



- RESISTANCE 101
- RESISTANCE TESTING IN CLINICAL PRACTICE
  - LIMITATIONS IN TESTING
- CONSIDERATIONS WHEN CHANGING DRUGS



## EDITOR'S NOTE

# Trying to Pin Down a Shape Shifter

**H**IV is a wily foe. It seems that no matter what we throw at it, the virus changes and gets around it. We truly are dealing with a shape shifter.

And now as we're learning more about the new class of co-receptor inhibitors, it seems that resistance will take a whole new turn. Instead of drugs binding to an enzyme and blocking HIV that way, it may be the case that the co-receptor blockers make the receptor change shape. Shape shifter indeed!

I'd like to thank *Positively Aware* for giving me the opportunity to edit this special issue on resistance. I learn a lot when I write, and this case is no exception. Each time I checked, articles kept popping up with a focus on some new wrinkle in resistance testing or its interpretation. For example, a study presented at a conference last fall reported on the successful use of artificial intelligence (computer analysis) to develop treatment recommendations based on genotypic resistance test results.

There's no question that resistance testing is a significant advance in HIV care and has made a very positive difference in treatment. At the same time, as you'll see in the articles in this issue, it's continuing the push towards specialization. Doctors who aren't HIV specialists may still deliver good care, but I honestly don't know how they can keep up with all of the various considerations on resistance testing and how to use it in their practice—along with all the other important developments in HIV treatments and their side effects! As we learn more about resistance, and as the tests become more sophisticated, it becomes more and more difficult to read and interpret them.

The first article in this issue is kind of a "nuts and bolts" introduction to resistance testing. It defines many of the terms that are used in the later articles, including wild type, mutation, genotypic and phenotypic testing, and cross-resistance. Hopefully it will equip you to better understand resistance and the articles that follow.

The second article, by Dr. Chad Zawitz, describes how resistance testing is used in regular clinical practice. It discusses treatment guidelines on the use of resistance testing (which change every few months), some considerations on payment for genotypic and phenotypic resistance testing, and special concerns for incarcerated populations.

The third article, by Dr. Trevor Hawkins, goes into more detail on the limitations and challenges in using resistance testing. What do clinicians need to keep in mind when looking at resistance test results? What about new mutations that can reduce or reverse the effect of existing mutations? Are the interpretations that come with resistance test results reliable? What aspects of patient behavior can overwhelm the impact of viral mutations? Can drug level boosting make a difference?

The final article, by Dr. Andrew Zolopa, discusses making treatment decisions for patients with virus that is already resistant to medication. This is unfortunately becoming more and more common. A recent analysis showed that 13% of patients had virus that already had resistance mutations affecting nukes, NNRTIs, and protease inhibitors. This obviously complicates the selection of the next treatment regimen and highlights the need for resistance testing.

Viral resistance is truly a case for me, as the song says, that "the more I learn, the less I know."

I hope you find this issue informative and helpful.

Sincerely,

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# RESISTANCE OVERVIEW

## A look at the basics

by Bob Munk

**R**etrovir (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 multi-

plies, 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.

#### GENOTYPIC 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 some 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* ☛

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# Using Resistance Testing in Clinical Practice

U.S. guidelines, cost, limitations  
—and usefulness

by Chad Zawitz, MD

**A**s our body of knowledge continues to grow regarding HIV resistance, we have begun to fine-tune the use of both genotypic and phenotypic testing to help determine the most effective combinations of medicines that the virus should respond to in an individual patient.

There have been a number of excellent studies published which support the use of resistance testing to help providers choose the best possible options that are available. The DHHS (Department of Health and Human Services) has embraced this information and has added the use of resistance testing to its HIV guidelines.

Currently, the DHHS recommends that resistance testing be performed or considered in several situations:

- 1) Virologic failure during combination HIV antiretroviral (ARV) therapy.
- 2) When the viral load is not fully suppressed after ARV initiation.
- 3) In acute (initial) HIV infection if ARV treatment is going to be started.
- 4) In chronic (long-term) HIV infection if it is possible the patient was infected by someone with drug resistant HIV.

The reason for testing someone at baseline before they have ever even taken a single pill of HIV medicine is because resistant HIV can be transmitted between sexual partners, from mother to child, or between IV drug users who share needles. If the source person has virus that is resistant to one or more of the HIV medicines (either because they have been on a failing regimen or they themselves were infected with resistant virus), there is a reasonable chance this same resistant virus will be the strain that causes infection in the new “host” or patient.

Since resistance is carried in the genes of the virus, it is not lost during transmission; it is passed along to the new patient. It is useful to know if this has occurred because the single best chance for long-term control of HIV is with the first regimen. If a mutation is detected with the genotype, the medications that are affected by it can be avoided right from the start.

The reason for obtaining resistance testing after treatment failure is because in most cases failure is associated with the development of resistance mutations which then accumulate over time. The longer someone is left on a failing regimen, the more likely it is that the virus will “collect” more and more mutations that allow it to evade more of the HIV medicines—even medications you may not have ever taken.

Genotype tests are most useful to clinicians when there are only a few mutations present. This is because when there are only a few (early) mutations present, their effect on the virus is easier to predict.

Once there are multiple mutations present (such as after failing the second, third, or more regimens), they begin to have complicated and unpredictable effects on the virus and each other.

Sometimes two mutations “cancel each other out”; sometimes their effects are additive (one mutation alone isn’t bad, but two or three or more together lead to resistance).

Occasionally mutations occur that are beneficial (weakening the virus or making the virus more susceptible to a different medicine).

One of the limits of genotype testing is that it requires a detectable viral load. Ideally the test is used when the viral load is greater than 1,000 copies/ml, although results can sometimes be acquired with lower levels.

Some people who experience viral breakthrough (a detectable viral load) develop lower-level viremia with copies only in the hundreds. It is possible that resistance is occurring at this time but the genotype test is not sensitive enough to detect it.

Further, in order for the genotype to detect a mutation, at least 20% of the viral population must contain the change.

The use of phenotype testing is limited by a few factors. First, it is more expensive than genotyping. In resource limited settings (such as public health clinics or with uninsured patients), use of this test may be restricted to contain costs. Second, the results typically take several weeks to return (whereas genotype results often can be reported in about a week). It too requires a viral load of about 1,000 copies/ml and is less sensitive/useful with lower levels.

The advantage of phenotyping over genotyping usually occurs once a patient has failed more than one regimen. As mentioned

before, once multiple genotypic mutations have accumulated, their effects on each other become more complicated and more difficult to predict using algorithms or computer models.

With a phenotype, there is very little guesswork about the interplay of the mutations because the test is simply looking to see whether or not the virus can grow in the presence of the medicine. The test doesn't know or care which or how many mutations are present. In a perfect world, this test would be widely available, very cheap, give rapid results, and be used together with a genotype.

With a newly infected or newly diagnosed patient, among the myriad of baseline tests that are ordered, a genotype should be considered based on the guidelines. In population centers where there is a higher prevalence of resistance (such as large cities) or patients who are infected from a higher risk group (such as IV drug users who share needles), this sort of genotypic testing is recommended.

## CASE STUDY

A 38-year-old detainee at Cook County Jail has been incarcerated since 2001 awaiting trial for a very serious crime. He had participated in voluntary HIV testing at least three times since being arrested, and until 2004 all of those tests were negative. In the fall of 2004, his HIV ELISA test and confirmatory Western Blot came back positive. The patient was referred to me for evaluation and management of his new diagnosis.

When asked, the patient believed his risk factor was making a homemade tattoo in his cell with the help of his cellmate (who was known to be HIV-positive and on medications but not known to have resistant virus). He denied any sexual contact with other detainees.

His baseline CD4+ count was 234 with a viral load of approximately 40,000 copies/ml. Because the jail is a resource limited setting, baseline genotype testing was not performed prior to starting treatment. Following extensive counsel and discussion over the need to consider treatment, the patient agreed to begin medications. He was started on a once-daily regimen of Truvada (combination pill of Emtriva and Viread) plus Sustiva (efavirenz) because he specifically wanted a regimen with the fewest possible pills the fewest number of times per day. The importance of strict adherence to this (and all regimens) was emphasized.

Six weeks following initiation of this regimen, the patient's viral load fell to undetectable (less than 75 copies/ml) and his CD4+ count rose to 458. This sug-

gested that the virus he was infected with was more than likely wild type (naturally susceptible to all medications) or at least lacking mutations that would limit the effectiveness of his first regimen.

Three months later, another CD4+ count and viral load were acquired. This time, the viral load was over 3,000 copies/ml and the CD4+ had decreased to 360. The patient insisted he was religiously adherent to his medications, stating he never missed a dose. Because of the easier threshold for acquiring resistance to Sustiva and the Emtriva component of Truvada if doses are regularly missed, the possibility of transmitted resistance from his cellmate, and the fact that I knew this patient would still be incarcerated by the time the results came back, a genotype test was ordered.

The results returned 24 days later revealing three mutations: K65R, K103N, and M184V.

The K65R mutation suggests probable resistance to the Viread component of Truvada. The M184V mutation conveys high-grade resistance to the Emtriva component of Truvada. The K103N mutation conveys high-grade resistance to Sustiva.

This genotype result easily explained the rebound of this patient's viral load after initially achieving an undetectable level within six weeks of starting his first regimen. More careful history with the patient revealed that in fact he was routinely missing some doses of his medications because he was smoking marijuana.

Due to intoxication, he slept through some of his evening doses.

Again, extensive counsel was provided regarding the critical importance of adherence. A second-line regimen was suggested but the patient refused, stating it contained too many pills. Despite my advice on which regimens offered the best chance for long-term suppression of HIV, the patient only agreed to take Combivir (a combination pill of Retrovir and Epivir) and Reyataz (atazanavir) without a booster dose of Norvir.

Follow-up CD4+ count and viral load testing was acquired six weeks later. Fortunately the viral load again suppressed to undetectable (less than 75 copies/ml) and his CD4+ increased to 402. To date, he has been able to maintain an undetectable viral load and he insists he is no longer missing doses or using illicit drugs.

This patient is an example of the utility of genotype testing within my correctional facility.

- 1) The patient was someone I knew would not be released before the results returned.
- 2) His viral load increased from undetectable to more than 1,000 copies/ml (meaning that there was enough virus for the genotype to detect mutations if they were present).
- 3) He was failing his first regimen, and the test was acquired early during viral rebound and while the patient was still on his medications.—Chad Zawitz, MD

In areas where HIV is much less prevalent or access to medications is limited (rural communities, developing nations, etc.) the use of baseline resistance testing may not be cost effective due to the lower baseline prevalence of intrinsic resistance in the general population. Once the result is available, it is carefully evaluated to determine the initial regimen that will have the highest likelihood of long-term success.

Recommendations for resistance testing during pregnancy are the same as for anyone else. They may have high CD4+ counts or low viral loads that would otherwise negate the need for immediate treatment. However, given the risk of perinatal transmission (the virus infecting the infant) during delivery, most providers will place the mother on antiretrovirals at some point prior to labor to minimize the risk of exposing and infecting the infant. Baseline genotype testing is particularly useful in this setting because the critical component of treatment before delivery is achieving an undetectable viral load. This is most likely to be achieved if the virus is treated with at least three medications to which it is completely susceptible.

For people on a regimen that is failing, resistance test results are most useful if the patient has the blood drawn while they are still actually taking the regimen and to which resistance is suspected to have developed. This is because the most “fit” virus is the wild type (naturally occurring virus). Without the selective pressure of the ARVs ongoing, both the resistant and wild type virus will replicate, but since the wild type is most fit, it will “out-populate” the resistant virus quickly, and the genotype test may be unable to detect the resistant strain.

The same principal applies to phenotypic testing. In practice, some providers will ask their patients to remain on the “failing” regimen while they await the results of the genotype (or phenotype) test. Others believe it is prudent to discontinue the failing regimen as early as possible to limit the chances of additional mutations accumulating while they await the return of the test results.

In clinical practice, I have found resistance testing to be an increasingly valuable tool in long-term management of my patients. Ideally all regimens would be ironclad and last for decades, but the reality is that not only are the medications imperfect, but it is extremely difficult for anyone to maintain perfect adherence to them over the long haul. Resistance is almost inevitable at some point in anyone’s treatment history and genotyping/phenotyping eliminates much of the guesswork and allows for greater precision in selecting the next regimen.

## JAIL SETTING

I work with a special population. My primary care site is the Cook County Jail in Chicago. It is one of the largest single-site correctional facilities in the United States. We house over 11,000 detainees on any given day with an HIV positive rate of 2.6% based on a serosurvey performed in 2001, a little over five times the prevalence rate for the U.S. as a whole. This equates to approximately 300 HIV-positive people behind our bars on any given day.

We are an exception to the traditional management rules used by most outpatient providers for several reasons.

- 1) We fall under the “resource limited” category, meaning that due to cost constraints we do not routinely offer the more expensive phenotype testing (there are rare exceptions).
- 2) As a “resource limited” site, we also do not offer genotypes at baseline for newly infected or newly diagnosed patients

(unless they provide a very solid history of exposure from a known or high-risk resistant source).

- 3) Since we are a jail (not a prison) our population is highly transient. The average length of stay is only nine days, so it is uncommon for either a genotype or phenotype result to be available before the detainee is released or sent to prison. Thus, the decision to use this type of testing is often deferred until the patient presents to their primary clinic as an outpatient.
- 4) Pregnant women are routinely offered genotype testing if we know they will remain in our facility long enough to receive the result.

Aside from being a resource limited setting, the single biggest differentiating factor in determining the practical use of resistance testing in corrections is the disposition of the inmate. In jail settings, detainees are held until their day in court. This means they remain under the care of the corrections healthcare team until they are either released, bonded out, or convicted and sent to prison.

In Cook County Jail, the average length of stay is only nine days, and more than 75% of those incarcerated are gone within 30 days. For this reason, there are only occasional instances where either type of resistance testing is practical because the detainee will more often than not be gone before the results ever return from the lab.

In prisons, the inmates have definitive sentences with known release dates. Knowing the patient will remain at one location long enough to observe trends in viral load testing, testing for resistance, waiting for the results, and then intervening is far more practical. The impact of proper use of genotype and phenotype testing is far more substantial in a prison population.

I also work at a “Continuity of Care” clinic at the CORE Center in Chicago. At this site, I am able to practice more traditional HIV medicine since the patients who attend this clinic will return to me for multiple visits just like any other primary HIV treatment setting. Although the CORE Center is still within the boundaries of a “resource limited” setting, the rules for ordering genotype testing are somewhat more relaxed compared to the jail.

Routine baseline genotypes are generally not performed excepting for the above mentioned special circumstances. All first-line treatment failures are genotyped as early as possible if the viral load levels rise above 1,000 copies/ml. Due to cost restraints, we do not offer phenotyping for advanced level treatment failures. Instead, genotyping is performed and the often complicated results are interpreted by a select committee of resistance experts within our facility who then suggest treatment options. ☒

*Chad Zawitz, MD completed his Infectious Diseases Fellowship at Rush University Medical Center, Chicago in June 2004, and started as attending physician at Cermak Health Services (Cook County Jail) in July 2004. He has been indirectly involved in HIV care since 1995, and began caring directly for HIV-positive patients as a senior internal medicine resident at University of Pittsburgh Medical Center in 2001-2002. At Rush during his fellowship, like all Infectious Diseases fellows, he had his own HIV clinic patients at the CORE Center (a joint venture outpatient HIV hospital associated with Rush and Cook County Hospital) for the past two years prior to starting full-time at the jail.*

# The Limitations of Drug Resistance Testing

## Many factors influence the results

by Trevor Hawkins, MD

In someone not taking antiviral therapy, HIV replicates at a rate of  $10^{10}$ – $10^{11}$  copies—or 10 to 100 billion—per day. As the virus seeks to construct a DNA version of itself from its RNA origins, prior to combining with the DNA of the host human cell, it makes every mistake possible every single day in the arrangement of its nucleotide bases. This is because the HIV enzyme reverse transcriptase that makes this change from RNA to DNA has no internal proofreader to make sure these mistakes don't happen.

In the presence of an only partially suppressive drug regimen, the mutations that allow the virus to replicate in the presence of the drug offers HIV an obvious benefit. The virus that contains these mutations, “resistant virus,” becomes the predominant species. This resistant virus may be passed on to another person and the number of people being infected with already resistant virus is rising in some areas.

In order to characterize this resistance, as discussed in earlier articles, two types of tests have been developed, called a genotype and a phenotype. They may be done separately or together, an example of the latter being the Phenosense GT from Monogram Biosciences.

The genotype looks at a population of viruses from the patient and determines

the genetic sequence in regions of the pol gene of the virus that codes for reverse transcriptase and protease enzymes, as well as those from its env gene that codes for proteins critical to the entry of the virus into the cell.

Specific mutations in these areas are known to confer resistance to certain drugs, and algorithms are developed to predict resistance or susceptibility. These algorithms are only as good as our knowledge of what those mutations are, something that is being constantly updated. Sometimes it takes months or even years for a new drug's resistance mutations to be clearly identified.

The phenotype measures the susceptibility of recombinant virus (HIV is one type of recombinant virus) from the patient directly to different drugs in cell culture. This is a direct test of resistance and can be performed even for drugs still in development. For both these tests, the expanding number of antiretroviral drugs, now 20, means that the tests get more complex as time passes.

Both of these methods have been shown to be useful in constructing drug regimens to treat resistant virus and have greatly increased our understanding of HIV and its natural evolution. However, there

are important limitations to both of these tests.

### 1. Viral load

Both viral load tests need a viral load (HIV-RNA) of at least 500 copies/ml of virus and preferably more than 1,000 copies/ml. This can create a problem when someone has a persistent low viral load of greater than 200 copies/ml but less than 1,000 copies/ml. Should the regimen be changed or intensified? Is it poor adherence? If we do nothing, will the virus develop more mutations, more resistance, and make it more difficult to construct a new regimen?

### 2. Minority species

The tests always miss virus that is present at less than 10-20% of the total viral load. They may even miss minority species that comprise up to 30% of the viral population. This means that mutations may be hidden and not be found, so-called archived mutations. These mutations may appear rapidly when a drug they offer resistance to is added to a new regimen. This is why a careful history of which drugs the patient has taken in the past is so important, especially in failing regimens that have resulted in viral rebound (an increase in viral load).

Methods are available to test for these minority species, such as single genome sequencing, where instead of populations of viruses being tested, single viruses are sequenced. The more viruses that are looked at, the more sensitive the test, even down to as little as 1% of the species. These tests are very expensive and time consuming and are reserved for research labs.

### 3. Mixtures of viral species

When a patient's virus is in the process of developing resistance, there may be a time when the viral population consists of wild type virus, the original infection (which could be wild type or a resistant strain), and virus with drug resistance mutations. Eventually, the resistant species become dominant, but a test done in the transition stage may show that the total population is "sensitive" on the phenotype, while the genotype shows the presence of both wild type and resistant virus. This can also occur in reverse when a patient stops taking drugs because of virologic rebound. While off medication, over time the predominant strain of HIV becomes the better growing wild type and there may be mixtures of both species if the tests are done at this stage. In this situation, the genotype is the best test to determine which drugs to use.

### 4. Re-sensitizing mutations

Combinations of mutations in a viral species may behave differently than they do when they occur separately. For example, the K65R mutation causes the virus to be resistant to Viread (tenofovir). However, if the

**Sometimes it takes months or even years for a new drug's resistance mutations to be clearly identified.**

M184V mutation is also present, the combination of the two mutations means that the virus is usually still sensitive to Viread. The two mutations together also make the virus hypersensitive (more sensitive) to Retrovir (zidovudine or AZT). A genotype would show both of the mutations noted above but only an experienced clinician would know how to interpret the combination. On the other hand, a phenotype would show the effect of the combination of mutations.

Other examples of hypersusceptibility include the Y181C and the L100I mutations that cause resistance to the NNRTI class of drugs. They give HIV increased susceptibility to AZT. HIV variants with multiple NRTI mutations such as K65R appear to be more susceptible to NNRTIs than wild type virus. Some studies have suggested that this hypersusceptibility improves treatment response to NNRTIs. In the protease inhibitor

(PI) class, the N88S mutation generated by pressure from Viracept and others causes the virus to be more susceptible to Lexiva (fosamprenavir), while the I50L mutation seen in Reyataz failures causes hypersusceptibility to most of the PIs. In these cases, the phenotype is the best test as it reflects these interactions.

### 5. Incomplete algorithms

The genotype predicts resistance or susceptibility based on a set of rules about which mutations cause resistance to which drugs. It does not

reflect interactions like those described above, nor does it reflect mutations that might occur in other parts of the viral genome like the gag gene. It is also always playing catch up as we identify new mutations for old and new drugs that we did not know about before.

We can rely on current algorithms to accurately predict drug susceptibility for single mutations like M184V, a mutation commonly seen in failing regimens containing Epivir (3TC) or Emtriva (emtricitabine, FTC), and the D30N seen with Viracept (nel-finavir) failures.

However, where there are complex patterns of mutations such as those seen with boosted protease inhibitors or most NRTIs, and because there is such a huge variety of HIV species, these predictions become a lot more difficult.

There have been attempts to correlate genotypic resistance patterns to outcomes by compiling so-called mutation scores. A listing of important mutations affecting a specific drug is developed and the mutations in a given viral sample are added up to

calculate the score. Cutoffs are suggested, such as a viral sample with a mutation score of less than four should be sensitive. These scores are specific for each drug and are useful, especially for new drugs, where our understanding of the mutational pattern is still evolving. However, they are only a general guide, and often we need a phenotype as well for clarification.

Also, even when the mutation commonly predicts resistance, the phenotype sometimes shows that the virus is still sensitive. An example is the virus with only the L90M mutation, which the genotype would predict as being resistant to Invirase (saquinavir). In fact, up to 30% of phenotypes show that this virus is still sensitive to Invirase.

#### 6. Phenotypic cutoffs

Fold change refers to the amount of drug needed to suppress any given virus. This is usually expressed as  $IC_{50}$  (the inhibitory concentration or drug level needed to inhibit 50% of virus replication). The  $IC_{50}$  fold change for wild type virus is defined as 1, so a resistant virus might require 2, 3 or 100 times as much drug—2, 3 or 100-fold resistance—compared to wild type.

By correlating baseline phenotype at entry into clinical trials with virologic outcomes at defined points, we are able to derive “clinical cutoffs,” which provide the greatest clinical relevance for phenotype assay interpretation. The lower cutoff or reduced susceptibility cutoff is where the response to that particular drug begins to decline, and the upper cutoff is where the response is considered to be negligible. Below the cutoff the drug is usually most effective. In other words the virus can be sensitive to the drug (fold change below the cutoff), partially resistant (fold

#### CASE STUDY

A physician is treating a 37-year-old female. She tested HIV-positive in 1990. In 1998, she was prescribed Crixivan (indinavir) plus Combivir (Retrovir plus Epivir). In 2000, her regimen was changed to Sustiva (efavirenz), Zerit (d4T), and Hivid (ddC). The physician saw her in March of 2003, when she presented with a CD4 count of 185 and a viral load of 33,610. He sent off a sample for combined genotypic and phenotypic testing.

The test showed no protease resistance mutations, but several NNRTI mutations, and the nuke mutations M41L, K65R and M184V. Interestingly, the test interpretation said that her virus was sensitive to Viread (tenofovir) according to the phenotype, but resistant according to the genotype. The doctor started her on Truvada (Viread plus Emtriva), Retrovir, and Lexiva (fosamprenavir) boosted with Norvir. After 3 months, her viral load was undetectable (less than 50 copies) and her CD4 count had risen to 312.

So—why did the Truvada work when the genotypic test showed resistance? In this case, the patient had what’s called a “re-sensitizing” mutation. The M184V mutation overcomes the negative effects of the K65R mutation that normally gives HIV resistance to Viread. Also, the K65R mutation combined with the M184V makes the virus hypersusceptible to AZT. In other words, the AZT works better against this virus than against the wild type.

This example highlights the importance of working with an experienced health care provider who understands some of the complex interactions among resistance mutations and can go beyond the resistance report results to choose an effective regimen. —Trevor N. Hawkins, MD

change between the lower and upper cutoff), or resistant (above the upper cutoff).

Clinical reduced susceptibility cutoffs have been defined for many drugs: 4.5-fold for Ziagen, 1.7-fold for Videx and Zerit, 10-fold for Kaletra and 1.4-fold for Viread. Attempts are being made to find clinical cutoffs for all drugs.

Where they have not been found or calculated, the cutoffs are based on laboratory testing averages rather than actual clinical outcome data. These biologic cutoffs, which are based on the natural variability of wild type viruses from patients, are the next best cutoff, while reproducibility cutoffs, which are based on assay variability with repeated testing of patient samples, are the least

sensitive. Clearly, it can be confusing to know which cutoff you’re dealing with and how helpful it might be in selecting an antiretroviral regimen.

Another important point to remember is that boosting the level of protease inhibitors, either by adding a small dose of Norvir to inhibit CYP3A, the liver enzyme that breaks down protease inhibitors, or by taking the drug with food in the case of Viracept and Aptivus (tipranavir), will increase the drug level and hopefully push the level above the  $IC_{50}$  of that virus. The clinical cutoffs do reflect boosting while the other cutoffs do not.

Also, the two main companies in the field, Monogram Biosciences and Virco Lab, each calculate their cutoffs differently and thus they are different. This can be confusing. Clini-

cians need to be sure that they're referring to the clinical cutoff produced by the company whose assay they're using, and not trying to take one company's cutoff and use it with another company's assay.

#### 7. Adherence

A regimen that fails because of very poor adherence (taking less than 50% of the prescribed doses) will often not show any mutations on the genotype or resistance on the phenotype. This is because there is not enough drug pressure on the virus for it to develop mutations. It is people who take their medication "fairly" well (adherence between 60-95%) who become resistant. Bottom line: adherence should be all or nothing to have the greatest chance of avoiding the development of drug resistance.

#### 8. Cost

Both tests are expensive, with ranges for the genotype from \$200-\$400 and \$800-\$1,000 for the phenotype/phenotype GT. This adds to the already mounting cost of providing care and medication to people living with HIV. However, the ability of the tests to help us avoid the use of expensive drugs that are not active against a particular patient's virus usually makes them cost effective in the long run.

#### 9. Time

Unless these tests are done right in the clinic's lab, it can take 1-3 weeks before the results are available. This delay may be significant if a patient is very sick.

These tests have proved themselves very useful to guide treatment decisions in

**Even when the mutation commonly predicts resistance, the phenotype sometimes shows that the virus is still sensitive.**

both treatment-naïve patients (those starting HIV medication for the first time) who may have been infected with resistant virus, and especially in patients whose regimens are failing. When a genotype done on a patient gives a different interpretation than the phenotype done at the same time, that is called "discordance." Discordance between the two types of tests is common but may improve as we update the genotypic algorithms to include new mutations and the potential for interaction between mutations.

#### 10. Is it worth it?

As noted above, there are some factors that can overwhelm resistance mutations and make resistance testing irrelevant. First is adherence. If a patient isn't taking enough of their pills, or taking them as prescribed, the regimen might fail even without resistance mutations being present. Doing resistance tests on this

patient's virus would probably be a waste of time and money. The most important thing is making sure that the patient understands the importance of taking the medications as prescribed, and working to find a regimen that is effective against their virus and fits their lifestyle.

A second factor is Norvir boosting of protease inhibitor levels. In some cases, the addition of Norvir to a protease inhibitor-containing regimen can result in blood levels that are far above the cutoffs, or far above the amount needed to suppress virus—even if it has some resistance mutations. Norvir boosting, in some cases, overcomes viral resistance.

#### SUMMARY

In deciding what regimen to choose, multiple individual factors have to be taken into account, including drug history, patient preference and the patient's medical history, side effects, adherence and dosing schedule. Resistance testing has taken a firm role in this decision making process, but the limitations described above need always to be accounted for. ☒

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# Resistance Testing in Experienced Patients



## Considerations when changing drugs

by Andrew Zolopa, MD

**D**rug resistance is a frequent challenge faced by both HIV-infected patients and their physicians. In a recent cohort study of over 1,000 patients followed in Vancouver, British Columbia, more than a quarter of the patients developed some degree of drug resistance over the first 30 months of their initial HAART (highly active antiretroviral therapy) regimens.

Furthermore, we are learning that newly infected patients who have never been on antiretroviral therapy are being infected with resistant forms of the HIV-1, which can result in suboptimal responses to their initial treatment regimens. According to recent estimates from a study sponsored by the Centers for Disease Control (CDC) across the U.S., about 15% of newly-diagnosed patients have drug resistance.

Antiretroviral (ARV) resistance testing is considered standard of care and is widely employed in the management of HIV-infected individuals. Current guidelines from expert panels recommend resistance testing in the setting of treatment failure and more recently have recommended that newly-infected individuals, those who have been infected for less than two years, have resistance testing prior to initiating antiretroviral therapy because of the increased prevalence and transmission of drug resistance.

Despite the widespread use of resistance testing in clinical practice, there remain a number of challenges to the clinician in applying these technologies to optimally manage the treatment of HIV infection, especially for the treatment-experienced patient.

When both genotypic and phenotypic tests are used in order to optimize treatment decisions, many times the results appear to be in conflict with one another—where the genotype is interpreted to show resistance while the phenotype does not show resistance, or vice versa.

Moreover, mutational interactions can lead to phenotypic “hypersusceptibility” which appears to have clinical relevance.

Again, the resistance picture becomes more complex as patients stay on therapy longer and their virus accumulates an increasing number of resistance mutations.

And finally, measurements of viral replication capacity (or fitness) are now available to the clinician. This is a measure of how capable the virus is of reproducing. Some resistance mutations may allow the virus to multiply in the presence of drug, but the virus pays a price: it multiplies at a lower rate than the wild type virus. The role this *in vitro* (laboratory) measure should play in the management of patients remains to be fully defined.

### CASE STUDY — PART I

JB is a 45-year-old interior designer who has been HIV-positive since 1989. He has been on a number of antiretroviral treatments over these years, starting with Retrovir (AZT) monotherapy and then Videx (ddI) monotherapy. His first PI was hard-gel saquinavir (Invirase) with Retrovir and Eпивir (3TC). He was also treated with Sustiva (efavirenz), Zerit (d4T) and Videx (ddI) in the past.

For the past several years he has been maintained on Retrovir/Ziagen/Epивir and Kaletra (lopinavir with a ritonavir boost), but he is complaining of progressive lipoatrophy (loss of fat in the arms, legs, and sometimes in the face) and ongoing gastrointestinal (GI) distress. JB's viral load has never been completely suppressed, and generally runs between 300 copies to 5,000 copies. His CD4 counts remain in the mid-300's after reaching a low of 50 in 1994.

JB's doctor orders both a genotype and a phenotype as she considers changing the patient to a new regimen. The genotype shows a large number of resistance mutations to the nucleosides (NRTIs or nukes) and a mixture of K103N/K, related to resistance to the NNRTIs. In addition there are also many PI-related resistance mutations.

The phenotype, however, shows that JB's virus is still sensitive to Zerit, Videx, and Viread. It also shows sensitivity to the NNRTIs. The PIs show high-level resistance which is in agreement with the genotype.

JB's doctor is now wondering if she can use Sustiva and Viread as part of a new regimen. She is confused by the results in which the genotype interpretation suggests that both Viread and Sustiva

would not be active while the phenotype report suggests these two drugs would be active.

### THE EVOLUTION OF DRUG RESISTANCE ON A FAILING REGIMEN

For some time now it has been clear that patients who have initial virologic breakthrough (increased viral load) on an antiretroviral regimen do not necessarily have resistance to all of the drugs in that failing regimen. At first this did not make sense to many clinicians, who asked, "How can a regimen be failing, if not all the drugs in that regimen are failing?" The likely answer is that only one or two active drugs are often not sufficiently potent to maintain high levels of viral suppression and therefore the viral load comes back.

Drugs in the regimen that have a relatively "low genetic barrier to resistance" (where only a single mutation results in loss of antiviral activity), such as the nucleoside reverse transcriptase inhibitors (NRTIs) Epивir and Emtriva or the non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as Sustiva and Viramune, will select for resistance rapidly in the early phase of virologic breakthrough.

Other drugs like Retrovir and the protease inhibitors (PIs) will have relatively slower evolution of drug resistance because of the requirement of accumulation of multiple mutations to confer high-level resistance. This means that clinicians may be able to recycle certain elements of a failing regimen if resistance testing is performed early enough in the course of virologic failure.

Boosting the protease inhibitor concentration using small doses of another PI such as Norvir appears to further protect the protease inhibitor component of the regimen from the early development of resistance in failing regimens. This was first demonstrated in a clinical trial where lopinavir boosted with Norvir (Kaletra) was compared to unboosted Viracept. The investigators showed that in the Viracept arm there was more PI resistance when virologic failure first showed up compared to the Kaletra arm.

This protective effect seemed to extend to the other drugs in the treatment regimen—such that Epивir resistance was detectable

**Clinicians may be able to recycle certain elements of a failing regimen if resistance testing is performed early enough in the course of virologic failure.**

in 29% of the Viracept arm compared to only 7% in the Kaletra arm after 96 weeks of follow up. This boosting effect is not unique to Kaletra—it has been demonstrated for boosted Lexiva (fos-amprenavir) and more recently reported for boosted Invirase.

#### **INTERPRETING GENOTYPES: THE ROLE OF THE “EXPERT”**

The first randomized controlled trial of resistance testing in the setting of treatment failure, the GART study, showed improved short-term control of HIV when treatment was guided by resistance testing compared to no resistance testing.

Since that time there has been controversy about the relative role of the resistance testing versus the expert advice that usually accompanies the resistance test results in improving outcomes. Patients who had resistance testing in the GART study also had the benefit of a resistance expert’s opinion that accompanied the test result. So what was responsible for the improved outcomes, the expert’s opinion, the resistance test, or the combination of the two?

The relative role of additional expert advice compared to practitioner-only genotype testing interpretation was clearly demonstrated in the HAVANA clinical trial. The investigators randomized patients on failing antiretroviral regimens to either receive genotype testing or not and either with or without expert advice. The group that had both genotyping results and expert advice had the best outcomes—69% of this group achieved a viral load of less than 400 copies/mL at 24 weeks. However, the group that had expert advice alone had outcomes that were comparable to the group that had genotyping alone, 49% compared to 46% achieving less than 400 copies/mL, respectively.

So given the complexity of interpreting resistance test results, it appears that having an expert help guide the treatment decisions improves treatment outcomes. However, one wonders how good the experts are at interpreting genotypes and how much agreement there is in the interpretation of genotypes among experts worldwide. We answered these questions in the GUESS study, in which we asked a panel of 12 international resistance experts to interpret 50 complex genotypes.

The experts had various levels of accuracy in predicting the phenotypic fold change based on the genotypic results for the 16 antiretrovirals commonly in use. For most drugs, the experts’ accuracy was roughly 25–40%. The exceptions were the NNRTIs and Efavir, where levels of accuracy reached 75%. Levels of agreement between the experts were also around 40% for most of the drugs.

Despite these relatively low levels of agreement, the expert panel agreed on the treatment recommendations about 80% of the time. So one can conclude that experts are not terribly accurate in translating genotypes into phenotypes or expected drug activity levels, but there is broad agreement in making treatment recom-

#### **RESISTANCE SCORES**

Stanford University: On the Stanford University web site at <http://hivdb.stanford.edu/> is a database, HIVdb. This is an expert system where users provide genotypic sequences and are provided with levels of resistance to approved anti-HIV drugs. For each drug, each resistance mutation is assigned a drug penalty score. The total score for a drug is the total of the scores of each mutation present that is associated with resistance to that drug. Using the total drug score, the program reports one of the following levels of inferred drug resistance: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance.

Aptivus: The recently approved protease inhibitor (PI) Aptivus (tipranavir) was developed specifically to deal with HIV that already has protease resistance mutations. But which ones and how many? How can a clinician know whether it’s worth trying Aptivus? The resistance score that the drug’s developer, Boehringer Ingelheim, has developed demonstrates how complex these analyses are getting to be.

Researchers analyzed the results of Phase II and Phase III trials and identified 21 mutations at 16 different codons that reduce susceptibility of HIV to Aptivus or reduce HIV’s response to Aptivus. About half of these mutations are unique for Aptivus, such that HIV often remains sensitive to Aptivus even when many PI mutations that came about from older PIs are present. The total number of these mutations generates the resistance score to Aptivus. Boehringer Ingelheim has determined the score at which Aptivus starts to lose its effectiveness, and a higher score where it seems to have no effect.

But will these complicated resistance scores ever be calculated and used in general practice? Will they be reflected in commercial genotypic tests? Or will the testing companies and HIV clinicians rely on simpler ways to predict whether or not to use a particular drug? There are two simpler approaches for Aptivus that will probably be used more frequently. The first is to count the number of “key” protease mutations present in a patient’s virus. The key mutations seen to affect Aptivus are at codons 33, 82, 84, and 90. A virus with two or fewer is sensitive to Aptivus; one with three of these mutations has decreased sensitivity; and one with all four is resistant. The other simple method is to obtain a phenotype. A baseline phenotypic test that shows a fold change below 3-fold indicates that HIV is still sensitive to Aptivus and is associated with a good treatment response defined as a 1 log (10-fold) or greater drop in viral load after six months of treatment. A fold change between 3 and 10 results in decreased sensitivity to Aptivus and somewhat less of a benefit. Finally, a fold change above 10 indicates resistance to Aptivus.

mendations based on the genotype—which in the end is what the average clinician seeks from a genotype interpretation algorithm.

The bottom line is which drugs should be used in the setting of resistance and on that the experts seem to agree pretty well.

### INTERPRETING THE PHENOTYPE

As mentioned earlier (see “Phenotypic test” on page 7), the interpretation of the phenotype comes in defining the “cutoffs” for drugs. The ARV drug concentration required to inhibit a patient’s virus strain compared to a reference wild type virus strain without ARV drug resistance is normally expressed as a fold change in IC<sub>50</sub> (the drug level or inhibitory concentration that inhibits 50% of virus growth), where the virus becomes less susceptible to a given drug.

There are two potentially important cutoffs for each drug. The lower cutoff defines when the susceptibility begins to decline but the drug still has partial activity. The upper cutoff would be the fold change where all drug activity is lost.

These so called “clinical cutoffs” have not yet been defined for most of the antiretrovirals in use today. Therefore the clinician depends on what are called “biological cutoffs.” These are based on the variation of fold changes seen in viral populations without drug

resistance. If a patient’s sample is outside that “normal” distribution, then it is considered to have reduced susceptibility.

The number of drugs with clinical cutoffs is few because of the challenges inherent in defining these cutoffs. For most drugs, resistance and response is on a continuum and therefore any cutoff is by its very nature somewhat arbitrarily drawn.

Secondly, most drugs are used in combination with other drugs, so teasing out the impact of a particular drug is difficult. Nonetheless progress is being made here as well. The PhenoSense assay by Monogram Biosciences and the Virco Antivirogram assays now report clinical cutoffs for several antiretrovirals. Having clinical cutoffs should improve the clinical utility of phenotype testing—particularly in experienced patients with complex genotype patterns.

### PHENOTYPE/ GENOTYPE DISCORDANCE

Since both phenotype and genotype are imperfect tests, many clinicians—like the one in our case example—order both tests to try to bring all information to bear when making the critical decision about a patient’s new antiretroviral regimen. Although there are no clinical trials that support the use of both genotyping and phenotyping together to improve outcome, this approach seems to

COMPARISON OF GENOTYPING AND PHENOTYPING		
METHOD	ADVANTAGES	DISADVANTAGES
GENOTYPIC TESTING	Rapid results (1-2 weeks)	Indirect measure of resistance
	Less expensive than phenotyping	Some mutations have questionable impact on resistance
	Mutations may show up before phenotypic resistance	Minority viral species are not detected
	Widely available	Mutational patterns can be difficult to interpret, especially for treatment-experienced patients
	Better than phenotype at detecting mixtures of resistant and wild type virus	Genotypic interpretation may not be relevant for non-B subtypes of HIV
PHENOTYPIC TESTING	A direct and quantitative measure of resistance	Susceptibility cutoffs vary for each testing company
	Can be applied to any antiretroviral, including new drugs for which resistance mutations haven’t been identified	Clinical cutoffs have not been defined for some agents; the level of fold change that matters may not be clear
	Interactions among mutations are reflected in test results	Minority viral species are not detected
	Accurate with HIV subtypes other than subtype B	Long turnaround (3-4 weeks)
		More expensive than genotyping

have clinical merit, particularly in the setting of highly treatment experienced patients with multi-drug resistant HIV-1 infection.

The problem with using both tests is discordant results—that is, the genotype appears to say one thing and the phenotype the opposite, as our case study illustrates. So the risk of more information is more confusion rather than clarity.

Discordance occurs commonly when both tests are ordered, where the results can be discordant as much as 25% -30% of the time. The causes of discordance can include mixtures of wild type and mutant virus; virus that has “back-mutated” to an intermediate form that doesn’t affect the phenotype but can quickly revert to a drug-resistant version if the drug is restarted; and interactions between mutations that can appear to cancel each other out in terms of the phenotype.

#### **HYPERSUSCEPTIBILITY**

Hypersusceptibility can be seen as the opposite of resistance or reduced susceptibility. Here the patient’s virus requires less concentration of drug to inhibit growth compared to the control wild type virus. This laboratory phenomenon has been shown to have clinical relevance, most clearly for the NNRTI class. There have been several cohort studies and clinical trials that demonstrate that patients with HIV that is hypersusceptible to the NNRTIs have a better short-term virologic response.

It has been shown that NNRTI hypersusceptibility is related to the presence of NRTI-associated resistance mutations, but the relationship is a complex one and difficult to predict from a genotype. Therefore, although it is not clear how a clinician could incorporate hypersusceptibility into a treatment plan, the phenotype has the advantage as a direct measure of this phenomenon compared to a genotype.

#### **REPLICATION CAPACITY AND VIRAL FITNESS**

Beyond phenotype and genotype tests, clinicians are now also faced with the question of how to incorporate measures of viral fitness into their treatment strategies for HIV-infected patients. This is particularly true for treatment-experienced patients in whom a

fully suppressible regimen is not possible. These patients may consistently have a low viral load with stable, but low, CD4 counts.

Viral fitness refers to the ability of the virus to multiply in a patient. It includes the effect of antiretrovirals, immune suppression and other factors. Although we do not have a true viral fitness measurement, replication capacity (RC)—a laboratory measurement—has been shown to correlate with measures of fitness in patients.

One study showed that resistant virus had a lower replication capacity than the wild type virus, which could explain why it was “overgrown” fairly quickly by the wild type virus. Replicative capacity may develop as a tool for predicting patient outcomes that is independent of viral load testing and resistance testing.

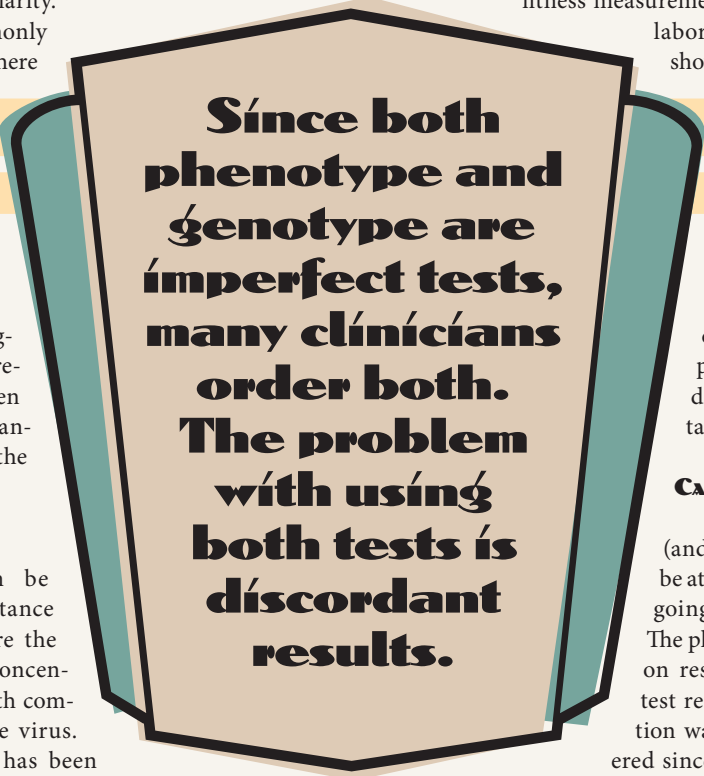
#### **CASE STUDY — PART II**

JB, the patient in our case study (and his physician) are fortunate to be at a hospital where there is research going on regarding viral resistance. The physician was able to get an expert on resistance to review the patient’s test results. The expert’s recommendation was that Viread should be considered since the patient had never taken it.

However, he didn’t trust the phenotypic result showing sensitivity to Sustiva—JB had taken it previously—and recommended against using it.

Another piece of good luck for JB is that one of the research studies being done at the hospital is a trial of a new NNRTI drug that is supposed to be active against HIV that already has NNRTI resistance. His doctor talked to the study coordinator and got JB enrolled in the study. However, she still has to consider how many “active” drugs the patient will have. Because of accumulated resistance, even a “new” drug may not be active against the virus. So with Viread and the experimental NNRTI, she still wants another active drug.

Videx and Zerit both showed up as possibilities on the phenotype, but JB has taken both of them before. Resistance might be archived and might come back out quickly. Also, Zerit has been linked to lipoatrophy, which JB is complaining about. The doctor discusses this situation with JB and he agrees to try Fuzeon (enfuvirtide) even though it requires injections twice a day. Since it



**Since both phenotype and genotype are imperfect tests, many clinicians order both. The problem with using both tests is discordant results.**

## ONLINE RESISTANCE RESOURCES

**Antiviral Mutations** at <http://www.mediscover.net/antiviralintro.cfm>

Mediscover Infectious Diseases is provided as a resource center for researchers and clinicians that specializes in the study and management of infectious disease.

**HIV Resistance Response Database** at <http://www.hivrdb.org/>

The aim of the HIV Resistance Response Database Initiative is to improve the clinical management of HIV infection by developing and making freely accessible a large clinical database and bioinformatic techniques that define with increased precision and reliability the relationships between HIV resistance and virologic response to treatment.

**HIV Resistance Web** at <http://www.hivresistanceweb.com/>

HIVresistanceWeb is an independent, educational resource dedicated to the advancement of anti-HIV therapy through information sharing and expert discussion of current issues in antiretroviral drug resistance and clinical HIV virology.

**HIV Sequence Database** at <http://hiv-web.lanl.gov/content/hiv-db/mainpage.html>

(From Los Alamos National Laboratories.) The HIV Sequence Database focuses on five primary goals:

- Collecting HIV and SIV sequence data (since 1987)
- Curating and annotating this data, and making it available to the scientific community
- Computer analysis of HIV and related sequences
- Production of software for the analysis of (sequence) data
- Publication of the data and analyses on this site and in a yearly printed publication, the HIV Sequence Compendium, which is available free of charge.

**Stanford University HIV Drug Resistance Database** at <http://hivdb.stanford.edu/>

See the site home page for information on the resistance tools available here.

## COMPANIES OFFERING RESISTANCE TEST PRODUCTS

**Monogram Biosciences** at <http://www.monogrambio.com/200HIVProducts.aspx>

Genotypic testing, phenotypic testing, replication capacity

**Virco Lab** at <http://www.vircolab.com>

Genotypic and phenotypic testing, virtual phenotyping

attacks a totally different part of HIV's life cycle than any drug JB has taken, it will almost certainly be an active drug for him.

As part of their discussion, JB's doctor told him that in several recent clinical trials of new drugs, the patients who added Fuzeon along with an experimental drug did better than others in the trial who did not use Fuzeon. This approach will also let JB get off of Kaletra, which might be contributing to his stomach problems—and, since it is still working and there is no sign of resistance to it, JB will still have the possibility of going back on it if the experimental drug doesn't pan out.

The results were very good for JB. He tolerated the twice-daily injections of Fuzeon, and the experimental drug worked well. He was very careful about not missing any doses of his new regimen, and within three months, his viral load was undetectable (less than 50 copies/ml) for the first time in years. His CD4 count had not gone up too much, now about 400, but JB and his doctor were hopeful that it will continue to climb.

## SUMMARY AND CONCLUSIONS

The management of HIV infection is getting increasingly complex and specialized as more drugs become available, patients are treated for longer periods of time, and more complex tests become available to manage patients and ARVs. Resistance tests are now standard of care and a clinician must have a solid understanding of the tests, including their limitations, to be considered qualified to manage HIV-positive individuals. Although the technology behind resistance testing is reliable and fairly standardized at this point,

the interpretation of resistance testing is still evolving. There are many online resources available for clinicians to consult and that patients should know about as well. The Stanford University database is one such resource that provides comprehensive and up to date information about drug resistance.

Test results need to be interpreted with a good understanding of their limitations, and how to assess conflicts between genotypes and phenotypes. Incorporating new tests like replicative capacity to optimize management will continue to challenge even the most expert clinician. In the end, all tests need to be interpreted in the clinical context of the patient, which includes assessment of the treatment history, viral load and CD4 profile, adherence patterns, and toxicities and other adverse events. It is only in this broader context that optimal use of resistance tests can be achieved. ☒

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