# Mechanisms of HIV Drug Resistance: A Primer

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Resistance of HIV to antiretroviral drugs is one of the most common causes for therapeutic failure in people infected with HIV. Sadly, the emergence of drug-resistant HIV variants is a common occurrence—even under the best of circumstances—given that no antiretroviral drug combination studied as of yet is completely effective in shutting down viral replication. And there is no shortage of data indicating that the emergence of HIV drug resistance is clearly associated with adverse treatment outcomes.

Fortunately, the availability of drug-resistance testing has improved the ability of clinicians to deal knowledgeably with HIV drug resistance head on. On the research front, drug-resistance testing has enabled investigators to more effectively develop and study both novel and older therapeutics for the sake of tailoring treatment for patients with varying resistance profiles. In this respect, therapy can now be individualized, based on our evolving knowledge of drug resistance, drug-resistance testing, and state-of-the-art treatment approaches.

But if clinicians are to fully appreciate the epidemiology, prognostic tests, and various treatment choices related to HIV drug resistance, it is important to understand the mechanisms by which HIV drug resistance evolves. To explain this, and to illustrate some of the recent advances elucidating the mechanisms of HIV drug resistance, Dr. François Clavel took the podium at PRN's annual holiday dinner in December to provide a basic and not-so-basic overview.

## Selection of Drug-Resistant Mutants

To understand how mutations associated with drug resistance arise, it is necessary to start at the beginning. At the time of infection, a single virus may be introduced and goes on to replicate with the help of a single CD4+ cell. A number of new virions are produced, each of which goes on to use other CD4+ cells to replicate further. During this process, even in its earliest stages, different strains of the virus are produced. These strains differ from one another by random mutations in their genetic structures. Some of these mutations are minor and are called base substitutions or amino acid substitutions. Other mutations are more significant, as they involve combinations of amino acid substitutions, deletions, or insertions.

"Some of these mutations are good for the virus, as they can help the virus to escape the pressure of the immune system, providing it with a survival advantage," Dr. Clavel explained. "Other mutations are harmful to the virus. In fact, most mutations are harmful to HIV, as they can cause the virus to create stops or changes in proteins that are essential for replication. In turn, these species of virus quickly disappear and are overgrown by strains that have better replicative capacity. But overall, as time progresses, there is constant diversification of HIV."

With the start of a single antiretroviral agent, it is likely that treatment will be initially effective in reducing the dominant, usually "wild type," strain of HIV. However, among the diverse population of virus, there will likely be at least one strain harboring a particular mutation that confers a small survival advantage in the presence of the particular antiretroviral drug. If this variant strain is permitted to continue replicating, it will continue to diversify, with some progeny virus accumulating additional mutations that may confer greater resistance to the antiretroviral agent being used. Eventually, a variant will likely emerge that harbors enough key mutations to fully resist the agent being used, thereby rendering it the uncontested dominant strain. "If we use a second drug to combat virus that has become resistant to the first drug, the process repeats itself," Dr. Clavel said.

This is usually a slow process, as it can take numerous rounds of replication and competition among the diversified strains to render one variant that has a strong survival advantage compared to other variants in the presence of the antiretroviral agent being used. But in some cases, high-level drug resistance can be almost instantaneous. This is certainly the situation with the non-nucleoside reverse transcriptase inhibitors and lamivudine (Epivir). Upon initiating any of these drugs as monotherapy, high-level resistance emerges within days to weeks because of the proliferation of variants containing the K103N and M184V mutations respectively. With other drugs, such as the protease inhibitors and nucleoside analogues, several mutations are necessary and, as a result, take longer to confer high-level resistance.

With the advent of combination antiretroviral therapy, it was believed that the selection of drug-resistant mutations could be halted or slowed considerably. First, Dr. Clavel explained, "it's very unlikely that you will find a variant in patients who have never been treated that is resistant to all three drugs being used in a combination. It's just basic statistics; it's statistically impossible to find such a virus. Second, because a combination of drugs is more powerful than single-agent therapy, we end up blocking the capacity of any breakthrough virus to acquire additional mutations that can lead to high-level resistance seen in the sequential monotherapy scenario."

Unfortunately, no drug combination studied to date has been shown to completely shut down viral replication. In turn, the virus continues to diversify and eventually accumulate enough key mutations to pose a challenge to the antiretroviral drug regimen being used. "This tends to be a slow process," Dr. Clavel added. "It may begin with resistance to one drug and subsequent diversification that leads to resistance to the second drug. And with resistance to two drugs complete, we're looking at the monotherapy scenario, which can quickly translate into resistance to all three drugs being used. However, the virus has an easier time resisting some medications than others when used in combinations. Resistance to lamivudine usually occurs quickly and is often the first evidence of resistance to emerge. Resistance to stavudine [Zerit] is much more complex and can take much longer. Resistance to the protease inhibitors falls somewhere in between."

To understand this, Dr. Clavel contends that it is necessary to explore

the methods of resistance that occur in each of the classes of currently available antiretroviral drugs.

## Resistance to Nucleoside Reverse Transcriptase Inhibitors

THERE ARE ESSENTIALLY TWO MECHANISMS BY WHICH RESISTANCE TO nucleoside reverse transcriptase inhibitors (NRTIs) can occur. The first involves mutations (e.g., M184V, K65R, Q151M) that occur at or near the drug-binding site of the reverse transcriptase gene, resulting in increased drug discrimination by this gene. This is the primary mechanism of resistance to most of the NRTIs.

The second mechanism that is of concern when discussing NRTIs involves key mutations that essentially work to undo the action of these drugs, even if they do manage to bind correctly within the RT gene. NRTIs exert a blocking effect by plugging a nonextendable nucleoside analogue monophosphate to the 3' end of the growing proviral DNA chain. This effectively terminates chain extension and, ultimately, inhibits replication of the virus. However, this process can be reversed by a reverse transcriptase reaction that removes the chain-terminating residue and reinstates an extendable primer. This reverse reaction of DNA polymerization, termed pyrophosphorolysis, enables reverse transcription and DNA synthesis to resume.

Pyrophosphorolysis can be enhanced by key mutations, most notably those selected by zidovudine (Retrovir) and stavudine. These mutations are sometimes referred to as thymidine analogue mutations (TAMs), as both zidovudine and stavudine are thymidine analogues and appear to select—and are hobbled by—many of the same mutations in reverse transcriptase that confer drug resistance. While pyrophosphorolysis is believed to be the primary mechanism of resistance to zidovudine and stavudine, the process is not drug-specific in the way that discriminatory mutations tend to be. Consequently, these pyrophosphorolysis-enhancing mutations can confer reduced susceptibility to all of the NRTIs. These drugs, however, are not equally prone to the effect of pyrophosphorolysis-enhancing mutations: resistance to zidovudine is a much easier biochemical process than resistance to stavudine or didanosine (Videx). "This is why we tend to see resistance developing much more quickly and effectively to some NRTIs than to others," Dr. Clavel explained.

See Table 1 for a basic look at key mutations associated with resistance to NRTIs.

**TABLE 1. Nucleoside Reverse Transcriptase Inhibitor Resistance Mutations at a Glance**

| Mutation Position | Zidovudine | Stavudine | Tenofovir | Abacavir | Didanosine | Lamivudine |
|---|---|---|---|---|---|---|
| 41 | | | | | | |
| 67 | | | | | | |
| 70 | | | | | | |
| 210 | | | | | | |
| 215 | | | | | | |
| 219 | | | | | | |
| 184 | | | | | | |
| 69 | | | | | | |
| 65 | | | | | | |
| 74 | | | | | | |
| 75T | | | ? | | | |
| 62 | | | | | | |
| 75I | | | | | | |
| 77 | | | | | | |
| 116 | | | | | | |
| 151 | | | | | | |
| 69SS | | | | | | |
| 44 | | | | | | |
| 118 | | | | | | |
| 115 | | | | | | |

**LEGEND**

High-level resistance
Intermediate resistance
Low-level resistance
Contributes to resistance
No Resistance
Hypersensitivity

Source: Stanford HIV Drug Resistance Database. Last updated: July 9, 2003. Reprinted with permission.
http://hivdb.stanford.edu/cgi-bin/NRTIResiNote.cgi

## Resistance to Non-Nucleoside Reverse Transcriptase Inhibitors

THE MECHANISM OF RESISTANCE TO NNRTIs is much more straightforward. All of the available NNRTIs are designed to bind to amino acids nestled in a hydrophobic binding pocket within reverse transcriptase. "This pocket is not really part of the active site of the enzyme," explained Dr. Clavel. "It's next to—very close

**TABLE 2. Non-Nucleoside Reverse Transcriptase Inhibitor Resistance Mutations at a Glance**

| Mutation | Nevirapine | Delavirdine | Efavirenz |
|---|---|---|---|
| 98G | | | |
| 98S | | | |
| 100I | | | |
| 101EPQ | | | |
| 103NS | | | |
| 103R | | | |
| | | | |
| 106A | | | |
| 106M | | | |
| 106I | | | |
| 108I | | | |
| | | | |
| 179D | | | |
| 179I | | | |
| 181CI | | | |
| 188L | | | |
| 188C | | | |
| 188H | | | |
| 190A | | | |
| 190S | | | |
| 190E | | | |
| | | | |
| 225H | | | |
| 227L | | | |
| 230L | | | |
| 236L | | | |
| 238TN | | | |
| 318F | | | |

**LEGEND**

- High-level resistance
- Intermediate resistance
- Low-level resistance
- Contributes to resistance
- No Resistance
- Hypersensitivity

Source: Stanford HIV Drug Resistance Database. Last updated: July 25, 2003. Reprinted with permission. http://hivdb.stanford.edu/cgi-bin/NNRTIResiNote.cgi

to—the active site. This pocket actually doesn't exist when the drug is not present. The NNRTIs open the pocket and, in the process, block some of the movements of the enzyme. Without these movements, DNA synthesis cannot occur."

Various mutations—such as L100I, Y181C, G190S/A, and M230L—that can confer NNRTI resistance involve amino acids that form the hydrophobic binding pocket. Interestingly, the K103N mutation, which causes high-level resistance to all of the available NNRTIs, has a different mechanism. Position 103 is not part of the hydrophobic NNRTI binding pocket. However, it is near the entrance to the pocket. The K103N mutation does not alter the structure of the complex between reverse transcriptase and NNRTIs. Instead, K103N creates a hydrogen bond in unliganded reverse transcriptase. This additional hydrogen bond helps keep the entrance to the pocket closed, making it more difficult for NNRTIs to enter the pocket.

"What's interesting about this region of reverse transcriptase is that it is fairly polymorphic," Dr. Clavel said. "We know that these drugs don't work on HIV-2. They simply don't work on a variety of viruses. We also know that mutations in this region can confer degrees of resistance that vary widely from one HIV isolate to another. For example, HIV isolates carrying the K103N mutations can have degrees of resistance to efavirenz [Sustiva]. In some viruses, the K103N mutation can produce a 10,000-fold increase in the $IC_{50}$, whereas in other viruses we see an $IC_{50}$ fold change of less than 100. It all seems to depend on all of the polymorphisms present in this region of reverse transcriptase."

Key reverse transcriptase mutations associated with resistance to NNRTIs are reviewed in Table 2.

## Resistance to Protease Inhibitors

THE HIV PROTEASE GENE FUNCTIONS AS A HOMODIMER—A COMPLEX OF two identical protein molecules, both consisting of two chains made up of 99 amino acids—that processes gag (p55) and gag-pol (p160) polyprotein products into functional core proteins and viral enzymes. During or immediately after budding, the polyproteins are cleaved by HIV protease at nine different cleavage sites to yield the structural proteins (p17, p24, p7, and p6), as well as the viral enzymes reverse transcriptase, integrase, and protease.

All of the approved protease inhibitors are based on amino acid sequences recognized and cleaved in HIV proteins. They all bind to active-site amino acids within a pocket at the center of the homodimer. Most protease inhibitors contain a synthetic analogue of the phenylalanine-proline sequence at positions 167 and 168 of the gag–pol polyprotein that is cleaved by the protease. In turn, protease inhibitors prevent cleavage of gag and gag-pol protein precursors in acutely and chronically infected cells, thereby arresting maturation and blocking the infectivity of nascent virions.

Interestingly, many mutations in HIV's protease gene that are known to confer resistance to the protease inhibitors involve amino acids that are not near the binding sites for these drugs. "Several mutations are nowhere near the active site of the protease enzyme or the binding sites for the protease inhibitors," Dr. Clavel explained. "What these mutations end up doing is changing the actual structure—the overall shape—of the enzyme, which can lead to resistance."

In the simplest terms, the development of key mutations force the pocket at the center of the homodimer to widen, causing the inhibitor to "float around" the stretched cavity, unable to effectively bind to the gene and block cleavage. "Unfortunately," Dr. Clavel added, "this expansion of the cavity does not render the protease gene useless in terms of cleaving natural substrates. While it does lose some affinity for the natural substrates, the loss of affinity isn't nearly as profound as the loss of affinity for the inhibitor."

Table 3 details the key protease gene mutations that confer differing degrees of resistance to protease inhibitors.

**TABLE 3. Protease Inhibitor Resistance Mutations at a Glance**

| Mutation Position | Nelfinavir | Saquinavir | Indinavir | Ritonavir | Amprenavir | Lopinavir | Atazanavir |
|---|---|---|---|---|---|---|---|
| 30 | | | | | | | |
| 48 | | | | | | | |
| 50V | | | | | | | |
| 50L | | | | | | | |
| 82 | | | | | | | |
| 84 | | | | | | | |
| 90 | | | | | | | |
| 46 | | | | | | | |
| 47 | | | | | | | |
| 53 | | | | | | | |
| 54 | | | | | | | |
| 24 | | | | | | | |
| 32 | | | | | | | |
| 73 | | | | | | | |
| 88 | | | | | | | |
| 10 | | | | | | | |
| 20 | | | | | | | |
| 33 | | | | | | | |
| 36 | | | | | | | |
| 63 | | | | | | | |
| 71 | | | | | | | |
| 77 | | | | | | | |
| 93 | | | | | | | |

**LEGEND**

High-level resistance
Intermediate resistance
Low-level resistance
Contributes to resistance
No Resistance
Hypersensitivity

Source: Stanford HIV Drug Resistance Database. Last updated: November 30, 2003. Reprinted with permission. http://hivdb.stanford.edu/cgi-bin/PIResiNote.cgi

## The Selective Advantage of Protease Mutants

VIRUS RESISTANCE IS USUALLY CALCULATED BY MEASURING THE CONCENtration of drug that is required to inhibit 50% ($IC_{50}$) of virus infectivity. For each HIV variant, the level of resistance is calculated relative to the virus's infectivity in drug-free conditions. Dr Clavel and his group have measured resistance in a slightly different way. Rather than calculating mutant virus inhibition relative to drug-free conditions, they have measured replication of mutant relative to wild-type virus over a range of drug concentrations. This amounts to measuring the selective advantage of that mutant as a function of the concentration of drug, thus defining a unique and characteristic "fitness profile." The fitness profiles that were calculated for viruses representing each of the mutational pathways studied were fully consistent with the observations made *in vivo* regarding the order of appearance of the mutations in treated patients. By integrating the main parameters of the selection for drug resistance, drug-free infectivity, resistance, and drug concentration, Dr. Clavel argues that it is possible to map out the pattern of accumulation of protease inhibitor-associated resistance mutations.

To conduct this exploration, Dr. Clavel's group reconstructed a large series of HIV mutants carrying single or combined protease mutations, retracing the pathways of resistance observed in patients treated with ritonavir (Norvir), saquinavir (Invirase), indinavir (Crixivan), and nelfinavir

(Viracept). First taking a look at ritonavir resistance, Dr. Clavel's group found that most of the mutations that are usually combined in ritonavir-resistant strains of HIV did not confer any selective advantage when present alone in the virus, no matter what concentration of the drug was used. However, there was a notable exception. The V82A mutation appeared to confer a small level of selective advantage in the presence of low ritonavir concentrations. Perhaps not coincidentally, this is usually the first mutation to emerge, *in vivo*, during ritonavir therapy.

With the gradual accumulation of resistance mutations, there was a notable increase in the extent and the range of the selective advantage. With ritonavir, variants harboring combinations of A71V and V82A among other mutations were the most efficient virus variant, with the maximal advantage obtained for the mutant carrying all four of the tested mutations (46I, 54V, 71V, and 82A) in combination.

The experience with saquinavir told a similar story. Only one single mutation conferred selective advantage to the virus: L90M. Interestingly, the G48V mutation, which results in significant drug resistance using standard testing, did not appear to confer a selective advantage when present alone. With continued evolution toward higher levels of saquinavir resistance, the most favorable combination of mutations—L10I, G48V, and L90M—markedly outperformed the other mutants both in the extent of the replicative performance and in the range of drug concentrations over which this advantage could be perceived.

The same held true for nelfinavir. Resistance to nelfinavir will move either along the D30N pathway or the L90M pathway. According to Dr. Clavel, mutants harboring either mutation alone did have a selective advantage, with the D30N mutation resulting in the most profound selective advantage. Neither the N88D nor the A71V mutation, alone, conferred any selective advantage to the virus. With a combination of mutations, most notably D30N and A71V, the selective advantage of the mutant virus increased substantially.

## Minority Virus Populations

AS IS DISCUSSED EARLY ON IN THIS ARTICLE, HIV INFECTION IS NOT A SINgle virus but rather a flurry of different populations of virus that evolve independently and can replace each other. In other words, while there may be one dominant strain of HIV, there are countless minority populations waiting for their selective advantage. To learn more about the behavior of minority species, with respect to the evolution of drug resistance, Dr. Clavel's group conducted another round of experiments (Charpentier, 2002). These experiments called for the use of a highly sensitive PCR—capable of detecting minority species constituting 0.1% of the virus population—to evaluate isolates collected from seven patients who were failing a protease inhibitor and had genotypic evidence of the L90M mutation being present.

As explained by Dr. Clavel, this mutation did not appear immediately before virologic failure occurred in these seven patients. "In some patients, the mutation was seen in a very small number of minority populations and then quickly increased to be present in close to 100% of the virus populations. In other patients, the L90M mutation would be present in 5% of the mutant populations, then 30% of the populations, then go back down to 10% of the populations, and then constitute close to 100% of the populations. For others, L90M could be found in a small number of virus populations and gradually increased to 100% of the populations. All of this was occurring before standard genotyping could detect the mutation." In the seven patients as a whole, the L90M mutation could be detected in minority species in as little as two months, and as long as 34 months, before it became detectable using a standard genotypic assay.

Over time, with the addition or switching of antiretroviral drugs used, there was a shifting of drug pressure. With continued therapy, a number of distinct resistant genotypes could be documented among the minority species. And while the number of resistant minority mutants grew in time, the growth was not linear. Rather, some mutations were lost in some minority populations and some mutations were gained in other minority populations.

"What this tells us," Dr. Clavel explained, "is that HIV, at any time point, has a library of different genotypes that it can select from and then use, in whatever capacity, for its own evolution. We're simply not dealing with one single genotype that evolves over time. We're dealing with minority virus populations that have a variety of distinct genotypes, including those that have accumulated multiple drug-resistant mutations. These virus minorities persist through treatment and constantly evolve. And they can rapidly replace the prevalent majority virus, behaving as a reservoir from where new genotypes can be recruited depending on the pharmacological pressure. The bottom line here is that the mutations we see using a standard genotypic assay may be the mutations that a minority variant has used to resist the current drugs being used. However, this doesn't provide much in the way of information regarding which mutations will arise using the next combination of drugs. Those key mutations may have already been selected by a minority variant, which won't be detected using standard assays."

## Conclusion

ON THE SURFACE OF THINGS, DR. CLAVEL'S PRESENTATION PAINTS A rather grim picture—that resistance is predestined and that there's little that can be done to effectively detect minority variants and to prevent them from causing a rebound in viral load. However, there is reason to remain hopeful, which was Dr. Clavel's message in addressing some of the more panicky questions of clinicians in attendance. "Tremendous progress has been made in terms of developing drugs with high antiviral activity that are also easier to take than their less potent predecessors," he said. "By developing drug combinations that have high barriers to resistance, that are less toxic, and are easier to take, we are potentially talking about regimens that won't inevitably lead to resistance. Attempting to combat resistance, once it has already occurred, is most difficult, given the presence of minority variants that may have a selective advantage upon changing a regimen. The key is to develop regimens that limit the growth of these variants, which means developing regimens that are capable of suppressing virus for a very long period of time. If we can start patients on regimens that are very strong and have durable effects, and are easy for the patient to adhere to, we'll be one step ahead." prn

## References

Charpentier C, Dwyer DE, Lecossier D, et al. **Co-evolution and competition of viral populations with distinct resistance genotypes in patients failing treatment with protease inhibitors** [Abstract 48]. *Antiviral Therapy* 7(suppl 1):S55, 2002.