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# Review of HIV Antiretroviral Drug Resistance

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Currently, there are 6 classes of antiretroviral (ARV) agents for treatment of human immunodeficiency virus type 1 (HIV-1) infection: nucleoside reverse transcription inhibitors (NRTIs), non-nucleoside reverse transcription inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5 receptor antagonists, and integrase inhibitors (Table 1). The ultimate goal of ARV therapy is to achieve virologic suppression and immune reconstitution. Virologic suppression is defined as a reduction in plasma HIV RNA (viral load) to below the limit of detection or <50 copies/mL (cpm). Immune reconstitution is reflected by an increase in the CD4 T cell count.

The use of potent combination ARV therapy (2 or more classes of ARVs) has dramatically improved the quality of life and overall survival of individuals infected with HIV-1. However, current therapies are unable to eradicate HIV infection from cellular reservoirs. Suboptimal exposure to ARVs can rapidly select for drug resistance mutations (DRMs). HIVs extraordinary replication rate ($10^{10}$ rounds of replication per day) and high mutation rate (because of error prone HIV reverse transcription) permit the selection of DRMs at an unprecedented rate.[1] Even before ARV therapy, every single point mutation is present within the quasi-species. Without selective pressure from ARVs, these viral variants remain below the level of detection. With incomplete viral suppression, these resistant variants can rapidly emerge and ultimately lead to virologic failure (defined as the inability to achieve virologic suppression within 16–24 weeks of initiation of ARVs or persistent viral load >1000 cpm). Given the dynamics of HIV viral evolution, almost complete (>95%) adherence to combination therapy is required to maximally suppress viral load and avoid selection of resistant strains.[2,3]

## WHO SHOULD RECEIVE RESISTANCE TESTING?

The presence of DRMs markedly decrease the likelihood of virologic suppression; therefore, knowledge of the ARV susceptibility of circulating HIV is important before ARV initiation. Transmission of drug resistant HIV is not rare and is increasing. The prevalence of genotypic ARV resistance among ARV-naive, HIV-positive pregnant women is estimated at 2.3–25% and was recently found to be 24% in newly infected adolescents.[4,5] Infants with perinatally-acquired HIV infection are also at risk for DRMs, particularly if they or their mothers have received ARVs for prevention of perinatal transmission. Because the tempo of disease progression is much faster in infants, it is critical that they receive appropriate ARVs.

In the absence of ARVs, the relative proportion of resistant clones may decrease to below the level of detection, but these clones can quickly emerge and decrease ARV efficacy. For

these reasons, resistance testing is recommended for all persons newly diagnosed with HIV, regardless of age or whether therapy will be initiated immediately. If therapy is deferred, testing should be repeated at the time of ARV initiation. Routine resistance testing is also recommended for all pregnant women before the initiation of therapy and for those with detectable HIV RNA levels (>1000 cpm) while on therapy. Finally, patients experiencing virologic failure and those with suboptimal virologic suppression may also benefit from resistance testing.[6,7]

Children seem to be more prone to selection of drug resistant variants for both biologic and behavioral reasons. Plasma viral loads are much higher in children compared with adults. As such, children require more potent ARV regimens to achieve virologic suppression. Young children also have less robust antiviral immune responses. Furthermore, drug absorption and pharmacokinetics are highly variable and change with age. Together, these factors place children at greater risk of subtherapeutic ARV levels. This is further compounded by difficulties in adherence given unpalatable liquid formulations and frequent dosing.[6,8] Thus, treatment and management of pediatric HIV infection requires knowledge of the limitations of therapy and ARV resistance.

## ANTIRETROVIRAL DRUG RESISTANCE ASSAYS

There are 2 major methods of assessing ARV drug resistance: phenotypic assays and genotypic assays. These assays provide complementary information and, depending on the clinical context, one may be preferable to the other. Both assays require amplification of the HIV genome and a minimum HIV RNA 500–2000 cpm. However, amplification of low-level HIV RNA is problematic and most experts do not recommend resistance testing if the plasma RNA is <1000 cpm.

Phenotypic assays directly measure ARV resistance, analogous to antibiotic resistance testing for bacteria. Unfortunately, in vitro culture of HIV is more complex and variable, takes many weeks, and is more expensive than bacterial culture. As a result of innate differences in peripheral blood mononuclear cells among individuals and variable reproducibility, this traditional approach has been limited to research laboratories. The use of engineered cell lines and recombinant molecular biology techniques has led to the development of rapid and reproducible testing. These assays selectively amplify a portion of the HIV genome targeted by a particular ARV class (eg, the gag gene for reverse transcription and protease inhibitors). The amount of ARV that suppresses viral replication by one-half (IC50) is compared with the value obtained for an HIV reference strain. The difference in IC50 between the patient and reference strains is reported as a fold-change in susceptibility, thereby permitting a quantitative assessment of the degree of resistance.

Phenotypic testing is able to account for the overall effect of mutational interaction (within a region) and does not require a priori knowledge of DRMs. Although laboratory results are easily interpreted, the correlation between these laboratory values and clinical cut-off values for susceptibility are limited.[9–11] Nevertheless, phenotypic testing is preferred for patients who have failed multiple ARV regimens, patients with a complex history of ARV regimens, and chronically-infected patients.

Genotypic assays detect changes in the nucleotide sequence of the relevant gene targeted by the ARV. Most genotypic assays sequence the entire gene (commonly gag). Known DRMs, other uncharacterized mutations, or mutational patterns associated with resistance are reported. Genotypic assays are more readily available, cheaper, and have a faster turnaround time compared with phenotypic assays. However, this approach assumes detailed knowledge of the mutational pathways associated with resistance. For some drugs, the relationship is straightforward. For example, nevirapine selects for a single amino acid change from lysine

(K) to asparagine (N) at position 103 [the K103N mutation], which confers very high-level resistance to nevirapine and other NNRTIs.

Other single amino acid changes such as the M184V mutation at position 184 are associated with high-level resistance to some drugs, but increased susceptibility to others (Tables 2 and 3). More complex mutational patterns are associated with resistance to other NRTIs and PIs. The degree of resistance to NRTIs increases with sequential accumulation of 6 mutations (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) referred to as thymidine analog-associated mutations (TAMs).[12]

The use of each PI is associated with major “signature” DRMs that confer resistance. Under selective pressure, however, “minor” mutations can also arise probably to compensate for major DRMs. These minor mutations can increase cross-resistance to other PIs. Consequently, some experts recommend phenotypic testing when PI mutations are detected by genotypic resistance testing or PI resistance is suspected.

The dynamic and complex nature by which resistance evolves makes interpretation of a genotype difficult, particularly in ARV-experienced subjects. There are several public access websites with listings of DRMs[13] (Table 4) and most commercial laboratories provide an interpretation. However, these algorithms may not be up-to-date and may not accurately predict the overall effect of mutational interaction. Consultation with specialists in HIV drug resistance is recommended because studies show it improves virologic outcome. Genotypic analyses can be problematic for newer agents (eg, CCR5 receptor antagonists), where no clear associations between mutations and decreased susceptibility have been established. The cost and accessibility of genotypic assays make them the preferred test for ARV naive individual.[7]

Neither assay reliably detects viral variants present in low frequencies (<20% of the quasi-species), nor variants archived in cellular reservoirs. Many mutations revert to wild-type within 4–6 weeks after ARV discontinuation, and viral replication dynamics often lead to reversion to wild-type as the dominant quasi-species. However, clinical trials have shown that reinstitution of ARVs rapidly selects for resistant variants that can result in virologic failure. For this reason, resistance testing is recommended before or within 4 weeks of discontinuing ARV therapy.[14]

Genotyping assays can also be used to generate a “virtual phenotype” by using databases that list paired genotypes and phenotypes of clinical specimens. The genotype of the test sample is compared with those within the database, and the matching phenotypes of viruses in the database that have similar mutation patterns are reported. However, this method provides limited representation of samples from a particular geographic region and can include many samples from the same patient over time. In addition, it may skew data toward older ARVs because few samples in the database will represent newer ARVs.

## SUMMARY

HIV can develop resistance to all currently available ARVs. To maximize the likelihood of successful virologic suppression, resistance testing should be performed in all patients newly diagnosed with HIV infection, including pregnant women, adolescents and perinatally infected infants. It should also be performed in individuals who fail to achieve virologic suppression or immune reconstitution despite appropriate ARV therapy (whether treatment naive or experienced).

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## TABLE 1

Recommendations for Drug Resistance Testing

| |
|---|
| Drug resistance testing should be performed (assuming VL >1000 copies/mL): |
| When HIV-1 infected patients enter into care regardless of whether therapy will be initiated immediately |
| Repeat testing at the time of ARV therapy initiation should be considered if therapy is deferred |
| Genotypic testing is generally preferred for ARV-naïve persons |
| To assist in selecting active drugs when changing ARV regimens in cases of virologic failure or when a change in clinical, immunologic, or virologic status is noted that might warrant a change in ARV regimen |
| Virologic failure is defined as the inability to achieve virologic suppression within 16–24 wk after initiation of therapy |
| Testing should be performed while the patient is taking the failing ARV regimen or within 4 wk of discontinuing therapy |
| When managing suboptimal viral load reduction (<3-fold reduction or <0.5 $\log_{10}$ copies/mL change in viral load) |
| For all pregnant women before initiation of therapy and for those entering pregnancy with detectable HIV RNA levels while on therapy |

Modified from Recommendations on Antiretroviral Drug Resistance Testing from “Guidelines for the Use of Antiretroviral Agents in HIV-1-infected Adults and Adolescents,” January 29, 2008 and “Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection,” February 28, 2008.

## TABLE 2

Current FDA-approved Antiretroviral Agents and Commonly Associated Drug Resistance Mutations

| Antiretroviral Drugs | Commonly Selected Mutations After Exposure |
|---|---|
| Nucleoside reverse transcriptase inhibitors (NRTI) | |
| abacavir (*Ziagen*, ABC) | K65R, L74V, Y115F, M184V |
| abacavir/lamivudine (*Epzicom*, ABC/FTC) | K65R, M184V |
| abacavir/lamivudine/zidovudine (*Trizivir*, ABC/3TC/AZT) | M184V |
| didanosine (*Videx*, DDI) | K65R, L74V |
| emtricitabine (*Emtriva*, FTC) | K65R, M184V/I |
| emtricitabine/tenofovir (*Truvada*, FTC/TDF) | K65R, M184V |
| lamivudine (*Epivir*, 3TC) | K65R, M184V/I |
| lamivudine/zidovudine (*Combivir*, 3TC/AZT) | M184V, thymidine analog mutations |
| stavudine (*Zerit*, D4T) | M41L, D67N, K70R, L210W, T215Y/F, K219Q/E |
| tenofovir (*Viread*, TDF) | K65R, K70E |
| zidovudine (*Retrovir*, AZT) | M41L, D67N, K70R, V118I, L210W, T215Y/F, K219Q/E |
| Non-nucleoside reverse transcriptase inhibitors (NNRTI) | |
| efavirenz (*Sustiva*, EFV) | L100I, K103N, V106M, V108I, Y181C/I, Y188L, G190S/A, P225H |
| etravirine (*Intelence*, ETR) | V90I, A98G, L100I, K101E/P, V106I, V179D/F/T, Y181C/I/V, G190S/A |
| nevirapine (*Viramune*, NVP) | L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A |
| Protease inhibitors (PI) | |
| atazanavir (*Reyetaz*, ATV) | L10F/I/V/C, G16E, K20R/M/I/T/V, L24I, V32I, L33I/F/V, E34Q, M36I/L/V, M46I/ L, G48V, I50L, F53L/Y, I54L/V/M/T/A, D60E, I62V, I64L/M/V, A71V/I/T/L, G73C/S/T/A, V82A/T/F/I, I84V, I85V, N88S, L90M, I93L/M |
| darunavir (*Prezista*, DRV) | V11I, V32I, L33F, I47V, I50V, I54M/L, G73S, L76V, I84V, L89V |
| fosamprenavir (*Lexiva*, FPV) | L10F/I/R/V, V32I, M46I/L, I47V, I50V, I54L/V/M, G73S, L76V, V82A/F/T/ S, I84V, L90M |
| indinavir (*Crixivan*, IDV) | L10I/R/V, K20M/R, L24I, V32I, M36I, M46I/L, I54V, A71V/T, G73S/A, L76V, V77I, V82A/F/T, I84V, L90M |
| lopinavir/ritonavir (*Kaletra*, LPV/r) | L10F/I/R/V, K20M/R, L24I, V32I, L33F, M46I/L, I47V/A, I50V, F53L, I54V/ L/A/ M/T/S, L63P, A71V/T, G73S, L76V, V82A/F/T/S, I84V, L90M |
| nelfinavir (*Viracept*, NFV)<br>ritonavir (*Norvir*, RTV) | L10F/I, D30N, M36I, M46I/L, A71V/T, V77I, V82A/F/T/.S, I84V, N88D/S, L90M |
| saquinavir (*Invirase*, SQV) | L10I/R/V, L24I, G48V, I54V/L, I62V, A71V/T, G73S, V77I, V82A/F/T/S, I84V, L90M |
| tipranivir (*Aptivus*, TPV) | L10V, I13V, K20M/R, L33F, E35G, M36I, K43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D, I84V, L90M |
| Fusion inhibitors | |
| enfuvirtide (*Fuzeon*, T20) | G36D/S, I37V, V38A/M/E, Q39R, Q40H, N42T, N43D |
| CCR5 antagonists | |
| maraviroc (*Selzentry*, MVC) | |
| Integrase inhibitors | |
| raltegravir (*Isentriss*, RAL) | Q148H/K/R, N155H |
| Combination drug(s) | |
| efavirenz/emtricitabine/tenofovir (*Atripla*, EFV/FTC/TDF) | |

Drugs no longer being manufactured: zalcitabine (DDC, HIVID), delavirdine (Rescriptor), or amprenavir (Agenerase). From the International AIDS Society-USA (Johnson, et al, *Top HIV Med.*, 2008).

## TABLE 3

Common Drug Resistance Mutations and Their Effects

| Drug Resistance Mutations | Selected by Exposure To | ↑ Susceptibility | ↓ Susceptibility | Other Effects |
|---|---|---|---|---|
| M184V | 3TC (lamivudine)<br>FTC (emtricitabine) | AZT (zidovudine)<br>D4T (stavudine)<br>TDF (tenofovir) | ABC (abacavir)<br>DDI (didanosine)<br>3TC (lamivudine)<br>FTC (emtricitabine) | Delayed TAMs |
| TAMs | AZT (zidovudine)<br>D4T (stavudine) | | To all nRTIs based on no. of TAMs | |
| Q151M complex | AZT/DDI<br>DDI/D4T | | Resistance to all NRTIs except TDF | |
| T69 insertion | AZT/DDI<br>DDI/D4T | | Resistance to all NRTIs | |
| K65R | TDF (tenofovir)<br>ABC (abacavir)<br>DDI (didanosine) | AZT (zidovudine) | TDF (tenofovir)<br>ABC (abacavir)<br>DDI (didanosine)<br>3TC (lamivudine)<br>FTC (emtricitabine) | |
| L74V | ABC (abacavir)<br>DDI (didanosine) | AZT (zidovudine)<br>TDF (tenofovir) | ABC (abacavir)<br>DDI (didanosine) | |
| E44D, V118I | DDI (didanosine)<br>D4T (stavudine) | | Increased resistance to NRTIs | |

Modified from the International AIDS Society-USA (Gallant, *Top HIV Med.*, 2005).

**TABLE 4**

Web Resources for Drug Resistance Mutations

| |
|---|
| Drug Resistance Mutation Summaries |
| International AIDS Society-USA Drug Resistance Mutation List, http://www.iasusa.org/resistance_mutations/mutations_figures.pdf |
| The Los Alamos National Laboratories HIV Sequence Database, http://resdb.lanl.gov/Resist_DB/default.htm |
| The Stanford University HIV Drug Resistance Database, http://hivdb.stanford.edu |
| Public Systems for Interpretation of Genotypic Resistance Assays |
| Geno2Pheno *German National Reference Center*, http://www.geno2pheno.org/cgi-bin/geno2pheno.plr |
| Antiretroscan *Italian Antiretroviral Resistance Cohort Analysis Multicenter Collaboration*, http://www.hivarca.net/hiv_resistance.asp |
| The Rega Institute System *Katholieke Universiteit (Leuven, Belgium)*, http://www.rega.kuleuven.be./cev |
| The Agence Nationale de Recherches sur le Sida (ANRS) System *French ANRS (National Agency for AIDS Research) AC11 Resistance group*, http://www.hivfrenchresistance.org |