Primer on Human Immunodeficiency Virus (HIV)-Drug Resistance

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Antiretroviral Therapy

Highly active antiretroviral therapy (HAART) is the standard of care for treating HIV-infected patients.[1] HAART is composed of 3 antiretroviral drugs; 2 of them are nucleoside/nucleotide reverse transcriptase inhibitors, and for the third one we can use either non-nucleoside reverse transcriptase inhibitors or protease inhibitors.

Antiretroviral drugs currently available for use in developing countries are from the first 3 classes.

* The first one, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), includes zidovudine (AZT), stavudine (d4T), didanosine (ddI), lamivudine (3TC), abacavir (ABC), tenofovir (TDF), and emtricitabine (FTC).

* The second class, non-nucleoside reverse transcriptase inhibitors (NNRTIs), includes delavirdine (DLV), neverapine (NVP), and efavirenz (EFV).

* The third class, protease inhibitors (PIs), includes ritonavir (RTV), indinavir (IDV), saquinavir (SQV), amprenavir (APV), lopinavir (LPV), and atazanavir (ATV).

* The fourth class of antiretroviral drugs is the fusion inhibitors (Enfuvirtide or T-20), which have just become available for clinical use recently.[2-3]

HIV Evolution and the Emergence of Resistant Variants

HIV is an RNA virus with a typical characteristic of high replication rates.[4] However, HIV reverse transcriptase lacks proof-reading capability during its replication cycle, resulting in a high mutation rate calculated to be about one mutation per one newly produced viral genome. These mutated HIV variants can have replication capacity that is better, equal to, or worse than wild-type variants.[5] Several quasi-species, therefore, exist in an HIV population. The mutated variants could become a dominant population in an environment with selective advantages favoring the mutated variants. Given these specific characteristics of HIV, drug-resistant variants will evolve as a result of incomplete viral suppression.[6] The selective pressure of failing antiretroviral therapy will select out the resistant variants, resulting in virological failure (defined as HIV viral loads of more than 50 copies/ml).[7]

The rational of HAART is to have drugs with different mechanisms of action to suppress viral replication completely and as a result, prevent resistant strains of HIV emerging and becoming a dominant population while on therapy. However, drug-resistance problems can still be seen when combination regimens are not potent enough to achieve complete viral suppression or when some components of the HAART are below therapeutic levels.[8] In addition, usage of medication outside the concept of HAART, such as using one or two drugs in preventing maternal-fetal transmission, will contribute to the resistance problem as well.[9-10] As an increasing threat, a primary HIV infection with resistant strains has recently been reported in several areas.[11-12]

Therefore, physicians, providing care to HIV-infected patients, will need to consider the possibility of HIV-drug resistance when designing effective HAART regimens in order to achieve the goal of complete viral suppression. Continuing viral replication in the presence of less effective HAART regimens will select out drug-resistant variants of the organism, leading to virological failure.

Several factors have been implicated as causing virological failure in treating HIV infection.[13] One of those factors is HIV-drug resistance, a consequence of incomplete viral suppression. In this article, we will focus on the resistance mutations as a cause of the virological failure. Of course, in approaching infected patients with a detectable viral load, physicians will need to consider other factors such as drug compliance or the pharmacokinetic issue. HIV providers should not focus only on the HIV-drug resistance problem until other causes of virological failure have been ruled out.

HIV Life Cycle

The HIV life cycle will be described briefly to help comprehend the mechanism of action/resistance of each drug class.[14] HIV virions will bind, via the gp120 Env protein, to the CD4 molecule on T cells, macrophages, and microglial cells. Through further interaction of HIV virions with the 2 co-receptors, CXCR4 and CCR5, on infected cells, the HIV entry process will be successful. Once in the cytoplasm, the process of reverse transcription will start to form DNA particles from the viral genomic RNA. In this reverse transcription process, the virions will require the enzyme reverse transcriptase and substrates nucleotides to form double-stranded DNA, which

will be integrated into the host cellular DNA. Using the host cell transcription and translation processes, pro-viral RNA and viral proteins will be produced. The process of virus packaging and assembly will take place afterward in the cytoplasm. The assembled virions will bud from the infected cell. Lastly, the maturation process will occur within the core, where the enzyme protease will cleave the Gag and Gag-Pol poly-proteins into structural proteins and enzymes such as reverse transcriptase or integrase.

Mechanisms of Action of Antiretroviral Therapy

In order for antiretroviral therapy to inhibit HIV replication completely, the design of therapy has focused on inhibiting the activity of 2 viral enzymes, the reverse transcriptase and protease. Other enzymes involved in different steps of the HIV life cycle have also been the target of currently in-development drugs, such as entry inhibitors or integrase inhibitors.

The enzyme reverse transcriptase is an RNA-dependent DNA polymerase. The enzyme has 2 subunits, p51 and p66 subunits.[15] The p66 subunit contains the active site in processing the reverse transcription. This subunit has 5 sub-domains, including the fingers, the palm, the thumb, the connection, and RNase H sub-domains. Nucleotide sequences of the fingers and palm sub-domains start from the first codon to codon 235 (a codon is a group of 3 nucleotides, which codes for a particular amino acid in the protein. For example, the nucleotide sequence at codon 184 is ATG, which codes for amino acid methionine). The first 3 sub-domains will participate in the polymerization or DNA chain extension process. NRTIs will act as a chain terminator in the process of nucleotide chain extension[16]. The inhibitors will compete with natural substrates for incorporation into the newly synthesized DNA chains and stop the reverse transcription process.

Another class of antiretroviral therapy, NNRTIs, also acts on the enzyme reverse transcriptase to inhibit the normal function of the enzyme. But the inhibitors instead act on the hydrophobic pocket near the polymerase active site (palm sub-domain at codons 110, 185, and 186) of the enzyme. The binding of NNRTIs to the pocket will decrease flexibility of the enzyme and prevent the reverse transcriptase from functioning properly.[17]

The second drug-target viral enzyme, protease, has a function of cleaving the Gag and Gag-Pol poly-proteins to derive both structural proteins and enzymes such as reverse transcriptase or integrase before the virions complete the maturation process.[15] The enzyme is composed of 2 structurally identical monomers (99 amino acids in length) with a central symmetric, substrate-binding cavity or cleft. This cleft is covered with a mobile flap. The third class of antiretroviral therapy, PIs, will inhibit the function of this enzyme by binding to the protease active site (at codons 25-27) and precluding the enzyme from cleaving the poly-proteins.

Mechanisms of HIV-drug Resistance

Because of the absence of a proof-reading mechanism in the process of reverse transcription, residual replication, as a consequence of incomplete viral suppression, will lead to an accumulation of more mutations. When mutations occur at the action sites of HIV drugs, HIV will become resistant to the medication and often other drugs in the same class. Two resistance mechanisms to NRTIs have been described.[16] One is impairment of the ability of reverse transcriptase to incorporate the inhibitors into viral DNA. This mechanism is usually seen with the M184V, K65R (selected by TDF and ABC, resistant to all NRTIs except AZT), and Q151M complex (selected by d4T or ddI, and resistant to all NRTIs except TDF and 3TC) mutations. The second mechanism is removal of the inhibitors from the terminated DNA chain, which is usually seen with a group of mutations (including M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) referred to as thymidine analogue mutations (TAMs, resistant to all NRTIs including TDF).[7] Sometimes they are called nucleoside analogue mutations or NAMs. All above-mentioned codon positions, at which resistance mutations occur, are either close to the polymerase active site or involved in the reverse transcription process[15]. For NNRTIs, resistance mutations occur at the binding sites of drugs, the hydrophobic pocket. The mutations will decrease the binding affinity of the medication to the action site. Mutations occurring near the protease active site or the cleft (D30N, G48V, I50V, V82A/F/T/S, I84V, and L90M) will decrease the binding affinity of PIs to the substrate-binding cleft of the protease enzyme, resulting in drug resistance.[16] Resistance mutations to PIs also can occur far outside the protease active site or on the flap region; however, their magnitude of resistance to PIs is less important than the mutations near the cleft.

Clinical Implications of Commonly Seen Resistance Mutations

1) NRTI resistance mutations:

Commonly seen and clinically important resistance mutations to NRTIs are M184V, K65R, L74V, and TAMs. The T69S insertion and Q151M complex also cause high resistance to NRTIs, but are rarely seen in patients failing therapy. M184V is selected by 3TC and FTC.[18-19] This is usually the first mutation emerging when failing the combination regimens with 3TC or FTC as a component. M184V induces high resistance to both drugs. However, the variant with this mutation has low replication capacity. Therefore, maintaining patients on these drugs might slow the pace of immunological failure.[20-21] ABC can also select M184V. However, without further development of resistance mutations such as L74V, K65R, or Y115F, patients with M184V still can be treated with ABC because of its low-level resistance to ABC[22-23]. M184V also deters the development of TAMs or reverses the resistance effect of TAMs. The presence of M184V can deter or decrease the removal of NRTIs from the terminated DNA chains, which is the resistance mechanism of TAMs.[19,24]

K65R is seen in patients taking ABC, TDF, or ddI. Variants with K65R also have low replication capacity.[19,23] The mutation decreases viral susceptibility to most of NRTIs except AZT. In fact, K65R results in hypersusceptibility to AZT. K65R is, therefore, usually seen after NNRTIs or M184V have emerged, but hardly ever co-exiting with TAMs.[25-26] With the current availability of a combination of FTC/TDF and 3TC/ABC in one pill, patients who fail on these combinations are likely to develop M184V with either K65R (in a case of FTC/TDF) or L74V (in a case of 3TC/ABC), but not TAMs.[23] These two combinations can be called thymidine-sparing backbones.

TAMs are a group of resistance mutations, including M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E. They are selected for by AZT or d4T, but induce resis-

tance to all NRTIs except 3TC and FTC.[16] Variants with TAMs have a relatively high replication capacity. The mutations will accumulate over time, but treatment failure will be observed when more than 2 TAMs has occurred.[27] TAMs development therefore can be avoided by preventing patients, who are on thymidine analogues, from having residual viral replication. Two pathways of TAMs development can be observed with a clinical implication.[20] One is a group of M41L, L210W, and T215Y, which usually induce high-level resistance to AZT, TDF, and ABC. The other one, less commonly seen than the first one, is a group of D67N, K70R, T215F, and K219Q, which usually induce low-level resistance and are less likely to have cross-resistance to other NRTIs. M184V is more likely to reverse TAMs-induced resistance when there are no more than 3 TAMs present or the second pathway of TAMs (D67N, K70R, T215F, and K219Q) has developed.[19,28]

L74V is selected during therapy with ABC or ddI. However, to cause ABC failure, further development of M184V or Y115F is required. L74V alone can cause ddI failure, but using ddI with AZT will postpone treatment failure because of the hypersensitivity of L74V variants to AZT.[22] L74V also re-sensitizes variants with TAMs to AZT. L74V variant remains susceptible to TDF.[19]

The Q151M complex is selected for by ddI or d4T, but is rarely seen in clinical samples. It usually starts with Q151M and is followed by F116Y, F77L, V75I, and A62V. The complex causes resistance to all NRTIs except 3TC and TDF.[16]

The T69S insertion is started with the mutation T69S and followed with an insertion of 2 amino acids. Variants with the T69S insertion emerge only in patients with TAMs and confer high resistance to all NRTIs.[16]

2) NNRTI resistance mutations:

For NNRTIs, only one resistance mutation occurring in the hydrophobic pocket can cause cross-resistance to all drugs in the class.[16] However, the resistance level of each resistance mutation seems to be drug-dependent. The most common resistance mutation seen in this class is K103N, conferring high-resistance to all NNRTIs.[16,29] This mutation is selected for usually by EFV. L100I is also selected for by EFV, and additively increases resistance to EFV when co-existing with K103N. Y181C induces high-resistance to NVP, but low-level resistance to EFV. However, only a transient response to EFV can be achieved when prescribing EFV to patients with Y181C. Y181C is selected for by NVP (when not taking with AZT). Variants with Y181C are hypersensitive to AZT. Therefore, patients, who failing on NVP and AZT or d4T, will develop K103N, instead of Y181C.[19]

Other mutations that can be seen in patients failing NNRTIs are V106A, Y188C, G190A/S, and M230L. In the presence of viral replication, NNRTIs-resistant variants can emerge rapidly because of a pre-existing mutant population in a patient. Variants with resistance mutations to NNRTIs also have an intact replication capacity. Therefore, patients, who fail NNRTIs with the presence of residual viral replication, should not be kept on drugs of this class.

3) PI resistance mutations:

Mutations in the substrate-binding cleft of the protease enzyme, or primary mutations, lead to either drug-specific resistance to PIs or cross-resistance to several drugs in the PIs class.[16,30-31] D30N is selected for by NFV, and induces resistance to only NFV.[22] G48V is selected for by SQV, and induces high-level resistance to only SQV. I50L is selected for by ATV, and induces resistance to only ATV. I50L may enhance susceptibility of variants to other PIs. I50V is selected for by APV, and induces resistance to APV and LPV. V82A/F/T/S is selected for by IDV & RTV, and by themselves, induces resistance to IDV, RTV, and LPV. When occurring with other PI mutations, V82A/F/T/S can induce resistance to all PIs. V82A can be selected for by SQV, following G48V. I84V is selected for by IDV, RTV, LPV, and APV. This mutation also induces resistance to all PIs. L90M is selected for by NFV and SQV, and causes cross-resistance to all PIs.

Mutations in the protease flap region (codons 46-56), or secondary mutations, contribute to PI resistance when occurring with the primary mutations. They have no resistance effect when occurring alone.[16] The secondary mutations probably increase the replication capacity of variants with the primary mutations.[7]

The unique characteristic of PI resistance is a high genetic barrier, in a manner similar to TAMs. Both primary and secondary mutations will accumulate over time before variants become highly resistant to PIs. When using non-boosted PI regimens especially with mono-therapy, resistance mutations to PIs will develop over time due to the poor pharmacokinetics (short half-life) of drugs and the compromised replication capacity of variants with the primary mutations (less fit than wild-types).[22] The current recommendation is to use boosted PI regimens which provide higher drug levels and avoid a condition with selective advantages to mutants. Patients, who are failing on boosted PI regimens, are not found to have resistance mutations.[32] This makes it possible to cycle to other PIs or to only encourage patients to be more compliant with medication when failing ART regimens with PIs. Moreover, patients, who are failing on combination regimens of NRTIs and boosted PIs, are less likely to develop NRTI resistance mutations.

Indications of Resistance Testing

The benefit of testing for drug resistance before starting antiretroviral treatment or changing the regimens has been examined and demonstrated in several clinical trials.[34-39] A reduction of plasma HIV-1 RNA at 6 months is greater when modifying the regimens based on resistance testing than when adjusting based on the standard of care (treatment history or clinical guidelines). The EuroGuidelines Group for HIV Resistance, the International AIDS Society (IAS)- USA Consensus Panel on Resistance testing, and the Panel on Clinical Practices for Treatment of HIV Infection of the US Department of Health and Human Services (DHHS) have recommended the use of resistance testing on the following indications; primary infection, before initiating antiretroviral therapy in chronic infection, before changing therapy in treatment failure, and before initiating therapy in pregnancy.[40-42] Treatment failure can be defined in 3 levels; virological, immunological, and clinical failures.[13] Resistance testing should be performed when patients develop virological failure, defined as repeated viral load measurements above 400 copies/mL after 24 weeks or above 50 copies/mL after 48 weeks of treatment.

Resistance Testing

1) Phenotypic assays:

There are 2 kinds of assays to detect HIV-drug

resistant variants: phenotypic and genotypic assays. The phenotypic assays are performed by generating recombinant viruses expressing the protease and reverse transcriptase genes of HIV isolates from patients, who fail antiretroviral therapy.[15,18] The recombinant viruses are grown in cell culture at different drug concentrations. Once the assays obtain a drug concentration at which virus replication is inhibited by 50% (IC50 or inhibitory concentration 50%), this IC50 will be compared to IC50 of a wild-type laboratory strain to derive a fold-change. Based on clinical cut-offs of resistance, the fold-change value will determine which antiretroviral drugs cannot be used. The phenotypic assays are a more direct measure of resistance and can be used to test any drugs.[42] However, the assays are expensive and not widely available. The standard clinical cut-offs of fold-change to interpret resistance for several antiretroviral drugs are still not defined. The assays can be performed with only one drug at a time and do not take activities of other drugs in combinations into account.

2) Genotypic assays:

The genotypic assays measure resistance indirectly by detecting resistance mutations in the protease and reverse transcriptase genes. There are 2 commercially genotypic assays available: the TRUGENE assay and the ViroSeq assay.[15,18] Viral RNA in patients' plasma is extracted, reverse-transcribed into single-stranded DNA, amplified the DNA, and sequenced in order to obtain the genomic nucleotide sequences of the entire protease gene and codons 1- 247 of the reverse transcriptase gene (codons 1-320 if using the ViroSeq). The assays will report the resistance mutations found in patients' plasma. The assays are widely available, less expensive (compared to the phenotypic assays), and have a short turnaround time. However, the assays are not able to detect minority variants (<20% of viral population).[42] The report of resistance mutations will need to be translated into drug resistance, which sometime is not straight forward due to the additive or synergistic interactions of several mutations. However, with several clinical studies of resistance mutations, predictable correlations of commonly seen resistance mutations and treatment failure have been defined which help physicians to modify treatment regimens for patients who have failed their first or second combination regimens.

3) Point-mutation assays:

Several point-mutation assays, alternative to sequencing methods, have been examined and used clinically.[43-46] This kind of assay will detect only the most common resistance mutations in both protease and reverse transcriptase genes. The advantage of point-mutation assays is that the assays are even less expensive than the sequencing methods because there is no need for sophisticated equipments or trained personnel to perform the assays. This is a very appropriate alternative to the sequencing methods in resource-limited clinics. Given that certain patterns of resistance mutations commonly seen in clinical samples have been studied and their clinical significance defined in many clinical trials as described previously, these point-mutation assays would be adequate and affordable for both physicians and patients to use as a tool to modify the treatment regimens when virological failure occurs. Currently we are in the process of modifying one of the point-mutation assays, the Oligonucleotide Ligation Assays (OLA), in order to implement the assay in our HIV clinic and use it with samples from Thai patients.

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