RESISTANCE OVERVIEW

A look at the basics

by Bob Munk

Retrovir (AZT), the first HIV drug on the market, in 1987, seemed to work well—for about a year and a half. Then it stopped working, and no one understood why. Later we learned about viral resistance, when HIV changes its shape just enough to prevent antiretroviral drugs (ARVs) from binding to it and slowing down its multiplication. We also learned that different HIV drugs need to be taken together to help prevent this from happening.

Soon the first genotypic resistance test was developed. Alongside the new viral load tests (first available in the mid-1990s), this resistance test documented the changes in HIV's genetic code that might explain the failure of an antiretroviral drug. Later came phenotypic resistance tests.

Within the next few years, clinical trials examined the impact of resistance testing on patient outcomes. Resistance tests quickly made their way into HIV treatment guidelines. It seemed that we had gained a powerful new tool for managing HIV therapy.

This article introduces the resistance topics covered in this special issue of *Positively Aware*.

Mutant virus

The "wild type" virus is the most common form of HIV. When a change, or mutation, in HIV's genetic code occurs the virus is now considered a mutant. The wild type virus is "sensitive" (the opposite of resistant) to all HIV antiretroviral drugs (ARVs), which means that they should work well to slow down the virus from multiplying.

HIV mutates almost every time it replicates (makes a new copy of itself). Unlike human cells, HIV does not have a "proofreading" function to correct mistakes. It has been estimated that even when a patient is not taking medication, a mutation will occur every day at every position in HIV's genetic code!

When a patient is taking ARVs and mutations occur in HIV, it becomes "resistant" to one or more of the ARVs. This is called "selective pressure." An ARV won't control HIV that is resistant to it. The resistance allows the virus to "escape" from the drug's control. If you keep taking the drug(s), the resistant strain of virus will continue to multiply since the drug(s) are not working. Pretty soon, the resistant strain of virus will be the most common strain in your blood. However, if you stop taking medications, there is no selective pressure so the wild type virus will multiply the fastest, and eventually overgrow the resistant strain.

What happens inside the virus is that the mutations cause slight changes in the shape of HIV's enzymes, such as reverse transcriptase and protease. Molecules of HIV drugs fit very precisely into these enzymes, so it doesn't take much of a change of shape to block the "docking" of the meds with the HIV enzyme. Just a slight change in the configuration of the enzyme can put the drug out of business.

Unfortunately, even though there may not be resistant virus detected in the blood by a resistance test, it might be "archived" or hiding out inside cells or elsewhere in the body. Also, you need a certain amount of detectable virus for a resistance test to work. People with so-called "undetectable" viral load may have drug resistance that can't be picked up by a resistance test.

Not every mutation causes resistance—in fact, most mutations are fatal to the virus. The more that HIV multiplies, the more mutations show up. These mutations happen by accident. The virus doesn't "figure out" which mutations will resist medications.

There's a special case for what are called "polymorphisms." These are mutations that don't seem to have any impact on the ability of ARVs to work. You might think of them as coding for brown-haired or black-haired virus.

How does resistance develop?

HIV usually becomes resistant when it is not totally controlled by ARVs someone is taking. The best advice for avoiding resistance is to take all antiretroviral medications on schedule and to follow any directions about food intake and storage.

In several studies, researchers tried to figure out just how precise a patient has to be. How many doses can be missed? At first, they were thinking about other diseases where, in many cases, taking 80% of prescribed medication doses is enough to control the disease.

This is not the case with HIV. The best estimate they came up with was that patients need to take 95% of their ARV doses! This is called being "adherent." This can be very difficult even if you're just thinking about the number of doses. However, it gets even harder for drugs with complicated storage requirements or food requirements.

Fortunately, there are fewer of these as the pharmaceutical companies work hard to improve convenience.

Also, not every drug gets into the body the same way or achieves the same blood levels in every person. Low blood levels can contribute to the development of resistance. Genetic factors and personal metabolism may play a role here. For more information on factors that affect blood levels (pharmacokinetics), see "A PK Primer" by Tim Horn in the *Positively Aware* Special Fall '05 Issue. The bottom line for the adherence researchers seemed pretty clear: the more doses you miss, the easier it is for resistance to develop.

But like just about everything related to HIV, it's more complicated than it seems. If you're not taking enough of your doses, there may not be enough selective pressure to favor the development of resistant virus. This is probably because the virus has to pay a price for resistance: the mutant virus usually doesn't multiply as well as the wild type. The wild type multiplies better and becomes the dominant strain. This might happen at an adherence level of, say, 60%. There's not enough pressure on the virus to make it worth its while to pay the price for developing a resistance mutation.

And then it turns out that different rules seem to apply to the three major classes of HIV drugs currently on the market. The NNRTIs (Sustiva, Viramune, and Rescriptor) stay in your bloodstream for a long time, and the virus doesn't pay much of a price for NNRTI resistance mutations. Any level of adherence that doesn't really shut down the virus can lead to resistance.

However, for the nucleosides and protease inhibitors, the more adherence the better—up to a point. More recent research suggests that the most common resistance mutations, at least for the nukes and protease inhibitors, show up at fairly high levels of adherence—around 80%. This doesn't mean you should slack off, because those mutations that show up at high levels of adherence might cost the virus a lot in terms of its ability to multiply.

Genotype test

There are two very different types of resistance test: genotypic and phenotypic. A genotypic test analyzes the genetic code of the patient's enzymes that are necessary for HIV to multiply, and looks for any changes or mutations—parts of the code that are different from the wild type.

The HIV genetic code is a chain of nucleotides. Every group of three nucleotides defines a particular amino acid that the virus will create when its code is "read." These groups of nucleotides are called "codons."

Researchers refer to resistance in the specific codons that are part of the reverse transcriptase or protease enzyme genes of the virus.

Mutations are described by a combination of letters and numbers, for example K103N. The first letter (K) is the code for the amino acid in the wild type virus. The number (103) identifies the position of the codon—the 103rd codon in the reverse transcriptase gene. The second letter (N) is the code for the "changed" amino acid in the mutant virus.

Over the past 10 years or so, researchers have carefully kept track of the specific mutations that are associated with viral resistance to individual drugs. They have developed lists of mutations that are used to interpret genotypic test results. These rules used to interpret the viral mutations and their effect on ARVs are called algorithms.

As new drugs are developed, new algorithms have to be developed. Often, the rules for older drugs have to be updated. For example, a lot of work will be necessary to develop a good sense of resistance mutations to the new entry inhibitors, since they bind to the *envelope* portion of HIV, totally separate from the reverse transcriptase and protease enzymes. Resistance to Fuzeon (T-20), the only available entry inhibitor, occurs when mutations occur in the HIV envelope which surrounds the entire virus.

Just one mutation can make HIV resistant to one or more drugs. This is true for the nucleosides Epivir (3TC) and Emtriva (FTC) and the current class of non-nucleoside reverse transcriptase inhibitors (NNRTIs), Sustiva, Viramune, and the rarely-used Rescriptor. If your genotypic test shows the following mutations, it's pretty certain that your virus is highly resistant to these drugs. The mutations are M184V for Epivir (and Emtriva) and K103N for the NNRTIs.

However, HIV has to develop a series of mutations to develop resistance to other drugs, including most protease inhibitors and nucleosides (or nukes for short). It can be much more difficult to read a list of mutations and know whether a drug will work or not. This helps explain why, in many cases, resistance is not "all or nothing." So for most nukes and the protease inhibitors, having one or two mutations might make the virus partially resistant to the drug, but not totally, meaning that the drug may still have some ability to slow down HIV multiplication.

Our understanding of resistance mutations is not perfect. Until recently, we were told that the mutations associated with resistance to Zerit (d4T) were unknown. Then it came out that they overlapped significantly with Retrovir's resistance mutations.

Kaletra (ritonavir-boosted lopinavir) resistance mutations have so far proved elusive. Researchers are having a hard time identifying the primary resistance to Kaletra, so at the moment, there isn't general agreement on which mutations are linked to lopinavir resistance. When HIV developed resistance in people taking lopinavir, it has virtually always been resistance to the other drugs in their regimen—not to lopinavir.

Different resistance algorithms can give different results. One set of genotypic interpretation rules might tell you that the virus is resistant to a specific drug while another set could say it's still sensitive. It is very difficult in some cases to interpret genotypic test results, and even resistance experts may disagree on what they mean. And no matter how expert the interpretation, genotypic tests are still an indirect measure of resistance. They tell you which codons will produce mutant amino acids, which are associated with resistance, but they don't tell you if the virus is definitely resistant to a particular drug. This is where the phenotype test comes in.

Phenotypic test

The second type of resistance testing is phenotypic testing. This *does* tell you if the virus is actually resistant to a drug. A benefit of phenotypic tests is that they directly measure viral resistance. That is, they tell

you how sensitive the virus is to various ARVs. However, they are more expensive and take longer than genotypic tests.

A sample of HIV is grown in the laboratory and a patient's HIV enzymes are added to the virus. Then increasing amounts of an ARV are added to see how much of the drug is needed to "suppress," or stop, the replication of the virus. The amount of drug needed to suppress the patient's HIV is then compared to the amount needed to suppress a standard sensitive wild type virus. If more drug is required for the patient sample than the wild type, it is resistant to the medication. Phenotypic resistance is reported as "fold change" resistance. If the patient sample requires 20 times as much drug as the wild type, there is "20-fold resistance."

Virtual phenotype

One company, Virco Lab, developed a way to interpret genotypic test results that provides some of the benefits of phenotypic testing without the expense or delay of a phenotypic test. They call it a "virtual phenotype." They have a large database of samples of HIV for which they have genotypic test results and phenotypic results. Instead of relying on rules to interpret genotypic mutations, the virtual phenotype finds samples of HIV in the database that have a similar genotypic test profile to the patient's genotype. It then looks at the phenotypic "behavior" of these samples to predict resistance in the patient sample. This type of test interpretation may be just about as good as a full phenotypic test.

Cross resistance

Sometimes a mutant version of HIV is resistant to more than one drug. When this happens, the drugs are called "cross-resistant." For example, most HIV that is resistant to Viramune (nevirapine) is also resistant to Sustiva (efavirenz). This means that Viramune and Sustiva are cross-resistant. Cross-resistance is important when you change medications. You need to choose new drugs that are not cross-resistant to drugs your virus is already resistant to.

We do not fully understand cross-resistance for the nukes and protease inhibitors. However, many drugs in these classes can become at least partly cross-resistant. As HIV develops more mutations, the virus develops more cross resistance and gets harder to control. If your virus has accumulated several protease resistance mutations, for example, it becomes more difficult to find a protease inhibitor that will work for you. That is why new drugs in development are so important. New protease inhibitors and other drugs are being developed to work in spite of the current resistance mutations.

Resistance "pathways"

As we've gained experience with viral resistance, researchers have learned that the virus often acquires resistance mutations in a certain order or grouping.

For example, the mutations that confer resistance to the thymidine analog reverse transcriptase inhibitors (Retrovir and Zerit) are referred to as Thymidine Analog Mutations (TAMs), and they usually develop a specific pattern.

Another totally different mutation is the K65R, which affects the susceptibility of HIV to Viread (tenofovir), Videx (ddI) and Ziagen (abacavir).

An interesting fact is that once the virus has several TAMs, it seems to be much harder for it to get the K65R mutation. In come cases, the TAMs overcome the K65R, which improves your chances of using Viread, Videx and Ziagen. Going the other direction, if the virus already has a K65R mutation, it is usually harder for it to develop the TAMs, so the thymidine analog drugs (Retrovir and Zerit) may be useful.

Sequencing

Clinicians are trying to learn which meds to use for patients whose virus has already developed some resistance. The idea is that after drug "x" you could still use drug "y." This is called sequencing. There are two goals of sequencing: one is to position the ARV to be used first, because it doesn't compromise the use of other ARVs. Another goal might be to save the drug for later because it has special strength against HIV that is already resistant to other ARVs.

For example, the most common mutation associated with resistance to Viracept (nelfinavir) is the D30N protease mutation. This is an unusual mutation not shared by other protease inhibitors. The manufacturer of Viracept argued that clinicians could use Viracept first, because resistance to their drug would not prevent them from using other protease inhibitors as follow-up. However, this is only true if the D30N is the only protease inhibitor mutation that arises when a patient fails a regimen containing Viracept.

A similar argument is being made by the makers of Viread (tenofovir) because the most commonly occurring mutation, albeit rare, is the K65R reverse transcriptase mutation. This does not contribute to resistance to the thymidine analog drugs. In fact, it can make HIV more sensitive to them, as explained above.

At the other end of the experience spectrum, drugs in development are showing some promise in overcoming existing resistance. The recently approved Aptivus (tipranavir) can often overcome high-level resistance to most other PIs. Tibotec is studying TMC-125 and TMC-278, both designed to overcome existing NNRTI resistance, and TMC-114 for PI-experienced patients.

However, the concept of sequencing doesn't really seem to extend beyond choosing the next drug. That is, there really aren't any multi-regimen sequences that clinicians are generally prescribing to minimize the effects of resistance.

Treatment-experienced patients

Once a patient has been on any ARVs, they are considered "treatment experienced." A growing challenge is the treatment of patients who have already used most ARVs, sometimes referred to as "salvage" therapy. This is especially an issue for patients who began treatment before triple-combination therapy was widely used. Many of them were started on just two or even just one ARV. This made it easier for HIV to develop resistance.

Over the years, as the patient failed subsequent treatment regimens and their virus became highly resistant to more and more drugs, new drugs were added to the patient's regimen to try to suppress viral replication. By the time someone had been on multiple treatments for five or more years, the virus had likely accumulated quite a collection of resistance mutations. When these show up on a genotypic test report, they make interpretation very complicated. Pheno-

typic testing is probably ideal for this type of treatment-experienced patient.

One more thing to consider is that resistance mutations can seem to disappear if a patient stopped taking a drug a long time ago. However, the resistant strain might be "archived"—that is, hiding out, or at a very low level where the resistance test can't see it.

That's why most physicians think that having a patient's treatment history and prior resistance test results is a critical part of decision making. It can be risky to "recycle"—that is, prescribe again—any drug that a patient has used, unless they stopped using it before resistance developed (due to toxicity or other reasons.)

The pharmaceutical companies are working hard on the development of "second generation" drugs in each of the existing classes. They hope to develop new nukes, NNRTIs, and protease inhibitors that will be effective against HIV that already has resistance to one or more drugs in the same class. So far, this effort has had some success, but not always. For example, there is still no NNRTI that works against HIV that has the most common NNRTI resistance mutations, although progress is being made.

Difficulties with resistance testing

Resistance testing is a valuable tool for choosing ARVs. It can help avoid having someone take medications that won't be effective in controlling HIV. However, it's far from perfect, for several reasons:

- **Availability:** Resistance tests are not available everywhere. They are expensive. They are not always available to all patients (such as incarcerated people.) However, they are becoming more common, faster and cheaper, and are reimbursed by most insurance plans.
- **"Minority species":** Some resistance mutations can make up less than about 20% of the total virus population in a person's blood, and the tests aren't good at detecting these minorities. These might be mutations that are starting to emerge against a particular drug. If there aren't enough of the mutants for the test to detect, the test might say that the virus is sensitive to the drug when in fact it won't be within just a few weeks.
- **Sensitivity:** Resistance tests have gotten much more sensitive. But they still work better when the viral load is higher. If your viral load is very low, it might not be possible to get a result from a resistance test. They usually cannot be run if the patient's viral load is less than 500 to 1,000 copies per ml.
- **Interpretation:** Test results can be difficult to understand. Drugs that should work according to the tests sometimes don't work, and vice versa. Sometimes genotypic and phenotypic tests give conflicting results for the same patient because they measure resistance differently.
- **Global validity:** Resistance tests have been developed using "clade B" virus, which is the most common in North America and Europe. However, the strains of HIV that are common in other parts of the world—especially Africa—may not have the same resistance profile. This is important for people in other parts of the world, and for people who have gotten infected by people from other parts of the world.

Despite these problems, many researchers and most treatment guidelines recommend resistance testing as a normal part of HIV treatment. More physicians are now even obtaining resistance testing before choosing someone's first antiviral medications to see if they were infected with a drug-resistant virus.

The silver lining of resistance?

There are a few unusual aspects of viral resistance mutations. Some of them can actually be good to keep around—not that you would want to go out and get them, but if you have them, your doctor might choose ARVs that will keep that mutation active.

For example, some mutations carry a high price for the virus in terms of viral fitness. That is, the virus might be able to continue to multiply in the presence of a particular drug, but it will have a harder time multiplying or infecting new CD4 cells.

This can be a good thing in the big picture of dealing with HIV. In fact, a recent study showed that patients with the M184V mutation (associated with Epivir and Emtriva resistance) who continued to take Epivir or Emtriva to maintain the M184V mutation, had a lower viral load and less of a drop in CD4 cells than those who stopped taking it.

Another example is that patients who have taken nukes for a long time and developed NRTI resistance, but who have never taken NNRTIs, may have a virus that is hypersusceptible to the NNRTIs—that is, the virus is even more sensitive to the ability of NNRTIs to control it.

A deeper look

The other articles in this issue focus on three aspects of HIV resistance. The first, by Dr. Chad Zawitz, describes how resistance testing is used in regular clinical practice. The second, by Dr. Trevor Hawkins, goes into more detail on the limitations and challenges in using resistance testing. The third, by Dr. Andrew Zolopa, discusses making treatment decisions for patients with virus that is already resistant to ARVs. See also "Why HIV Drug Resistance Matters: An Overview" by James Learned in the September/October 2005 issue of *Positively Aware*

Robert Munk, PhD, has been involved in AIDS activism since 1987, the year he tested positive. He was the first Development Director for The NAMES Project in San Francisco before founding the AIDS Service Providers Association of the Bay Area. He moved to New Mexico in 1992. In 1997 he founded and continues to coordinate the AIDS InfoNet, an Internet-based patient education resource providing HIV treatment information in English and Spanish. His main focus is on providing current HIV/AIDS treatment information in non-technical language. He is a frequent writer and speaker on HIV treatment topics. He participates on numerous advisory boards and international scientific conference planning committees, including the New Mexico Medications Advisory Committee and the U.S. FDA Antiviral Drugs Advisory Committee.