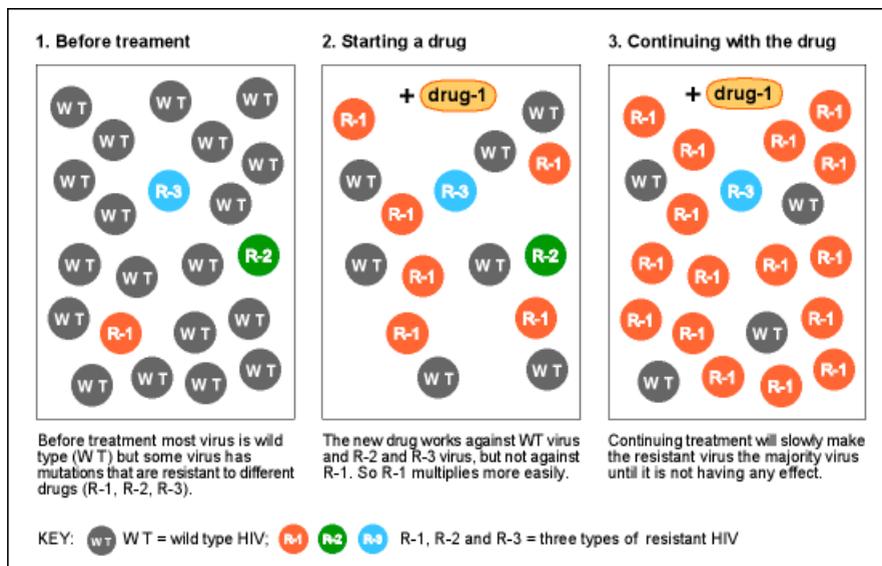
Now think about how the pool of viruses will change if you start taking this drug.

- The drug will be able to kill most of the viruses in the pool. However, it will not be active against the virus that is resistant to the drug.
- The drug resistant virus will continue to replicate. This resistant virus now has an advantage over the other viruses. It is relatively more fit.
- Slowly, the drug resistant HIV will become dominant in the pool.
- In the presence of the drug, further mutations can develop and make the resistance even stronger.

Before taking treatment the resistant HIV had no advantage over wild-type. Now, it is stronger than the other viruses. See Figure 2.

This major concept is called ‘survival of the fittest’.

Figure 2: How resistant mutations respond to treatment



3.4 Survival of the fittest

‘Survival of the fittest’ is central to the concept of evolution.

Whether talking about how life developed from tiny organisms into plants, fish, birds, animals and humans, or how a virus changes, life evolves. It has taken millions of years for humans to evolve, but HIV does this in days.

Each generation evolves and adapts in relation to the surrounding environment.

This is the same for HIV. HIV evolves and when the environment changes this affects how HIV evolves. By taking treatment, the environment for the virus changes because the virus is now in the presence of drugs.

This idea is sometimes explained another way. Treatment is referred to as exerting a *selective pressure* on the virus to change.

- This **pressure** is encouraging the resistant virus to reproduce.
- It is a **selective** pressure, because it is encouraging the mutations linked to resistance to that drug to be selected over those without resistance.

3.5 Selective pressure

Sections 3.1 to 3.4 involved important ideas. Take a break. Let these ideas sink in.

These ideas will be the foundations for the next sections, which should be easier. Take time to recap the following key points.

Key point 1: HIV drugs do not initially cause resistance. Mutations occur because the virus makes mistakes. However, a drug exerts selective pressure for resistance to develop and expand.

Although the first mutations occur by chance, often before treatment, if the virus continues to replicate in the presence of a drug, this can now generate new and more complicated patterns of resistant mutations.

The second and third mutations would not be likely to occur naturally if the treatment wasn't present. This means that if you stay on a failing treatment then more complicated mutations can occur.

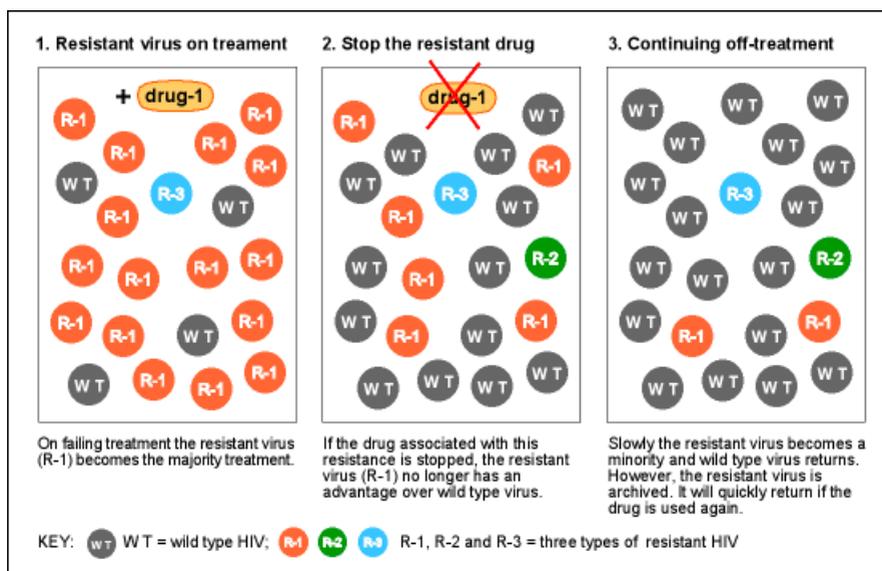
Key point 2: is that selective pressure of drugs can work in two ways.

If you stop or switch treatment then the drug pressure is taken away.

This will reduce the amount of resistant virus in the pool. Without the drug, the resistant HIV no longer has a relative advantage over wild type.

- Wild type virus is better at replicating than resistant HIV, when there are no drugs.
- When the drug pressure is removed, wild type HIV then becomes the majority virus.
- The time this takes depends on the specific mutation. Mutations that develop easily are sometimes the fastest to reduce when a drug is stopped.

Figure 3: Resistance reduces if treatment is stopped but it remains at low levels



Question: Does the resistant virus disappear when you stop taking a drug?

Answer: No. Drug resistant HIV becomes a minority in the pool (see Figure 3) and is archived in CD4 cells that are sleeping. Sometimes this can happen within weeks and sometimes it might take years. The resistant HIV then becomes too difficult to detect with a standard resistance test. (See Section 5: When to use resistance tests).

Key point 3: A resistance test only tells you about resistance to the drugs that were being taken when the blood sample was taken. When looking for transmitted drug resistance, the sample closest to the infection is most likely to show resistance.

This point is important when advocating for resistance testing.

If you are infected with drug resistant HIV, this will initially be your dominant strain. Over time, some mutations become a minority at a level that is too low to be detected.

Even though the majority population may become wild type HIV, drug resistance will still be present. This is called 'archived resistance'.

- UK guidelines recommend resistance testing for everyone who is newly diagnosed with HIV.
- If a resistance test is not provided, a blood sample needs to be taken and stored. This is so it can be tested before starting treatment.
- UK guidelines also recommend resistance testing when viral load has rebounded on treatment and when treatment has never reduced viral load to undetectable.

Key point 4: Drug resistant HIV can be transmitted in the same ways as wild type HIV. This includes sexually, through shared injecting equipment, from needlestick injuries and from mother to baby. HIV positive people can be reinfected with a different drug resistant strain.

Many cases of re-infection are only detected because the new infection was with drug-resistant HIV.

About 10% of new infections in the UK are with HIV that has one or more major mutations. A smaller percentage are infected with HIV that is resistant to two or more drugs. Although rare, some people are infected with HIV that is resistant to three or more drugs or drug classes.

- The longer the time from infection to diagnosis, the more difficult it is to detect some types of transmitted resistance.
- Some mutations drop below the levels that can be detected by resistance tests within a few months but some mutations can still be detected after several years.

Question: What happens if someone was infected with HIV that was drug resistant and starts treatment that includes one of those drugs?

Answer: If they are using a combination with three drugs, then only two of those drugs will be fully active. This increases the risk that the combination may not be strong enough. It will be more difficult to get an undetectable viral load and resistance to the other drugs can develop.

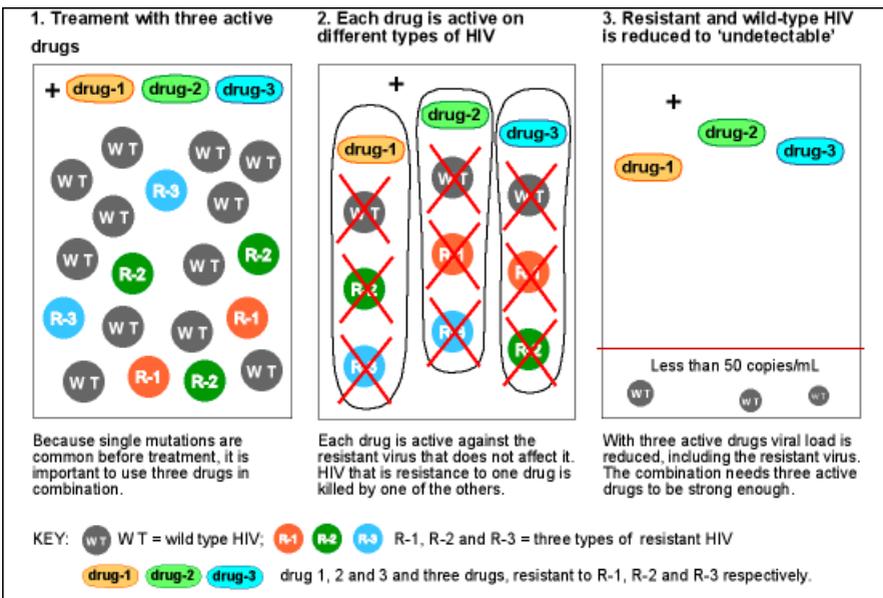
3.6 Using three drugs in HIV treatment

The example in Figure 2 shows that using a single drug provides pressure for pre-existing resistance to become dominant.

This is one reason why combination therapy uses three drugs. See Figure 4.

Single mutations are all present when someone starts treatment. Some dual resistant mutations may be present on a few viruses. But triple resistance will not be present on the same viruses. Even with HIV being such a rapid and prolific virus, this is very unlikely.

Figure 4: Combinations with three drugs work against low-level pre-existing resistance



In Figure 4, virus R-1 is resistant to Drug-1, R-2 is resistant to D-2 and R-3 is resistant to D-3. Although R-1, R-2 and R-3 mutations are all present when starting treatment, these three different mutations are very unlikely to have all developed on the same virus.

HIV that is already resistant to Drug-1, will be killed by D-2 and D3. The virus resistant to Drug-2 will be killed by D-1 and D3.

Even if R-1 and R-2 have developed on the same virus (dual resistance) then D-3 will still be active against this virus.

D-1, D-2 and D-3 will also all be active against wild-type HIV, which is hopefully the majority population.

3.7 Section 3: Learning points

- HIV drugs do not initially cause resistance. Mutations occur because HIV makes mistakes and there is no proofreading mechanism.
- A drug exerts selective pressure for resistance to develop.
- Under continued drug pressure, more complicated patterns of resistance mutations develop that only occur because of this continued drug pressure.
- Results from resistance tests only accurately tell you about resistance to the drugs that were being taken when the blood sample was drawn.
- Without drugs, wild type virus is more fit than resistant virus.
- Drug resistant HIV can be transmitted in all the same ways the wild type virus can be transmitted. See section 6.5.
- Someone can be infected by HIV that is already resistant to one or more drugs.
- An HIV-positive person can be reinfected with a different strain of HIV. Cases of reinfection are only usually detected when the new infection (or superinfection) is with drug-resistant HIV. See section 6.8 and Appendix 11.
- Reinfection with drug resistant HIV can have serious health implications because there are fewer drugs to choose from.
- Combination therapy uses three active drugs to try to ensure it is active against pre-existing resistance.

4. Resistance tests and interpreting test results

4.1 Recap of previous section

The previous section looked at how the presence or absence of drugs interacts with the evolution of HIV and that HIV is a virus with a very rapid and prolific life cycle.

Instead of thinking about one virus, we have talked about how this is really a pool of thousands of slightly different viruses. Which viruses dominate depends on the drug pressure. When drugs are started, continued or stopped, this changes the environment.

When drug levels are too low to keep the viral load suppressed, HIV can develop resistance to those drugs. These resistant viruses are then fitter than wild type HIV and most able to survive. The pool then becomes mainly resistant virus.

More complicated patterns of mutations can then occur under the ‘selective pressure’ of the drugs. These combinations of mutations evolve in ways that would be unlikely if drugs were not present.

When on treatment, new resistance only seems to develop when viral load is detectable (greater than 50 copies/mL). If viral load is undetectable and adherence is good, then resistance is rare.

Learning how resistance tests work will help understand this.

4.2 Introduction to section 4

Section 4 explains two main types of resistance tests: genotype tests and phenotype tests. A third type of resistance test that is marketed as ‘a virtual phenotype’ combines both approaches.

Both genotype tests and phenotype tests work from blood samples but they work in different ways (see Figure 5). The results are also interpreted differently.

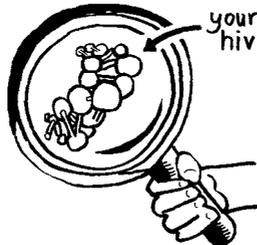
These two words are often used in other aspects of science. Genotype refers to a genetic sequence and specific changes. Phenotype refers to observations related to the changes (i.e. in a test tube).

Section 4 also includes technical information, so work through at your own pace. Please write down questions when anything is not clear.

Figure 5: How genotype and phenotype tests work

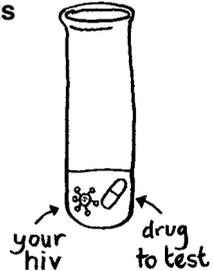
Genotype tests

Genotype tests look to see how the structure of a sample of your HIV may have changed.



Phenotype tests

Phenotype tests see whether HIV drugs still work to control your type of HIV.



4.3 Genotype tests and genotypic resistance: numbers and letters

Genotype tests (also called genotypic tests) look for changes in the structure of the virus.

The test compares genetic sequences to those seen in wild type HIV. Mutations are described with numbers and letters.

This is easier to imagine if you think of HIV as a long chain of amino acids.

Each group of three bases is an amino acid. The example in Appendix 2 shows that the three bases for the amino acid called methionine (M) only needs one base change to make the amino acid valine (V). This is a simple change.

If the three bases change from ATA to GTA, the amino acid at that junction changes from methionine to valine. Appendix 5 illustrates mutations in relation to the HIV genome.

The section of the genome targetted by nukes (and NNRTIs) is called reverse transcriptase (RT). If the change for ATA to GTA occurs at junction number 184 in the RT gene, this will affect how some drugs work. The mutation described above is written as M184V (in RT).

If the virus changes at junction 103 on the RT genome, from AAA or AAG to AAC or AAT, the amino acid at that junction changes from lysine (K) to asparagine (N). This mutation is written as K103N in RT.

You don't need to know all these names but a key to the abbreviation letters for different amino acids is in Appendix 3.

These two mutations are good examples to start with because:

- They only need one base change to change the amino acid.
- They are both very common mutations
- Their impact is like an on-off switch. Without the mutation the drug works. With the mutation the drug doesn't.

M184V results in high level resistance to 3TC and FTC. It stops both these drugs from working.

K103N results in high level resistance to NNRTIs like nevirapine and efavirenz. It stops both these drugs from working.

Table 1 lists key mutations and the impact they have on treatment. The integrase mutation is included as a more complicated example.

Table 1: Important drug resistance mutations

Mutation	Gene	Interpretation
M41L	RT	M41L usually occurs with T215Y. Together these mutations confer intermediate-to-high resistance to AZT and d4T, and a lower resistance to ddl, abacavir, and tenofovir.
K65R	RT	K65R causes intermediate resistance to ddl, abacavir, 3TC, FTC, and tenofovir, and low -level resistance to d4T. K65R causes AZT to be more active (called hypersensitive or hypersusceptible).
M184V	RT	High level resistance to 3TC and FTC and low level resistance to abacavir and ddl
K103N	RT	K103N causes high -level resistance to nevirapine, delavirdine, and efavirenz. By itself it does not affect etravirine efficacy. However, it increases the effect on etravirine from 3-fold to 15-fold reduced sensitivity when L100I is also present..
I50L	PRO	I50L causes intermediate-to-high level resistance to atazanavir/r and increases susceptibility to other PIs.
L90M	PRO	L90M causes resistance to nelfinavir, saquinavir/r, atazanavir/r, and indinavir/r. When present with other mutations it also reduces the activity of fosamprenavir/r and lopinavir/r (Kaletra).

Q148H/ K/R	Integrase	Q148H/K or R are mutations selected by raltegravir and elvitegravir. By itself Q148H reduces susceptibility to both these drugs by about 5-10 fold and Q148RK reduces susceptibility by >30-100 fold. With G140S, Q148HRK reduces susceptibility by more than 100-fold. Q148HKR alone have minimal effects on dolutegravir, but causes more than 10-fold reduced susceptibility in combination with E138K, with or without G140S.
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Key point 5: Some mutations stop a drug from working completely (high level resistance). However, some only have a moderate impact (intermediate) and some only have little impact (low level resistance).

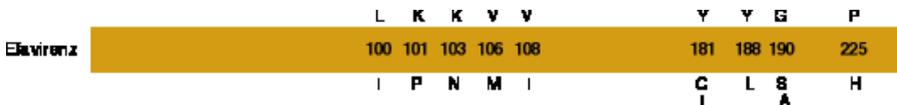
Key point 6: Mutations that are associated with resistance to one drug can also have resistance to similar drugs in the same family. This is called ‘cross-resistance’. For example, if you develop resistance to one NNRTI like efavirenz it is likely you will be cross-resistant to nevirapine, even though you have never taken nevirapine.

There are too many mutations to remember but it is good to know the common examples in Table 1.

Luckily, several research groups publish comprehensive tables and explanations online. Use these if you need to find about about a specific mutation or drug. See: Appendix 6: Stanford Drug Resistance Database online tables.

As you learn more about drug resistance, it gets easier to remember key mutations, especially knowing what the letters and numbers mean.

The IAS-USA guidelines illustrate resistance to each drug in a different way.



Each drug has a bar representing the section of the HIV genome where resistance develops. The numbers inside the bar are the junctions where mutations are linked to resistance. The letters on the top are the amino acids at that junction for wild type HIV. The letters underneath are the amino acid changes linked to drug resistance.

This is a good way to visually compare the resistance profile of drugs in the same class. It is a quick way to get an idea of cross-resistance. Appendix 7 includes these charts and links to the original resources.

Mutations that show resistance is reverting back to wild type are called **revertant mutations**.

For example, the T215Y mutation is associated with AZT resistance. If this mutation is transmitted to someone who is not taking AZT, the virus changes back closer to wild type. The mutations T215D, T215N or T215S are seen as ‘tracks’ in this change. They are interpreted as previously having T215Y and so imply resistance to AZT.

Question: How do researchers find out about the mutations associated with each drug?

Answer: Every new drug is tested in test tube studies to see which mutations occur (called in vitro passaging). These are usually similar to the mutations seen in human studies when treatment fails (ie in people whose viral load stays detectable). For example, in studies that include 3TC, the M184V mutation is one of the first changes seen if viral load is not reduced to undetectable (less than 50 copies/mL). Using 3TC without any other HIV drugs (i.e. 3TC monotherapy), results in M184V within a couple of weeks. This 3TC resistant HIV will be cross-resistant to FTC. New drugs are often developed to specifically work against resistant virus.

Question: Are resistance tests perfect? Are all mutations known?

Answer: Resistance tests are not perfect, but major mutations usually accurately predict when a drug will not work. Given the number of possible combinations of mutations, this is an area of research that is always developing.

4.4 Phenotype tests and phenotypic resistance: x-fold resistance

Phenotype resistance tests look at resistance in a very different way.

Instead of looking at mutations, phenotype tests measure how active a drug is in a sample of HIV compared to how active it is in a sample of wild-type HIV.

So, an HIV drug is added to a sample of HIV in a test tube, and the test measures how much HIV continues to be produced. The quantity of the drug is then slowly increased to see how much extra is needed to have the same impact on reducing viral replication compared to a normal dose on wild-type HIV.

Phenotype tests are more difficult to run. They are more expensive and take longer to get a result. For these reasons phenotype tests are mainly used when the result from a genotype test is unclear.

Phenotype results are given as a fold change (or cut-off) with an interpretation of what this means. For example, if a sample needed four times the quantity of drug to have the same impact on stopping the virus replicating, the result would be 4-fold resistance (or 4-fold loss in sensitivity). In practice, you would need to increase the daily dose by four times to get the same effect on viral load.

Sometimes resistance can be overcome by increasing the dose of medication in a person. In practice, this only tends to be when someone has complicated resistance and fewer drug choices. For most drugs and in most circumstances this would cause too many side effects to be an option. Some HIV drugs, including darunavir and dolutegravir, have higher doses for people that have drug resistance.

The **clinical** impact of phenotypic resistance varies depending on each drug. For example, 4-fold resistance to one drug may still be sensitive while 4-fold resistance to another may be resistant. **Each drug has a different cut-off for when a drug is sensitive, partially resistant or completely resistant.** Nukes generally become resistant at low fold-changes while PIs have higher thresholds.

Each make of phenotype test has its own reference chart for cut-off values for each drug. These numerical values can differ between tests.

4.5 How are genotype and phenotype resistance related?

The clinical interpretation of both genotype and phenotype resistance tests are based on results collected from large numbers of responses from real patients, collected in various databases.

These databases relate the average impact that a mutation (or pattern of mutations) has on the phenotypic resistance.

For example, M184V is associated with high level resistance to 3TC and FTC, and results in a more than 300-fold reduction in drug sensitivity. With this mutation, it would take a dose 300 times higher than the standard dose to have the same anti-HIV effect. This would be physically impossible and too toxic.

M184V may still have a benefit in continuing 3TC because M184V replicates less well than wild type and because it increases susceptibility to AZT, d4T and tenofovir. However, the benefit of continued 3TC will be less than the benefit of 3TC in patients with wild-type HIV.

This is an unusual feature of M184V that is not shared with most other mutations. Although other mutations also reduce fitness, this effect is usually overcome by new mutations that compensate for this.

Continuing to take 3TC or FTC to keep the M184V mutation may keep viral load a little lower. This is because the virus is less fit. It reproduces less well so there is less virus.

4.6 Virtual phenotype tests

Virtual phenotype tests are a third type of resistance test. They are really genotype tests, and the mutations that are detected are included in the results.

However, the pattern of mutations is also compared to a huge database of matched genotype and phenotype results and a phenotypic result is predicted from the database.

As with the other tests, an interpretation comes with the results that explains whether each drug is likely to be sensitive, intermediate or resistant.

These tests produced very sophisticated individual results. However, they are now rarely used because drug options to treat resistant HIV are now much better. One of the main virtual phenotype tests, produced by Virco, was withdrawn at the end of 2013 because of low use.

4.7 Primary and secondary vs major and minor mutations

The terms **primary** and **secondary** resistance mutations, are now rarely used.

Instead, **major** and **minor** mutations are used.

Major mutations have a big impact on drug resistance.

Minor mutations only have a small impact on drug resistance.

The terms primary and secondary are confusing because they sound like primary mutations occur first. Sometimes however, the first mutations make little difference.

For example, with protease inhibitors the first mutations have little impact on how well a drug works. Then, as more mutations accumulate, the impact becomes more important.

Finally, after 5 or 6 or more mutations, the clinical impact becomes more significant. With protease inhibitors the first mutations to occur are minor (secondary) mutations and major (primary) mutations occur later.

4.8 Resistance testing: practical issues

Resistance testing is widely used in most western countries. The information they provide helps choose drugs that have the best chance of working.

However, resistance tests are more accurate in showing which drugs will **not** work, than guaranteeing which drugs **will** work.

- Resistance tests can only detect resistance to drugs that you are currently taking or have recently been taking. Remember that when a drug is stopped, wild-type HIV becomes relatively more fit (see section 3) and many mutations reduce to levels that are too low to detect. Usually a mutation has to be present in more than 20% of viral population to be detected with routine tests.
- Treatment choice needs to be based on someone's lifetime history of treatment and resistance, not just the single result of one current resistance test.

- In addition to the report given by the test lab, results need to be interpreted by an expert who has your treatment and resistance history. Experts do not always agree and different databases sometimes report different results. Even though the tests may not all agree, it is better to have this information to inform your treatment choices.
- There is less information about resistance to newer drugs. This is because fewer people have developed resistance to those treatments. There is less information in resistance databases to predict how new or old mutations will affect how these drugs will work.

4.9 Section 4: Learning points

This has been a complicated technical section.

- There are three main types of resistance tests but genotype tests are used most frequently.
- Different mutations have different clinical implications. Some are associated with high level resistance, some with intermediate and some with low resistance.
- Resistance for some drugs develops on a sliding scale, but for some drugs it only takes one key mutation results in complete resistance.
- Genotype tests report mutations and phenotype tests report fold-changes. All resistance tests should include a detailed interpretation for each drug. You can ask for a copy of this report.
- Resistance to one drug can result in resistance to similar drugs in the same class. This is called cross-resistance.
- Viral load needs to be detectable to get a result. How ‘detectable’ depends on the specific lab and test. This used to be above 500 copies/mL but some labs can get a result when viral load is between 50 and 500 copies/mL.
- The interpretation of complicated results requires expert advice.

Resistance 5: When to use resistance tests

5.1 Recap of previous section

The last session looked at how resistance is measured and how test results are interpreted.

- There are two main types of resistance tests but genotype tests are used most often. Genotype tests report mutations (ie M184V) and phenotype test report fold-changes (ie 4-fold resistance). Both tests should include a detailed interpretation – ie whether each drug is likely to be active (sensitive), partially active (reduced sensitivity) or inactive (resistant).
- Each mutation has a different clinical implication. Some are associated with high level resistance and some with lower resistance.
- Resistance to one drug in a class often means you have resistance to similar drugs in the same class. This is called cross-resistance.
- Resistance can only be tested when viral load is detectable but different labs have different lower viral load cut-offs for the test to work.
- It is important to consider the history of resistance and not just the results of the current resistance test. This include previous treatment history and previous resistance tests.
- The interpretation of complicated results requires expert advice.

5.2 Introduction to section 5

This section look at when resistance tests should be used.

- UK recommendations are based the monitoring guidelines, adult guidelines and pregnancy guidelines produced by BHIVA and the paediatric guidelines produced by PENTA.

<http://www.bhiva.org/PublishedandApproved.aspx>

<http://www.pentatrials.org/guidelines.htm>

Other guidelines include:

- European HIV Drug Resistance Guidelines (2009)

http://regaweb.med.kuleuven.be/publications/european_guidelines

- US treatment guidelines (DHHS)

<http://www.aidsinfo.nih.gov/guidelines/>

Most treatment guidelines for Western countries have similar

recommendations for resistance testing. The resistance section of the BHIVA adult guidelines is reprinted in Appendix 8.

Section 5 also includes **access to resistance testing**.

- Why are these tests not always given?
- When to advocate for someone who has not been given a test.

5.3 When to use resistance tests

Genotype resistance tests are recommended when first diagnosed and before most treatment changes (see Table 3), including:

1. When first diagnosed (to check for transmitted drug resistance).
2. Before starting treatment (to help with the choice of treatment).
3. Before changing treatment, as long as viral load is detectable.

Table 3: When to use resistance tests

Stage	Recommendation	Reason
Newly diagnosed	Yes, all patients. Both for recent and older infections.	In the UK, 5-15% of newly diagnosed people have at least one mutation.
Starting first treatment	Yes, BEFORE STARTING. i) People who have never had a resistance test should have a sample tested before starting treatment. ii) People who may have been reinfected with a new strain of HIV since their first resistance test may be retested before starting treatment.	i) Test the earliest sample. If this is not available, a current sample should be used. ii) If someone has had other exposures since diagnosis, a resistance test will limit the chance that the first treatment will fail.
Changing treatment (viral failure)	Yes. If viral load rebounds on treatment, test for resistance BEFORE CHANGING treatment. Resistance testing can help determine if treatment failure is due to HIV reinfection.	A resistance test BEFORE CHANGING treatment will provide an indication of how much resistance developed while the treatment was failing. Some low level resistance may not be detected.

Changing treatment (side effects)	No. A resistance test is not needed if your viral load is undetectable. If this is soon after starting treatment and viral load is still reducing, resistance testing is not needed.	Resistance only develops on failing treatment. Never test when viral load is undetectable.
Pregnancy	Women who start treatment during pregnancy should be tested for drug resistance. Resistance testing should be done if viral load is still detectable at delivery. If the women decides to stop treatment after the birth, resistance should be tested six weeks after stopping treatment.	As for starting treatment. Testing if viral load remains detectable is important for future care. Although treatment should be stopped carefully to reduce the risk of resistance, this should be confirmed with a resistance test.
Children	Guidelines for resistance testing in children are the same as for adults. In the rare cases (in Western countries) of infants born with HIV, resistance testing should be included with the full panel of other tests.	Resistance develops in children in the same way as it does in adults. Any child on treatment with a detectable viral load is likely to have developed, or be developing resistance.
Before using a CCR5 inhibitor (ie maraviroc)	UK and European guidelines recommend using a type of genotype test to check viral tropism.	CCR5 inhibitor only work against CCR5-tropic virus.
PEP (Post-Exposure Prophylaxis)	PEP should be started as soon as possible. It should not be delayed waiting for resistance test results. If the HIV positive partner has drug resistance, this will affect the choice of drugs used for PEP. If resistance is discovered later the drugs can be modified.	PEP combination usually include protease inhibitors as transmitted PI resistance is less common. The urgency with PEP is to first start any combination.

5.4 Which tests to use: genotype or phenotype?

Recommendations for resistance testing always refer to using a GENOTYPE test first.

This is because genotype tests are cheaper (approximately £200 vs £700) and quicker (1–2 weeks compared to more than two weeks), compared to phenotype tests. Genotype results are also more widely understood.

A phenotype test is generally only recommended if the results from a genotype test are difficult to interpret.

Phenotype tests (including virtual phenotype tests) are only used in people who have very limited treatment choices. This is usually in a case where there is extensive resistance to several different classes of HIV drugs.

Question: Are resistance tests used to see if the type of HIV in different people is in some way linked – in prosecution cases of transmission, for example?

Answer: No. The tests comparing two different viruses are called phylogenetic tests. They are more complicated and expensive tests. It is important to remember that phylogenetic tests can show when people have a similar virus, but not the direction of infection (ie whether one partner infected another).

5.5 How to access tests if the guidelines are not followed

Although guidelines are clear, resistance tests are not always provided routinely. This is why it is important to know about the current UK guidelines.

If this is for cost reasons, then it is important to go back to the clinic to ensure that the test is included as part of current standard of care.

i-Base sometimes get calls from people who were newly diagnosed but didn't get resistance a resistance test until they asked for it.

- Usually it is sufficient to go back to the doctor and refer to the guidelines.
- Some clinics store a sample to test later, before starting treatment. In theory this may be okay, but sometimes samples get lost, or old samples may be difficult to test. In these cases, testing the current sample may not pick up resistance which is present at low levels. There is no real cost-saving from delaying this test.

- If your doctor or clinic will not agree to the test when it is clearly recommended, you can write to the head of your clinic and the head of your health trust. If this is still not provided you may want to register at another clinic to get this test. You can always change back to your local clinic in the future for routine monitoring or treatment.

Please call the i-Base phonenumber if you would like further information or support in accessing resistance tests.

5.6 Section 5: Learning points

This section has been more practical and should help connect the previous technical information to how this affects things in the clinic.

- Treatment guidelines are an important resource, because they state when tests should be used. Most guidelines agree on the use of resistance testing. Guidelines are free to access online.
- Genotype tests are used routinely. Phenotype tests are used when there is more complicated resistance and fewer treatment options. They are also used when results from a genotype test are unclear.
- Guidelines are not always followed, especially for newly diagnosed people.
- Resistance tests and the subsequent results often require active patient or advocacy involvement.

Resistance 6: Research into drug resistance

6.1 Recap from previous section

This last section looked at when to use resistance tests.

We referred to the UK treatment guidelines, though other guidelines for countries with access to resistance tests are similar. Even in the UK these tests are not always provided, so this is an advocacy issue.

Key times include:

- On first diagnosis (to test for transmitted resistance).
- Before starting treatment (if there is a risk of HIV reinfection).
- If viral load doesn't drop by more than 90% in the first month on treatment.
- If viral load rebounds to over 200-1000 copies/mL on treatment.
- Before any treatment change when viral load is over 1000 copies/mL.
- One month after discontinuing treatment (for women who only use treatment during pregnancy).

The reason for testing at each time is included in Table 3 in section 5.3.

6.2 Introduction to section 6

This section looks at some research studies. These provide examples of the ideas discussed in earlier sections.

They show some of the ways that research changed how we understand the way HIV acts. They also show how new information led to changes in guidelines and in the way that HIV positive people are treated.

6.3 Resistance with 3TC monotherapy

A good study to show how resistance to a drug develops in a person (rather than in theory or in a test tube) comes from early studies of the first HIV drugs.

This is the perfect way to get resistance! You have a high viral load and take a treatment that is not strong enough to reduce viral load to undetectable.

This was early research. Using only one or two drugs is not recommended now, other than in some circumstances with a boosted protease inhibitor.

In this example, 360 people with good CD4 counts (200-500) used one of four treatment.

The groups were:

- 1) AZT monotherapy (no other active drugs)
- 2) 3TC monotherapy (no other active drugs)
- 3) AZT + standard dose 3TC (150 mg twice-daily)
- 4) AZT + high dose 3TC (300 mg twice-daily)

This early AZT dose was 200 mg every 8 hours (ie three times a day). Placebo pills were used in the monotherapy groups so everyone in the study was taking the same number of pills.

CD4 and viral load results were recorded for one year, see Figure 6.

Figure 6: Changes in viral load with mono and dual therapy (adapted from Eron et al.)

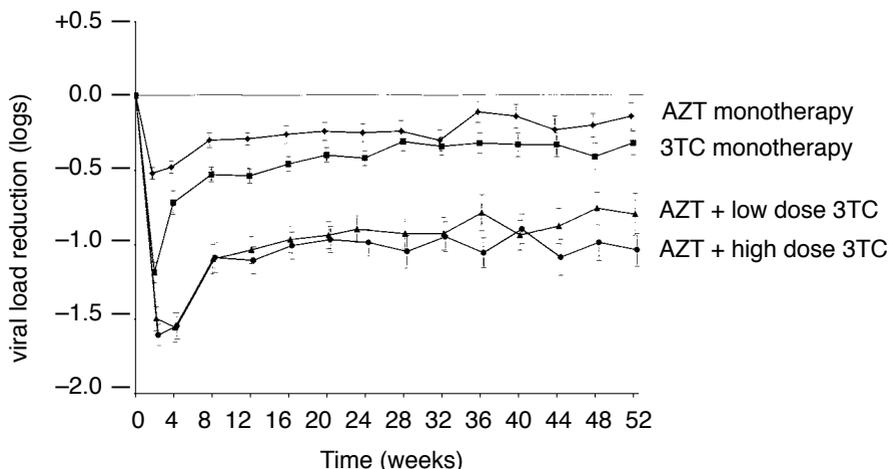


Figure 6 shows the impact of resistance when only one or two nucleosides are active. Within two weeks viral load dropped in all groups. But then with 3TC monotherapy viral load rebounded by week four and rebounded in the other groups by week eight).

Reference: Eron JJ et al. Treatment with 3TC, AZT or both in HIV-positive patients with 200 to 500 CD4+ cells/mm³. NEJM Vol 333 no 25 p1662-1669. (21 December 2005).

<http://www.nejm.org/doi/full/10.1056/NEJM199512213332502>

6.4 An undetectable viral load stops HIV evolving

In section 3 we looked at HIV not proofreading to check that new virus is always the same. This results in someone having a vast mixture of slightly different viruses before they start treatment.

This example is included to show the benefit of treatment driving viral load to undetectable. [1]

Lisa Frenkel is an American paediatrician who led a team that looked at viral load in 37 children who generally had undetectable viral loads on treatment. About 60% of children (21 out of 37) also had low-level blips (from 50 to 400 copies/mL) on treatment.

The group looked at the virus present during the blips and compared this to the sample before treatment (using a phylogenetic test). In 8 out of 11 children the virus was exactly the same. The only evidence of virus development was in two children whose viral load had blipped many times.

This was one of the first studies to show that ongoing replication effectively stops when on treatment. Some of these children had been on treatment for several years and their HIV was exactly the same as when they started treatment. This research supported the theory that treatment is usually as potent as it can be. Adding more drugs does not reduce viral load any further. This has been demonstrated in other intensification studies. [2]

This is important.

The HIV produced in most people with an undetectable viral load seems to come from resting or latent CD4 cells that went to sleep before treatment was started, and are periodically waking. These long-lived cells (some sleep for more than 60 years) are referred to as the viral reservoir. This has led to other researchers trying to find a way to target the resting CD4 cells. If this can be done there is the chance to cure HIV.

References

1. Frenkel, L. M. et al. Evidence that Low-Level Viremias during Effective Highly Active Antiretroviral Therapy Result from Two Processes: Expression of Archival Virus and Replication of Virus. *Journal of Virology*, August 2005, p. 9625-9634, Vol. 79, No. 15
<http://jvi.asm.org/cgi/content/abstract/79/15/9625>
2. See this HTB report on intensification studies. Lack of virological impact of treatment intensification in suppressed patients supports latent viral reservoir as source of residual viraemia. HTB August 2008.
<http://i-base.info/htb/596>

6.5 Trends in transmitted drug resistance over time

In section 3.5 we included the first reference to the fact that resistant HIV can be transmitted.

Lots of research groups keep databases of every resistance test in a country. They can then look at trends in transmitted drug resistance each year.

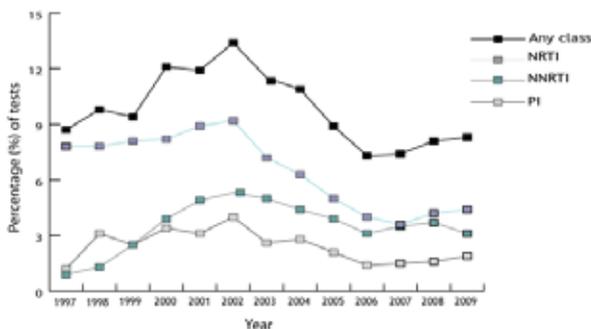
This study, from the UK HIV drug resistance database (UK-HDRD), is available online.

The group tracks and reports the results of resistance testing each year and shows how this changes over time.

The study reported trends in resistance over time and differences between newly diagnosed people and people with drug resistance.

This is only one of many studies produced by the group,

Figure 7. Percentage of tests showing drug resistance by drug class in the UK



Surveillance summary:

<http://www.ctu.mrc.ac.uk/hivrdb/public/surveillance.asp>

Publications and presentations from the UK HDRD:

<http://www.ctu.mrc.ac.uk/hivrdb/public/publications.asp>

6.6 Single dose nevirapine during pregnancy

This example shows the importance of timing when looking for resistance.

Section 4.3 showed how some mutations develop easily and that for some drugs and drug classes resistance develops easily - in this example NNRTIs.

Nevirapine is an NNRTI and key mutations - Y181C and K103N - only need one base change to develop. Once there they are like an on/off switch: the NNRTI stops having any impact on the viral load.

Nevirapine is good at getting nearly everywhere in your body very quickly. A single dose of nevirapine just before giving birth dramatically reduced the chance of passing HIV to the baby. From 25% down to 12% with a single dose. Previously, AZT needed to be taken twice-daily for several months to have the same impact on reducing transmission to the baby.

For women in countries with no access to treatment this was potentially very exciting. However, other researchers were cautious about the risk of resistance for the mother. Using only a single dose of a drug meant that the nevirapine was not supported by any other drugs. Viral load was still likely to be high, and nevirapine also takes several weeks to leave the body. This left a long time for HIV to develop NNRTI mutations by chance. They develop and reproduce because they are more fit when nevirapine is still there.

In an important single-dose nevirapine study (called HIVNET 012), the researchers first tested for resistance six months after the women used nevirapine, and reported that resistance was only seen in 20% of women (which was still very high). Resistance experts were more concerned. In section 3.5 we learnt that resistance that develops when a drug is present usually becomes difficult to find when the drug is stopped. Some researchers talk about resistance ‘fading’ but this is a confusing term because we know that when someone restarts treatment that resistance quickly comes back. It is nearly always archived. When the researchers went back and tested earlier samples - this time only two weeks after the women had used nevirapine - they found over 75% of mothers had nevirapine mutations.

Reference:

Eshleman EH et al. HIV-1 variants with diverse nevirapine resistance mutations emerge rapidly after single dose nevirapine: HIVNET 012. XII International HIV Drug Resistance Workshop, Los Cabos, Mexico, 10-14 June 2003. Abstract 79.

Reported in HTB, September 2003. Single-dose nevirapine resistance in over 75% of mothers.

<http://i-base.info/htb/11689>

6.7 Importance of resistance testing before starting treatment

This study is interesting because it shows the importance of resistance testing before starting treatment.

It looked at about 300 people who were treated during primary HIV infection (within 6 months of infection). Resistance testing was not used before starting treatment to choose drugs. However, 35 people were found to have resistance when their pre-treatment (baseline) samples were subsequently tested. Of these 35 people, 21 had resistance to one drug, 10 to two drugs and 4 people had resistance to all three drugs in their combination.

The resistance group had a significantly poorer response to treatment. Only 16% had undetectable viral load after three months compared to 40% of people with no resistance. After six months the difference was still significant: 57% vs 79%, even after allowing for age, gender and differences in CD4 count and viral load at baseline.

This study and similar others were used as evidence to support the recommendation to provide resistance testing before starting treatment in treatment guidelines.

Reference: Chaix1 ML, Desquilbet L, Cottalorda J et al. Sub-optimal response to HAART in patients treated at time of primary HIV-1 infection and infected with HIV resistant strains. Abstract 114. Antiviral Therapy 2005; 10:S126.

<http://i-base.info/htb/7232>

6.8 Reinfection/superinfection: catching HIV twice

This example is a report on reinfection from an important conference in 2010. These were certainly not the first studies to report reinfection (sometimes called superinfection) but it is a report of four different studies that each involved interesting cases.

The table in Section 5.3 includes testing for resistance in anyone whose viral load rebounds on treatment. It specifically mentions the circumstance for someone on stable treatment but who may have been reinfected with a different strain of HIV. One of the studies included a couple where both partners were HIV-positive and did not use condoms. However, one of the partners has no resistance and an undetectable viral load. The other had triple-class resistance and a generally detectable viral load.

In this case the partner with no resistance became infected with the resistant HIV. The initial viral load rebound prompted the doctor to test for resistance and to compare the results to earlier samples.

This case is important because it occurred in someone who had been HIV positive for many years (ie not in early infection). It also had a dramatic clinical impact - the person's previously active treatment stopped working.

Reference:

Castro E et al. HIV-1 superinfection with a drug-resistant strain in a patient successfully controlled with ART. Poster abstract 480.

<http://www.retroconference.org/2010/Abstracts/37374.htm>

See report in HTB, June 2010. HIV reinfection cases reported at CROI 2010.

<http://i-base.info/htb/10502>

6.9 Section 6: Learning points

- HIV drug resistance is an important and essential field of HIV research.
- Studies can change the way that resistance tests are used.
- Research can change treatment guidelines.
- This research often has a direct impact on the way that HIV drugs are prescribed and how HIV treatment is managed.

Appendix 1: Questions on resistance

The questions below were posted by participants on the i-Base course on HIV and drug resistance. All question are answered on online:

<http://i-base.info/home/appendix-1-questions-on-resistance/>

1. Which drugs can someone use if they have already developed resistance?
2. How are treatment choices made for someone with resistance?
3. Can drug resistance be passed from mother to child?
4. Can a person's genetic makeup contribute to them becoming resistant to a drug?
5. Is it possible to develop resistance to a drug even with perfect adherence?
6. Are some drugs easier to become resistant to than others?
7. I want to understand the terms used about resistance.
8. If viral load in the blood is less than 50 copies/mL, but is higher in sanctuary or compartment sites (ie the brain or genital compartments), can resistance develop in those sites?
9. What is "wild type" virus and what does it do?
10. You need virus present for resistance testing – so you can't test if the viral load is undetectable. How high does the viral load need to be to be able to use resistance testing?
11. Do you need to be currently taking a drug to see if you are resistant to that drug? Is resistance still detected if you have stopped or changed treatment?
12. If you have developed resistance to a drug, does that mean that you are resistant to all the drugs in that class?
13. Is poor adherence the only factor that leads to developing drug-resistance? Are there any other factors?
14. If your viral load is undetectable but your CD4 still low, could that be a sign of drug-resistance? Would the doctors consider doing a drug-resistance test?
15. What are the main signs of drug-resistance?
16. At what level is viral load considered undetectable?

17. What is a viral load ‘blip’ and what should you do about it?
18. When should you have a resistance test?
19. Seeing as HIV “makes mistakes” and does not have “proof reading” abilities when replicating, is it possible to introduce “defective or modified” genetic material that would render the virus ineffective?
20. What is the difference between the types of tests for resistance – phenotype vs genotype? Which is preferred?
21. Are resistance tests the same tests that are used to see if the type of HIV in different people is in some way linked – in prosecution cases of transmission, for example?
22. If a woman takes ART during pregnancy for prevention of mother to child transmission (PMTCT) and wants more children, is she likely to become resistant to those drugs?
23. What options does a woman have for future pregnancies?
24. Some people have never had resistance tests and would not know how to bring this up in a discussion with a doctor. How can this best be addressed?
25. With all clinics trying to cut back because of funding cuts, how do we make sure that important tests like the resistance tests are offered to patients when they need them?
26. How expensive (or cheap) are resistance tests?
27. Are resistance tests available in developing countries?
28. What happens to a pregnant woman with resistance to the drugs used for PMTCT?
29. Are the tests always 100% accurate?
30. Are all possible mutations known?
31. Why can boosted protease inhibitors be used as monotherapy without developing resistance?
32. Is resistance inevitable?
33. How does wild type virus prevent resistant virus from reproducing?
34. As a patient, what should I expect to hear back from my resistance test? What should the doctor tell me?

Appendix 2: Supplementary information about genetics

DNA as a recipe book – for making new HIV

Sometimes it helps to think of DNA as a recipe book.

- There are only four letters used in this book, (A, T, C and G: the four nucleotides or BASES)
- There are only 20 different words (the 20 common AMINO ACIDS). Each word only has three letters. The place that each of these words is printed is also called a CODON.
- Each sentence (a PROTEIN) is made up of chains of many 3-lettered words (AMINO ACIDS).
- Each recipe – a chapter – (a GENE) is made up of several thousand sentences.
- Each book (GENOME) is made up of many chapters (GENES)

The HIV genome has nine short chapters, using about 3,000 words (AMINO ACIDS/CODONS) and about 9,200 letters (BASES).

The human genome contains 23 large chapters (CHROMOSOMES), many thousands of sentences, around one billion words and three billion letters. If you read one word every second, 24 hours a day, it would take over 30 years to read the human genome.

In humans, only 10% of the 3 billion bases are thought to be important and active. So some changes in DNA may not make any difference. This means that a large percentage of human DNA is like advertisements or blank pages.

We know what some parts of the DNA chain relate to – ie one part will determine the colour of your eyes. Other sections of DNA have been linked to more critical functions including risk or protection from a range of hereditary health complications. So, many things about you are determined by the order of the four bases and 20 amino acids in your DNA.

Nucleotides (bases)

There are only four base chemicals that make up DNA:

A = adenine T = thymine C = cytosine G = guanine

The order of these bases determine the structure and function of all life. To make it easier, in DNA these molecules only have two pairs of bonds:

A always binds to T and C always binds to G.

Amino acids

Each group of three bases will be one of 20 different amino acids.

Amino acids are the chemical building blocks to make proteins and almost everything in the body is either made of proteins or needs proteins to make it.

See Appendix 3: List of amino acids and their abbreviations.

You do not need to learn these or know about the differences for each amino acid. It is important to understand that each letter stands for a different amino acid.

For example:

The three bases ATG is the code for the amino acid called methionine (M)

The three bases GTA or GTG are two of the codes for the amino acid called valine (V)

Most amino acids can be made up from different combinations of letters.

See Appendix 4: DNA codes for amino acids.

For example:

Valine can be made from four different base combinations: GTT, GTA, GTG and GTC

- Strings of amino acids make up different proteins.
- Strings of proteins make up different genes.
- Strings of genes (in humans) are called chromosomes.

In this example, notice that only one letter needs to change to get from methionine (M) to valine (V). This gives an indication that the mutation is a simple change.

Appendix 3: List of amino acids and their abbreviations

This table is included for reference only.

There is no need to learn these names and abbreviations. It is important that you understand that the letter in drug resistance refers to different amino acids. This table is included for future reference.

Table 4: Amino acids and their abbreviations

Amino acid	Single-letter abbreviation	3-letter abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamic acid	E	Glu
Glutamine	Q	Gln
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

Appendix 4: DNA codes for amino acid



Table 5 is only for reference. You do not need to learn any of these codes by heart.

It is included for two reasons.

1. Most amino acids can be made from more than one combination of bases.
2. That some mutations are easy and some are difficult. Easy mutations only need one letter to change. The most complex mutations need to change all three bases.

Table 5: DNA codes for amino acids

	T	C	A	G
T	TTT phenylalanine	TCC serine	TAT tyrosine	TGT cysteine
	TTC phenylalanine	TCC serine	TAC tyrosine	TGC cysteine
	TTA leucine	TCA serine	TAA stop	TGA stop
	TTC leucine	TCG serine	TAG stop	TGC tryptophan
C	CTT leucine	CCT proline	CAT histidine	CGT arginine
	CTC leucine	CCC proline	CAC histidine	CGC arginine
	CTA leucine	CCA proline	CAG glutamine	CGA arginine
	CTG leucine	CCG proline	CAA glutamine	CGG arginine
A	ATT isoleucine	ACT threonine	AAT asparagine	AGT serine
	ATC isoleucine	ACC threonine	AAC asparagine	AGC serine
	ATA isoleucine	ACA threonine	AAA lysine	AGA arginine
	ATG methionine	ACG threonine	AAG lysine	AGG arginine
G	GTT valine	GCT alanine	GAT aspartic acid	GGT glycine
	GTC valine	GCC alanine	GAC aspartic acid	GGC glycine
	GTA valine	GCA alanine	GAA aspartic acid	GGA glycine
	GTG valine	GCG alanine	GAG aspartic acid	GTG glycine

Valine is coded by four different combinations: GTT, GTC, GTA and GTG. This shows that some changes will not change the amino acid.

If one mutation changes the third base from a T to C – as in GTT to GTC – the amino acid at that junction will still be valine.

Some amino acid changes require one base change, some require two base changes and some require all three bases to change.

The number of base changes needed to change one amino acid to another is related to how quickly a mutation develops.

This chart shows that the M184V is an easy mutation - like getting one bell on a fruit machine.



To change from methionine (M) to valine (V) only requires a change at one base: ATG to GTG.

The mutation M184V that stops 3TC from working, is commonly the first mutation to be detected in any 3TC-containing combination if that treatment fails. As an easy mutation, it can occur quickly. With 3TC monotherapy (ie when 3TC is the only drug) resistance would be likely to occur within two weeks.



By comparison, the T215Y mutation is more complex - like getting three cherries.

Threonine (T) can be made from four different base combinations: ACT, ACC, ACA and ACG.

Tyrosine (Y) can be made from only two: TAT and TAC.

Importantly, to change from threonine to tyrosine needs at least the first two bases to change (from ACT to TAT or ACC to TAC). In some cases, it might need all three bases to change (from ACA or ACG to TAT or TAC).

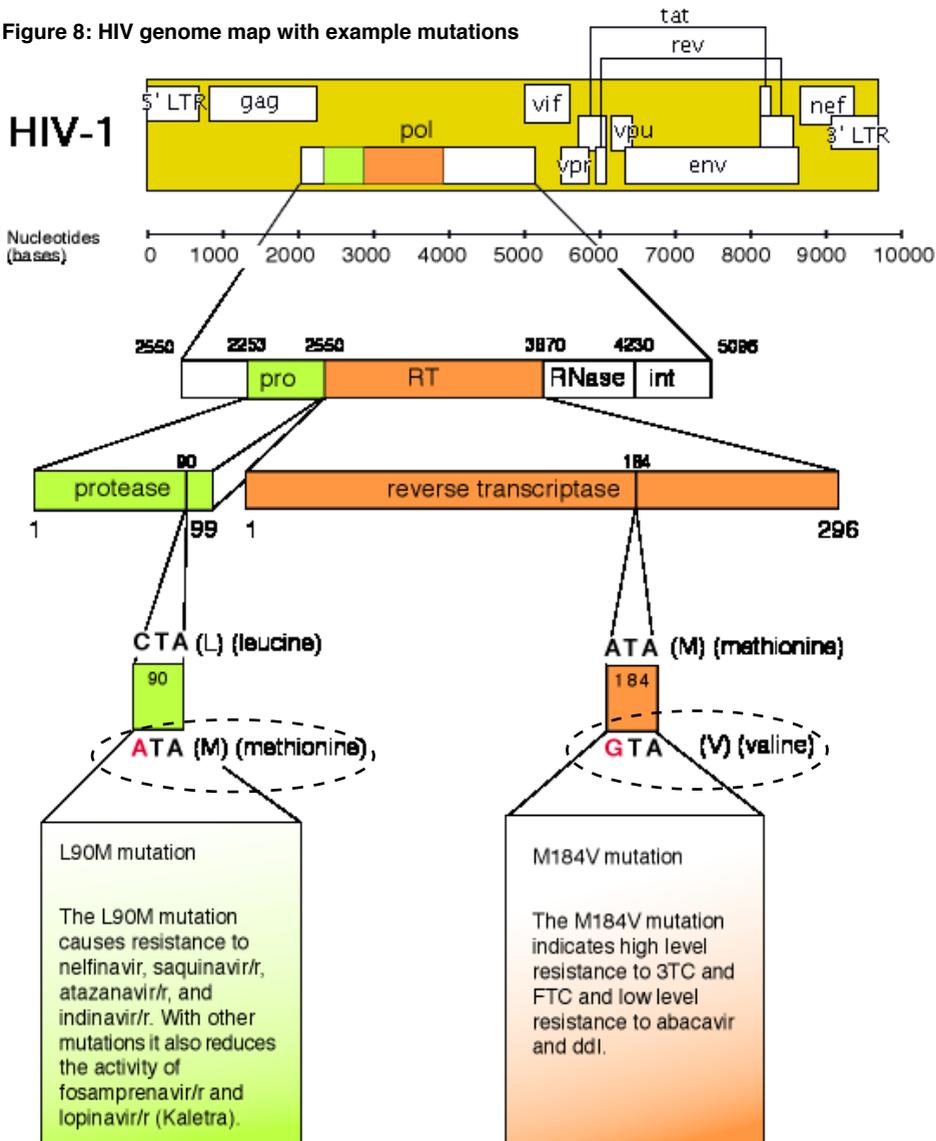
The T215Y mutation is one of the main mutations associated with resistance to AZT. However, as a more complex mutation, it is less likely to occur by chance. In practice, this mutation might take six months to develop in someone taking AZT monotherapy.

These mutations are like fruit machines: getting one bell is easier than getting a row of three cherries to show. You need to play more often and for longer to get the difficult combinations.

Appendix 5: HIV genome map with example mutations

This graphic is a map that simplifies HIV into nine main genes: *gag*, *pol*, *vif*, *vpr*, *vpu*, *env*, *tat*, *rev* and *nef*. The map shows roughly how these genes are laid out.

Figure 8: HIV genome map with example mutations



Each gene has a different role in the HIV life cycle. You don't need to learn all these functions, just recognise that we know roughly what many of the functions are. For example *env* is easiest to remember because it is the recipe for spikey proteins that stick out from the 'envelope' coating on the outside of the virus.

Pol is important when talking about drug resistance. This is because this section of the HIV genome that has the code for the enzymes that are targeted by many HIV drugs. These include protease inhibitors (PIs), reverse transcriptase inhibitors (nukes and non-nukes) and integrase inhibitors.

The numbers underneath the main picture relate to the 9700 bases (roughly 3,200 amino acids) that make up the genetic structure of HIV.

Pol starts at number 2550 and stretches to 5096.

The protease section stretches from 2253 to 2550. This is a chain of 297 nucleotides, which equals 99 amino acids (amino acids are groups of three nucleotides).

Therefore, the protease gene is numbered from 1 to 99. The example here is L90M which is a common PI mutation.

In a similar way the amino acids in reverse transcriptase are numbered from 1 to 296. A change at position 184 on the RT gene from ATA to GTA is the M184V mutation.

Genotype resistance tests do not need to look at the whole of the HIV genome. They only need to look at this short section to get information about resistance to nukes, non-nukes and PIs.

Resistance tests for entry inhibitors like T-20 need to look at a section of the *env* gene. Resistance to integrase inhibitors needs a test that looks at a small section on the integrase section of the *pol* gene.

A more detailed map of the HIV-1, HIV-2, and SIV genomes, available at <http://hiv-web.lanl.gov/content/immunology/pdf/2000/intro/GenomeMaps.pdf>

A more detailed explanation of what these different genes do is at this link: <http://i-base.info/qa/faq/hiv-genome-explained>

If you feel really geeky, then check it out, but while learning about resistance just to let the rest of the information in this section sink in first...

Appendix 6: Stanford Drug Resistance Database online tables

The Stanford Drug Resistance Database is one of several online research resources that contain a vast amount of support information about drug resistance.

One page in the resource includes links to each drug family (Drug resistance mutation tables) and the related mutations and individual pages for each HIV drug (Antiretroviral drug summaries).

<http://hivdb.stanford.edu/pages/drugSummaries.html>

For example the link to NRTI-associated mutations.

<http://hivdb.stanford.edu/cgi-bin/NRTIResiNote.cgi>

This link looks complicated but just needs explaining (see Table 4).

Table 4: Nucleoside RT inhibitor (NRTI) resistance mutations (Stanford database)

	184	Thymidine analogue mutations (TAMS)						Non-thymidine analogue mutations					Multi-NRTI resistance mutations					
		41	67	70	210	215	219	65	70	74	75	115	69	151	62	75	77	116
Cons	M	M	D	K	L	T	K	K	K	L	V	Y	T	Q	A	V	F	F
3TC	V/I							RN	EG				Ins	M	V			
FTC	V/I							RN	EG				Ins	M	V			
ABC	V/I	L	N		W	F/Y		RN	EG	VI	TM	F	Ins	M	V	I	L	Y
ddl	V/I	L	N		W	F/Y		RN	EG	VI	TM		Ins	M	V	I	L	Y
TDF		L	N		W	F/Y		RN	EG		M	F	Ins	M	V			
d4T		L	N	R	W	F/Y	Q/E	RN				TM	Ins	M	V	I	L	Y
AZT		L	N	R	W	F/Y	Q/E						Ins	M	V	I	L	Y

Cons stands for *consensus sequence* which means wild-type non-resistant virus. The letters in the *Cons* row refer to the amino acids that should be at those junctions.

The line of numbers above the ‘Cons’ row are the positions or junctions (codons) where important changes occur:

41 67 70 210 215 219 65 70 74 75 115 69 151 62 75 77 116

Each of these junctions in RT is linked to resistance to different members of the family of HIV drugs called ‘nukes’ (reverse transcriptase inhibitors). They are arranged in three sections.

The first section is for mutations linked to AZT (ZDV, zidovudine) or d4T (these drugs are both thymidine analogues). The second section relates to other nukes (non thymidine analogues, ie 3TC, ddl, tenofovir, abacavir etc). The third section includes mutations that have broad cross resistance to all nukes (multi-drug resistance).

Each row next to a drug name then shows the mutation at each junction that is associated with drug resistance.

So reading across from 3TC the letters V and I mean that M184V or M184I both indicate resistance to 3TC. **Red** letters indicate the resistance is high level (it will have a big impact on stopping the drug from working). Regular letters indicate lower level resistance, showing that the drug may still work a little, but less than if it was wild-type.

The letters in black (representing K65R, K65N, K70E, K70G, T69Ins, Q151M and A62V) list other mutation that all reduce how well HIV works.

Although this looks like a lot of mutations, amino acid changes at all the other junctions are not associated with resistance. For example changes at 42, 43, 44, 45, 46 etc in RT have not been seen to be clinically relevant.

NOTE: 'Ins' refers to insertion, which is a type of mutation where instead of one base switching to another (technically called a 'point mutation'), an additional base is inserted.

This shows the complexity of interpreting HIV drug resistance....Time for a break to let this information settle :)

The link to 3TC (lamivudine) drug summary explains the implications for each of the mutations that have been associated with 3TC resistance AND includes references for the studies which show this:

<http://hivdb.stanford.edu/pages/GRIP/3TC.html>

For example:

M184V or M184I	M184V causes high-level resistance to 3TC (>300-fold reduced susceptibility). In patients with viruses containing M184V, there is some benefit in continuing 3TC because viruses with M184V replicate less well than wild-type viruses and because this mutation increases susceptibility to ZDV, d4T, and TDF (Campbell et al. 2005; Larder et al. 1995; Nijhuis et al. 1997). However, the benefit of continued 3TC will be less than the benefit of 3TC in patients with wild-type virus.
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This explanation includes new terms which relate to phenotype resistance including 'fold changes' and 'reduced susceptibility'.

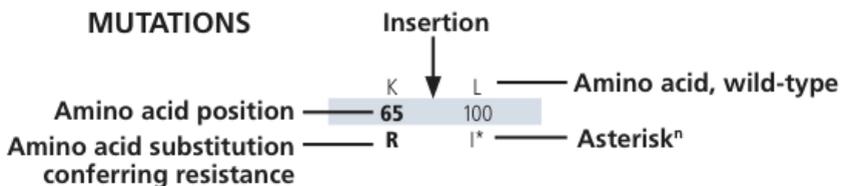
Appendix 7: IAS-USA mutations and cross resistance

The following tables from the International Antiviral Society USA (IAS-USA) show mutations for each drug and cross resistance between drugs in each class.

The IAS-USA website includes free download of the latest guidelines to HIV drug resistance published in 2013 in Topics in HIV Medicine that includes these tables.

<https://www.iasusa.org/sites/default/files/tam/21-1-6.pdf> (PDF)

Table 5: Key to IAS-USA drug resistance mutation tables (2013)



Appendix 8: Summary of UK Guidelines (BHIVA)

Section 14 in the UK monitoring guidelines (BHIVA 2011) includes the current recommendations for resistance testing.

They are summarised in Table 9 below. Please see the online version for full details and references.

Ref: Asboe D et al. British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011. *HIV Medicine* (2012), 13, 1–44.

<http://www.bhiva.org>

Table 9: Summary recommendations on when to perform resistance testing

When to test	Comments	Method	Level of evidence and grading
New diagnosis	Recommended	Genotype	a
Starting ART	Recommended, if not already carried out. Repeat testing not routinely recommended but can be considered if superinfection likely.	Genotype	a lb
After starting ART	Consider resistance testing if suboptimal response to first-line therapy (<1 log ₁₀ copies/mL reduction after 4 weeks of therapy). Consider resistance testing if viral load >50 copies/mL at 12–16 weeks after starting therapy. Recommend resistance testing if viral load >50 copies/mL at 24 weeks after starting	Genotype	V II a
ART failure	Recommended to guide treatment changes. Perform while on treatment (or not more than 2 weeks after stopping)	Genotype *	a lb

*Consider phenotype or virtual phenotype if multiple regimen failure and/or multiple mutations on genotype where interpretation is uncertain.

Source: BHIVA monitoring guidelines, 2011, Table 14.1.

Appendix 9: Example of a resistance test report

Most resistance test in the UK use the Stanford database to generate a resistance report.

The list of mutations is put into an online resource that generates the report.

The sample below shows the section of a sample report for nukes and NNRTIs. The online version includes PI resistance.

A virtual phenotype test result is also included online.

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A national public database of drug-resistant HIV-1 and HIV-2 sequences and their clinical outcomes. HIV Drug Resistance

HOME GENOTYPING GENOTYPING/PHENOTYPING GENOTYPING/CLINICAL HIV/AIDS PROGRAM

HIVdb: Genotypic Resistance Interpretation Algorithm

Summary Data

Sequence includes PR: codons: 1 - 99
 Sequence includes RT: codons: 1 - 341
There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

1. PR: CRF02_AG (98.3%)
2. RT: CRF02_AG (95.4%)

Drug Resistance Interpretation

NNRTI Resistance Mutations: **M41L, M184V, T215Y**

NNRTI Resistance Mutations: None

Other Mutations: V35T, T39A, D123E, I135V, S162T, K173T, Q174K, T200A, Q207E, V245Q, T286A, E291D, V292I, I293V, S322A, I326V, Q334P, G335D

Nucleoside RTI

3TC	High-level resistance
ABC	Intermediate resistance
AZT	Intermediate resistance
D4T	Intermediate resistance
DDI	Intermediate resistance
FTC	High-level resistance
TDF	Low-level resistance

Non-Nucleoside RTI

DLV	Susceptible
EFV	Susceptible
ETV	Susceptible
NVP	Susceptible

RT Comments

- M41L usually occurs with T215Y. Together these mutations confer intermediate-to-high level resistance to AZT and d4T and a lower level of resistance to ddI, ABC, and TDF.
- M184V/I cause high-level in vitro resistance to 3TC and FTC and low-level in vitro resistance to ddI and ABC. M184V/I increase susceptibility to AZT, TDF, and d4T.
- T215Y causes AZT and D4T resistance and reduces susceptibility to ABC, ddI, and TDF particularly when it occurs in combination with M41L and L210W.
- M184V partially reverses AZT, d4T, and TDF resistance caused by other AZT mutations (TAMs). AZT mutations in this isolate include: M41L, T215Y.

Glossary

The i-Base website glossary includes more detail for some of these terms.

active – an active drug is a drug that still works to reduce viral load. The virus is still sensitive to that drug.

amino acids – amino acids are the building blocks of proteins. DNA codes for amino acids. Three nucleotides (segments of the genetic code) make one amino acid.

baseline test – a blood test taken before treatment is started to see if there is any resistance.

CD4 count – a blood test that indicates the strength of the immune system. The CD4 test is one of the most important indicators for deciding when to start HIV treatment.

clinical cut-off (CCO) – a test result that is associated with an impact on clinical care. With resistance tests a lower CCO is the level below which a drug is still sensitive or active. An upper CCO is the level above which the drug is not considered active.

codon – the word for the junction on genetic material (DNA or RNA) occupied by three nucleotides (or bases) to form an amino acid.

combination therapy – three or more HIV drugs to treat HIV.

compensatory mutation – an additional mutation, that return the virus to a greater fitness.

cross-resistance – when resistance to one drug causes resistance to other similar drugs.

DNA – an abbreviation for the scientific word for genes and genetic material. It is the abbreviation for deoxyribonucleic acid.

drug resistant mutation – a mutation or change that occurs in the HIV genome that reduces a drugs ability to work.

escape mutation – a change in HIV that evades the immune system (rather than stopping a drug from working).

genome – the complete genetic information (RNA or DNA) of an organism.

genotype – the genetic makeup of a cell, an organism, or an individual.

genotype test – a test that looks at how the genetic structure of a sample of HIV and whether the virus has changed with drug resistant mutations.

high level resistance – when an HIV drug no longer works against the virus.

intermediate level resistance – when a drug still has some impact on HIV, but when this is reduced (compared to wild-type HIV) because there is some drug resistance.

low level resistance – when there is some resistance but it does not have any significant impact on how well a drug works.

major mutation – a drug resistance mutation that has a big impact on whether a drug continues to work. This used to be called a primary mutation.

minor mutation – a drug resistance mutation that has a small impact on whether a drug continues to work. This used to be called a secondary mutation.

monotherapy – using only one drug.

mutation – a change in the genetic structure of an organism (including a virus like HIV).

nucleotide – the building blocks of the genetic code (DNA/RNA). Also called a base.

partially active – a treatment that has some resistance and some sensitivity.

PEP (Post-Exposure Prophylaxis) – a course of HIV treatment (usually one month) taken after a potential exposure to reduce the chance of infection.

phenotype – relating to how an organism behaves, based on how its genotype relates to the environment.

phenotype test – a resistance test that looks at how well a drug works against HIV in a test tube.

reinfection – catching HIV a second time. Sometimes called superinfection.

resistance – when the genetic structure of a virus or organism changes so that treatment no longer works.

reverse transcriptase – an enzyme unique to HIV. It is used to convert single-stranded RNA into double-stranded DNA. This is needed before HIV's genetic material can be integrated in the human DNA.

revertant mutation – this term is used in two ways:

i) a genetic change that shows the virus is returning to a wild-type genotype; and

ii) a compensatory mutation as it compensates for the reduced fitness caused by the first mutations.

RNA – an abbreviation for the scientific word for genetic material found in some types of viruses (ribonucleic acid). It is very similar to DNA but is single-stranded rather than double-stranded.

selective pressure – this is when a factor in the environment causes one type of organism to develop and grow in preference to another. With HIV drug resistance, the presence of a drug exerts selective pressure for resistance to develop. It is based on evolution and the concept of 'survival of the fittest'.

sensitive – when referring to the activity of a drug, sensitive means that a drug still works.

viral load – the amount of virus (for example in blood, genital fluids of tissue sample).

wild-type – HIV without any drug resistant mutations.

Feedback

Your feedback on this guide helps us develop new resources and improve this resource. All comments are really appreciated. Comments can be posted free to: FREEPOST RSJY-BALK-HGYT, i-Base, 57 Great Suffolk Street, London SE1 0BB.

Or made directly online at: <http://www.surveymonkey.com/s/L8ZJM7P>

1. How easy was the information in this guide to understand?

Too easy Easy Difficult Too difficult

2. How much of the information did you already know?

None A little Most All

3. Did the information help you feel more confident speaking to your doctor?

Yes, a lot Yes, a little Maybe No

4. Which information did you find most useful?

5. Do you still have questions after reading this guide? Please give examples.

Please include a contact email address if you would like us to reply.

6. Any other comments?

Contact details (if you would like a reply): Name _____

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