INTRODUCTION

The first response to the HIV epidemic—identified as AIDS in 1981—was the feeling of helplessness. The cause of the illness was soon identified as Human Immunodeficiency Virus, the most widespread retrovirus ever known to mankind. Even though Azidothymidine (Zidovudine) was identified as an effective reverse transcriptase inhibitor, it was not very effective in the long run when used alone. Because of the complexities of viral dynamics, it took many years for a treatment strategy to be formulated. From a single drug the armamentarium has grown to many drugs under many groups. HIV replication is prone for frequent errors. Within a single individual, innumerable variants differing from the originally infected virus coexist. HIV diversity leads to emergence of resistance to drugs. As newer drugs were introduced, resistance of the viruses also emerged, necessitating the need for further research. First failures paved the way for more research for successes and the story is continuing. Here we analyse the phenomenon of drug resistance and the options for further treatments being available as second, third or many levels of alternate regimens. The present day ARTs act on the three products of HIV-1 env and pol, which includes reverse transcriptase, protease and the fusion glycoprotein gp 41. Mutations are also selected at different rates. When a single base change confers high-level resistance, such as M184V (methionine to valine at amino acid position 184) to lamivudine, the mutant can predominate within 2 weeks. But when multiple targets are required, it may take longer and longer periods for the resistant viruses to emerge.

EVOLUTION:

Despite adequate efficacy in suppressing viral replication, many ARV regimens fail ultimately. One of the major reasons is the development of resistance by the virus. These tend to be very common, as observed in studies quoting figures of 50 to 80% of these with detectable viral loads. It is postulated that many viruses acquire resistance because of selective drug pressure. However primary resistance, defined as that seen in patients who have not been exposed to antiretrovirals is also on the increase. This is thought to be due to transmission of viruses with resistance in the first instance. Viral mutants with resistance patterns evolve in an infected patient over a period of time even before treatment initiation, but once selective drug pressure reduces wild type viruses, the resistant strains predominate. ART using multiple drugs was envisaged to protect against this, as one mutation will not interfere with the effects of all the drugs used.

The summary of mutations associated with HIV-1 drug resistance is regularly updated and published by the International AIDS Society USA Drug Resistance Mutations Group and can be accessed at www.iasociety.org.

Nucleoside Reverse Transcriptase Inhibitors (NRTI): Mutations associated with resistance to nucleoside analogues are referred to as nucleoside-associated mutations (NAM). Thymidine analogues, such as zidovudine and stavudine, tend to select 1 of 2 groups of mutations: either M41L, L210W, and T215Y; or D67N, K70R, and K219Q. These mutations phosphorolysate and therefore allow the removal of chain-terminating nucleotide reverse transcriptase inhibitors (NRTI) from the elongating DNA, after which, reverse transcription of the RNA to DNA can continue. Viral replication under pressure from thymidine analogs allow NAM to accumulate, selecting for progressively greater resistance that extends to all NRTI, except for lamivudine and emtricitabine. Other less frequently selected mutations cause steric hindrance of ARV binding to RT. These mutations, including M184V, Q151M, L74V, and K65R, favor the binding of natural nucleotides. Among these mutations, M184V confers resistance only to lamivudine and emtricitabine, whereas Q151M confers resistance to all NRTI. Certain mutations are known to significantly impair viral
HIV Drug Resistance and Second Line of ART

replication. For example, M184V, selected by lamivudine and emtricitabine, decreases the replication capacity of virus by 40% to 60%. Mutations that have profound effects on viral replication can delay immune depletion. Furthermore, mutations that have been associated with hypersusceptibility of the virus to other NRTI and NNRTI have been linked to significant decreases in plasma HIV-1 RNA levels and increases in CD4 cell counts.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI): NNRTIs select mutations in a groove of RT that preclude the binding of NNRTI. One of 2 patterns of mutations generally predominate: K103N, V106M, and Y188L; or L100I, V106A, Y181C/I, G190S/A, and M230L. However, individual mutations can cause high-level resistance, as well as cross-resistance to all currently available NNRTI.

Protease Inhibitors (PI): Mutations encoding structural changes within the active site of protease that inhibit binding of PI are referred to as major mutations. In contrast, minor mutations encode changes located outside of the active enzymatic site, and at times outside of the protease gene. The minor mutations appear to compensate for the reduction in fitness caused by the major mutations. Protease inhibitor mutations can be specific for a certain ARV. For example, D30N appears to confer resistance only to nelfinavir. However, several mutations, including V82A/F/T/S/L, 84V, and L90M, confer broad cross-resistance to most PIs. In addition, as with other classes of ARV, hypersusceptibility has been reported. These observations have led to speculation regarding the optimal sequence for use of PI in treatment, to maximize the benefit from each. Susceptibility of HIV-1 to most PI (except nelfinavir) is affected by the use of ritonavir, which pharmacologically “boosts” concentrations of PI to levels that suppress replication of viruses with low-level resistance. In addition, certain non-peptidic PI, such as tipranavir and others in development, appear to be extremely potent, due to flexibility in their structure that allows tight binding to Protease even when it is mutated.

Fusion Inhibitors: HIV-1 resistance to fusion inhibitors (ie, enfuvirtide) is due to mutations in env encoding the ARV binding site on gp41, spanning positions 36 to 45, and 138. These mutations have a profound effect on the replication capacity of HIV-1. Thus, if viral replication is not suppressed to undetectable levels, the selection of virus highly resistant to enfuvirtide should decrease plasma viral load to some degree and allow CD4 cells to increase.

Resistance Mutations Considered Significant in Sequential Analysis

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Mutation</th>
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<tbody>
<tr>
<td>PI Major</td>
<td>30N, 33F, 46I/L, 48V, 50V/L, 82A/F/T/S/L, 84V, 90M</td>
</tr>
</tbody>
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RESISTANCE TESTING

HIV-1 resistance to antiretrovirals is evaluated by either genotypic or phenotypic testing. Commercial tests utilize HIV-1 RNA isolated from plasma. Genotypic assays detect drug resistance mutations present in relevant viral genes. The viral RNA is reverse transcribed into cDNA, PCR amplified, then sequenced directly or inserted into a replication competent viral construct for phenotypic testing. Commercial genotypic resistance assays directly sequence the PCR-amplified cDNA encoding protease and reverse transcriptase genes. Interpretation of test results requires knowledge of the mutations that different antiretroviral drugs select for and of the potential for cross resistance to other drugs conferred by certain mutations. The sequence data is analyzed based on the rules created by panels of experts (eg, HIV-1frenchresistance.org, Stanford database, and others) that interpret the available data regarding mutations and their associated clinical significance. Genotypic assays can be performed rapidly, and results can be reported within 1–2 weeks of sample collection.

Phenotypic assays measure the ability of a virus to grow in different concentrations of antiretroviral drugs. Reverse transcriptase and protease gene sequences derived from patient plasma HIV RNA are inserted into the backbone of a laboratory clone of HIV, either by cloning or by in vitro recombination. The cDNA of interest, generally pol, encoding reverse transcriptase and protease, is amplified and inserted into a recombinant virus that is grown in increasing concentrations of an antiretroviral drug. Viral replication is measured by a variety of reporter systems. Typically, the 50% inhibitory concentration (IC50) is determined for a panel of ARV. Resistance is reported as fold change in IC50, calculated by comparing the IC50 of the recombinant “patient” virus to the IC50 of a laboratory strain. The fold change is interpreted with respect to biologic and clinical cut-offs. The biologic cut-off is determined statistically based on the range of resistance measured on testing of virus from ART-naive individuals. Genotypic testing can be performed more rapidly (1 to 2 weeks) than phenotypic testing (2 to 3 weeks). Phenotypic testing is also less expensive. However, genotypic analyses only assess mutations that have been correlated with phenotypic resistance, virologic failure, or clinical outcome. Furthermore it is impossible for a rules based system to accommodate all of the possible mutation combinations, and it is often difficult to predict the combined effect of a collection of mutations. In contrast, phenotypic testing could hypothetically detect resistance not characterized by mutations.

Both geno- and phenotypic techniques have additional limitations. Further limitations of both genotypic and phenotypic assays include lack of uniform quality assurance for all available assays, relatively high cost, and insensitivity for minor viral species. If drug-resistant viruses are present but constitute <10%–20% of the circulating virus population, they probably will not be detected by available assays. This precludes testing of low-level viremias (50-500 copies/mL) that have infrequently been associated with the selection of drug-resistant virus. Resistance testing is of greatest
value when performed before or within 4 weeks after drugs are discontinued. Both methods are prohibitively expensive for the majority of individuals living with HIV-1.

**USEFULNESS OF RESISTANCE TESTING ON CLINICAL MANAGEMENT**

There is enough theoretical support for using resistance testing in developing a therapeutic regimen, but many randomized control trials (RCT) are inconclusive in suggesting direct application of the results to the patient. Virologic outcome was improved in association with resistance testing in few of the trials when assessed by the percent of subjects with plasma HIV-1 RNA below the limits of detection, and when the change in plasma viral load was compared. Notably, when benefits were observed, these were generally small and evaluated over a relatively short duration; 3 to 6 months. Subgroup analyses within the various trials identified populations that may be especially likely to benefit from drug-resistance testing; however, a consistent patient-profile was not observed across the studies. Importantly, among studies reporting changes in CD4 cell counts none observed improvements of immune status in association with testing for HIV-1 drug resistance. Many meta analysis reports concluded that genotypic testing offered some benefit, but that the effect was marginal; estimated as a 10% reduction in the percentage of subjects with a plasma viral below detection and a 0.2-0.3 log10 decrease in plasma viral load 6 months after changing ART.

Why has HIV Drug Resistance Testing not had a Greater Benefit on Treatment Outcome has also been discussed at length. Multiple factors impact on whether HIV-1 replication is suppressed by a particular ARV regimen. Of foremost importance is whether adequate ARV levels are achieved intracellularly, where the virus replicates. Therapeutic ARV levels depend on the correct dosing of ARV, the individuals adherence to prescribed ART, gastrointestinal pathology that limits absorption, and host polymorphisms that affect drug transport from the gut lumen into the blood and excretion from the cytosol. Viral factors (eg, the rate of replication, the presence of archived drug resistance mutations), and ARV factors (eg, number of mutations needed for high-level drug resistance, also known as the genetic barrier, penetration into tissues, and availability of agents that are not cross-resistant with previous ARV) also affect whether ART will suppress viral replication. Given the complex host-viral-drug relationships multiple factors can contribute to treatment failure. Nevertheless, poor adherence to ARV figures prominently in treatment failure. Unfortunately, adherence patterns, similar to other human behaviors, may be difficult to modify. In addition, measurement of intracellular ARV levels is not widely available. Thus, while resistance testing may guide the choice of appropriate ART, other changes may be essential for effective suppression of viral replication.

Several advisory panels have published recommendations on when to do and how to use HIV resistance testing. These include the International AIDS Society-USA Panel, the Euro guidelines group for HIV-1 resistance, and the Panel on Clinical Practices for Treatment of HIV-1 infection convened by the Department of Health and Human Services. The Department of Health and Human Services Panel (2008) recommends genotypic resistance testing for patients who have pretreatment HIV RNA >1,000 copies/mL, when the patient enters into care, regardless of whether therapy will be initiated immediately or not. The recommendations are summarized as follows.

- HIV drug resistance testing is recommended for persons with HIV infection when they enter into care regardless of whether therapy will be initiated immediately (AIII). If therapy is deferred, repeat testing at the time of antiretroviral therapy initiation should be considered.
- A genotypic assay is generally preferred for antiretroviral-naive persons.
- HIV drug resistance testing should be performed to assist in the selection of active drugs when changing antiretroviral regimens in cases of virologic failure and HIV RNA levels >1,000 copies/mL. In persons with >500 but <1,000 copies/mL, testing may be unsuccessful but should still be considered.
- Drug resistance testing should also be performed when managing suboptimal viral load reduction.
- Drug resistance testing in the setting of virologic failure should be performed while the patient is taking his/her antiretroviral drugs, or immediately (i.e., within 4 weeks) after discontinuing therapy.
- Genotypic resistance testing is recommended for all pregnant women prior to initiation of therapy and for those entering pregnancy with detectable HIV RNA levels while on therapy.

The following points are to be noted.

1. Transmission of drug-resistant HIV strains has been well documented and has been associated with suboptimal virologic response to initial antiretroviral therapy.
2. Recent studies suggest the risk that transmitted virus will be resistant to at least one antiretroviral drug is in the range of 6%-16%, with 3%-5% of transmitted viruses exhibiting reduced susceptibility to drugs from more than one class, depending on the prevalences that keep on changing.
3. The rate at which transmitted resistance-associated mutations revert to wild-type virus has not been completely delineated, but mutations present at the time of HIV transmission are more stable than those selected under drug pressure, and it is often possible to detect resistance-associated mutations in viruses that were transmitted several years earlier.

**USE OF RESISTANCE ASSAYS IN THE EVENT OF VIROLOGIC FAILURE**

Resistance assays are useful in guiding decisions for patients experiencing virologic failure while on antiretroviral therapy. Prospective data supporting drug-resistance testing in clinical practice at this time are derived from trials in which test utility was assessed for cases of virologic failure. These studies involved genotypic assays, phenotypic assays, or both. In general, these studies indicated that early virologic response to salvage regimens
was improved when results of resistance testing were available to guide changes in therapy, compared with responses observed when changes in therapy were guided only by clinical judgment. Thus, resistance testing appears to be a useful tool in selecting active drugs when changing antiretroviral regimens in cases of virologic failure with HIV RNA >1,000 copies/mL. In persons with >500 but <1,000 copies/mL, testing may be unsuccessful but should still be considered.

Resistance testing also can help guide treatment decisions for patients with suboptimal viral load reduction. Virologic failure in the setting of combination antiretroviral therapy is, for certain patients, associated with resistance to only one component of the regimen. In that situation, substituting individual drugs in a failing regimen might be possible, although this concept will require clinical validation.

**Use of Resistance Assays in Pregnant Patients**

In pregnant women, the goal of antiretroviral therapy is to maximally reduce plasma HIV RNA to provide appropriate maternal therapy and to prevent mother-to-child transmission of HIV. Genotypic resistance testing is recommended for all pregnant women prior to initiation of therapy and for those entering pregnancy with detectable HIV RNA levels while on therapy. Optimal prevention of perinatal transmission may require initiation of antiretroviral therapy before results of resistance testing are available.

**MANAGING A PATIENT WITH FIRST LINE FAILURE**

Majority of patients on first line with triple drugs will eventually develop failure, defined as suboptimal response to therapy. This is associated with virologic failure, immunologic failure and clinical progression. Many patient, physician, community related factors may play their roles in this situation. Data from some patient cohorts suggest that suboptimal adherence and toxicity accounted for 28%–40% of treatment failure and regimen discontinuations. Resistance to ARV is a major cause.

**Management of Virologic Failure.**

Ideally, one should design a regimen with at least two, and preferably three, fully active drugs on the basis of drug history, resistance testing, or new mechanistic class. Some antiretroviral drugs (e.g. NRTIs) may contribute partial antiretroviral activity to an antiretroviral regimen, despite drug resistance. Because of the potential for drug-class cross resistance that reduces drug activity, using a “new” drug that a patient has not yet taken may not mean that the drug is fully active. Drug potency and viral susceptibility are more important than the number of drugs prescribed. Many studies have shown that patients who received more active drugs (e.g., a ritonavir-boosted PI and a drug with activity against resistance viral strains with or without a new mechanism of action) had a better and more prolonged virologic response than those with fewer active drugs in the regimen. These studies illustrate and support the strategy of conducting resistance testing while a treatment-experienced patient is taking a failing regimen, designing a new regimen based on the treatment history and resistance testing results, and selecting active antiretroviral drugs for the new treatment regimen.

In general, adding a single, fully active antiretroviral drug in a new regimen is not recommended because of the risk of development of rapid resistance. However, in patients with a high likelihood of clinical progression (e.g., CD4 T-cell count <100/mm3) and limited drug options, adding a single drug may reduce the risk of immediate clinical progression, because even transient decreases in HIV RNA and or transient increases in CD4 T-cell counts have been associated with clinical benefits. Discontinuing or briefly interrupting therapy (even with ongoing viremia) may lead to a rapid increase in HIV RNA and a decrease in the CD4 T-cell count, and it increases the risk for clinical progression. Therefore, it is not recommended.

**Sequencing and Cross Resistance: The order of use of some antiretroviral agents may be important. Cross resistance among NRTIs is common but varies by drug. Most, if not all, mutations associated with efavirenz resistance cause cross resistance to nevirapine, and vice versa. Novel early mutations to some PIs (e.g., unboosted fosamprenavir, atazanavir, nefinavir, saquinavir) that do not confer cross resistance to other PIs may occur initially, but subsequent accumulation of additional mutations confers broad cross resistance to the entire PI class. Pharmacologic boosting of PIs with ritonavir markedly reduces the likelihood of PI resistance with failure in patients without pre-existing PI mutations. Tipranavir and darunavir are the two newest PIs effective for patients who are highly treatment-experienced or have HIV-1 strains resistant to multiple PIs based on demonstrated activity against PI-resistant viruses. However, with ongoing viremia and the accumulation of additional mutations, antiretroviral activity is time limited unless the regimen contains other active drugs (e.g., enfuvirtide, a CCR5 inhibitor, or an integrase inhibitor).

**RECENT ADDITIONS**

Maraviroc, the first approved CCR5 inhibitor, is an antiretroviral drug that specifically binds to the CCR5 receptor of the CD4 T-cell, thereby inhibiting HIV strains that use this co-receptor for cellular entry.

Raltegravir, the first approved HIV integrase inhibitor, specifically inhibits the final step in integration, strand transfer of viral DNA to host cell DNA.

Etravirine, an NNRTI, has activity in vitro against viral strains with mutations that confer resistance to efavirenz and nevirapine. Phase III clinical studies enrolled triple-class, treatment-experienced patients who experienced failure on their current antiretroviral regimens with detectable viremia. In these studies introduction of these new drugs resulted in significantly better virologic responses over 24 weeks compared with placebo when added to an antiretroviral regimen that was optimized based on treatment history and drug resistance testing.
CLINICAL SCENARIOS IN MANAGEMENT OF PATIENTS WITH ANTIRETROVIRAL TREATMENT FAILURE

1. Prior treatment with no resistance identified. Consider the timing of the drug resistance test (e.g., Was the patient off antiretroviral medications for more than 4 weeks?) and/or nonadherence. Consider resuming the same regimen or starting a new regimen and then repeating genotypic testing early (e.g., in 2–4 weeks) to determine whether a resistant viral strain emerges. Consider intensifying with one drug (e.g., tenofovir) (BII) or pharmacokinetic enhancement (ritonavir boosting for an unboosted PI, e.g. atazanavir, fosamprenavir).

2. Prior treatment and drug resistance. The goals in this situation are to resuppress HIV RNA levels maximally (e.g., to <50 copies/mL) and to prevent further selection of resistance mutations. With virologic failure, consider changing the treatment regimen sooner, rather than later, to minimize continued selection of resistance mutations. Discontinuing an NNRTI in a patient with ongoing viremia and evidence of NNRTI resistance to decrease the risk of selecting additional NNRTI resistance mutations is particularly important, because newer NNRTIs with activity against some NNRTI-resistant strains are available. A new regimen should include at least two, and preferably three, fully active agents.

3. Extensive prior treatment and drug resistance. The goal is to resuppress the HIV RNA levels maximally (e.g., to <50 copies/mL). With the availability of multiple new antiretroviral drugs, including some with new mechanisms of action, this goal is now possible in many patients, including those with extensive treatment experience and drug resistance. In some cases, however, viral suppression may be difficult to achieve. If maximal virologic suppression cannot be achieved, the goals are to preserve immunologic function and to prevent clinical progression (even with ongoing viremia). Even partial virologic suppression of HIV RNA >0.5 log10 copies/mL from baseline correlates with clinical benefits; however, this must be balanced with the ongoing risk for accumulating additional resistance mutations.

4. New regimen that contains at least two fully active agents cannot be identified. It is reasonable to observe a patient on the same regimen, rather than changing the regimen, depending on the stage of HIV disease. There is evidence from cohort studies that continuing therapy, even in the presence of viremia and the absence of CD4 T-cell count increases, decreases the risk of disease progression. Other cohort studies suggest continued immunologic and clinical benefits if the HIV RNA level is maintained <10,000–20,000 copies/mL.

Problems peculiar to developing nations with regard to second line therapy.

The most common first line regimen in resource poor setting will be a combination of 2 NRTIs (Zidovudine/ Stavudine plus Lamivudine and 1 NNRTI ( Nevirapine/Efavirenz)). In many cases of failure where resistance may not be the cause, unnecessary switches to second line is done which may happen prematurely or sometimes delayed. Late switches are associated with accumulation of TAMs which can cause cross resistance to many NRTIs being used in second line. This phenomenon can be reduced if Tenofovir is used in the first line, but that is not possible in many places. Many countries are also not capable of adopting many newer drugs which are expensive. The WHO has brought out an HIVResNet, a network of laboratories, clinicians and institutions for monitoring these and suggesting alternatives and capacity building. Early warning indicators and surveillance is being planned and implemented. These are expected to change the scenario of HIV resistance and make switches to second line more effective.

WHO Recommended second-line regimens in adults and adolescents

<table>
<thead>
<tr>
<th>First-line regimens</th>
<th>Second-line regimens for treatment failure</th>
<th>Alternative second-line regimens for treatment failure</th>
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<tbody>
<tr>
<td>ZDV/3TC/EFV or ZDV/3TC/NVP</td>
<td>d4T/ddI/RTV-PI, ab,c</td>
<td>RTV-Pla/ABC/ddI,d</td>
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<td>ZDV/3TC/NVP</td>
<td>d4T/ddIb,c/EFZ or d4T/ddIb,c/RTV-PIa,b,c RTV-PIa/ABC/ddIc,d</td>
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<tr>
<td>ZDV/3TC/ABC</td>
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<tr>
<td>ZDV/3TC/RTV-PI or ZDV/3TC/NNV</td>
<td>d4T/ddIb,c/EFZ or d4T/ddIINVP</td>
<td>ABC/ddI,d,EFZ or ABC/ddI/NVP</td>
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REFERENCES