31 *Emerging HIV Drug Resistance—How to Beat It?*

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Abstract: The management of HIV infection has posed significant problems. Management of patients with HIV infections has improved with the availability of a series of retroviral drugs. The cost of therapy is exorbitant, especially in the Indian context. Within a few years of their use, the medical fraternity has started encountering people with drug resistant viruses. This development of resistance is due to patient related factors like poor absorption of drugs, drug-drug interaction and nonadherence to treatment. The situation is confounded because of the improper use of these drugs by the doctors by not using adequate dosages and poor selection of the primary line of therapy.

It is important that the primary therapy which is chosen by the doctor is most effective in suppressing the viral load and bring it below 50 copies/ml and should be effective in maintaining this level of suppression of viral load over many years.

The corner stone of beating the drug resistance is to choose the therapy regime effectively and consciously and educate the patient about the regular usage of medicine and adequate dosages. The other modalities and combination of drugs used in patients who tend to develop resistance are discussed in detail in this Chapter.

EMERGING HIV DRUG RESISTANCE

All the organisms, macro as well as micro, have two goals—survival and replication. For both these functions, a Darwinian model of continuous selection of the 'fittest' is followed. Under the pressure of drugs which are directed against the survival as well as replication, every micro-organism alters itself in order to survive by developing drug resistance.

HIV drug resistance can be broadly defined as any change that improves viral replication and survival in the presence of inhibitors. It can appropriately be termed as 'altered drug susceptibility.' It is an altered phenotype resulting from a change in viral genotype.

HIV is a single stranded RNA virus, the genome being approximately 10,000 nucleotides in length. The virus lacks the correcting or proof reading mechanism.[1] The reverse transcription of viral RNA into DNA is error prone introducing on an average one mutation for each viral genome transcribed.

Moreover HIV has a very high rate of replication with a daily production of 10 billion virons, as a result of which large number of mutations are produced. Most of these errors are base substitutions, duplications, insertions and even recombination can occur. As a result, the patients have a complex and diverse mixture of viral quasi species each differing by one or more mutations. These mutations can confer some selective advantage to the virus such as decrease in its susceptibility to drugs (Darwinian model) compared to the wild type of virus. The rapidity of this process depends on the level of the selective advantage conferred by the mutation, the prevalence of the mutant within the virus population and the level of the drug at the site of HIV

replication. In some cases a substitution of a single amino acid can produce high level of resistance. In other cases only low level resistance can be induced by single mutation in which case high level resistance develops as a consequence of a stepwise accumulation of mutations.

ANTIRETROVIRAL (ARV) DRUGS

Available ARVs are targeted against viral enzymes viz. reverse transcriptase, protease and envelope glycoprotein gp120-gp41 (Annexure 1)

Mechanisms of Drug Resistance

Nucleoside Reverse Transcriptase Inhibitors (NRTI)

NRTI class of drugs arrest the synthesis of viral DNA by reverse transcriptase. After phosphorylation by cellular kinases, these compounds are incorporated into the nascent chain of viral DNA by reverse transcriptase. Because these drugs lack a 3' hydroxyl group, no additional nucleosides/nucleotides can be attached to them and the synthesis of viral DNA is arrested.[2,3]

Two distinct mechanisms are involved in HIV resistance to these drugs:

a. *Impairment of the incorporation of the analogue into DNA*[4,5,6] The first mechanism is mediated by mutations which allow the RT enzyme to discriminate against NRTIs during polymerization, thereby preventing their addition to the growing DNA chain.
b. *Removal of the analogue from the prematurely terminated DNA chain*[7,8] The second mechanism is also referred to as pyrophosphorolysis; nucleotide excision and primer unblocking. This mechanism is mediated by mutations that promote hydrolytic removal of chain terminating NRTI and thus enable to continue DNA synthesis. The hydrolytic removal requires a pyrophosphate donor which in most cells is ATP.[3]

In Panel A, the incorporation of a nucleoside analogue into drug-sensitive viruses results in the termination of the viral DNA chain. Mutations in drug-resistant viruses prevent the incorporation of the nucleoside analogue into the growing viral DNA chain. In Panel B, ATP in drug-sensitive viruses does not have access to a reverse transcriptase that has formed a complex with a nucleoside analogue. Mutations that cause resistance to nucleoside analogues, referred to as thymidine analogue mutations, allow ATP to bind reverse transcriptase near the 3' end of viral DNA terminated by the incorporation of a nucleoside analogue. ATP then excises the analogue from viral DNA, allowing reverse transcription to proceed normally.

Thymidine Analog Mutations (TAMs)[9]

Mutations at positions 41, 67, 70, 210, 215 and 219 are referred to as TAMs because they are most often selected by ZDV and STAV containing regimens. These mutations occur gradually and their order of emergence can vary. TAMS promote ATP or Phytophosphate mediated removal of nucleocide analog from 3' end of terminated DNA strand (Fig. 31.1). ATP then excises the analog from viral DNA allowing the reverse transcription to proceed normally. This observation helps to explain the reasons that the primer unblocking mutations cause highest level of phenotypic resistance to ZDV but also explains that these mutations can cause cross resistance to other NRTIs. Primer unblocking mutations at 215 position interfere with clinical response to ZDV, STAV, ABC, DDI and most dual NRTI regimens. The presence of 4 or more TAMs will typically cause more than 100 fold decreased susceptibility to ZDV, 5-7 fold decreased susceptibility to STAV, DDI, ddC and Tenofovir.

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)

Non-nucleoside reverse transcriptase inhibitors are small molecules having a strong affinity for a site close to the catalytic domain of RT, blocking its ability to synthesize viral DNA. The

mutations that are selected for, reduce the affinity of NNRTI drugs by (a) changing the conformation of the binding site and (b) by changes in RnaseH activity (Fig. 31.2).

Nonnucleoside reverse-transcriptase inhibitors (NNRTIs) have a strong affinity for a pocket next to the active site of reverse transcriptase. In drug-sensitive viruses, NNRTIs bind this pocket and block the polymerization of DNA by reverse transcriptase. In drug-resistant viruses, mutations prevent the binding of NNRTIs, allowing DNA polymerization to proceed normally.

Protease Inhibitors (PI)

HIV-1 protease enzyme is responsible for the post-translational processing of viral Gag-Pol polyprotein yielding the viral enzymes and the structural proteins facilitating the viral maturation. This enzyme is an aspartic protease. The three dimensional structure of wild type HIV-1 is shown in Figure 31.3. The PIs bind to certain sites and block the protease activity.

Mutations in the substrate cleft cause resistance by reducing the binding affinity between the PIs and the mutant protease enzyme. Most of these substrate cleft mutations cause a two to five fold reduction in *in vitro* susceptibility to one or more PIs.

Panel A shows the amino acid chains of both subunits of the protease. The protease inhibitor (ritonavir) occupies the central substrate-binding domain of the enzyme. The sites of amino acid residues most frequently involved in resistance to protease inhibitors are shown as red beads.

The designation of the corresponding residues is written in red for the first subunit of the protease and in blue for the other subunit. In Panel B, the substrate-binding cavity contains a protease inhibitor (lopinavir) in the context of either the sensitive protease (green) or the resistant protease (red). Modified from figures generated by Dr. Ladislau Kovari, Wayne State University School of Medicine, Detroit.

Fusion Inhibitors

HIV-1 enters the target cells by many intricate interactions with gp120-gp41 envelope glycoproteins thereby resulting into conformational change and facilitating fusion of viral and cellular membrane resulting into entry of viral core in the cell.

The fusion inhibitors destabilize this process by biding to hydrophobic region (HR1) thus blocking the infectivity of HIV-1. Viral resistance to fusion inhibitors results from mutations located in a stretch of 10 amino acids in HR1.

Convention for Describing Drug Resistant Mutation

Based on the amino acid sequences of RT and Protease, a standard numbering system has been developed in reference to wild type virus (mainly subtype B). For example, a change in genotype from ATG to GTG at codon 184 would be reported as a change from methionine to valine at residue 184 or M184V.

Methods to Test Resistance

1. *Phenotypic assay:* Phenotypic resistance of HIV is an *in vitro* test. HIV-1 isolates are cultured in presence of serial dilutions of inhibitor drugs. The concentrations required to inhibit virus replication by 50% Ic50 or 90% Ic90 are the most commonly used method. Resistance is roughly indicated by the increase in Ic50 to inhibit the growth of resistant virus compared to the wild virus. The process is expensive, cumbersome and time consuming.
2. *Genotypic assay:* The molecular mechanism underline the phenotypic susceptibility can be identified by studying the changes in the sequence of the gene coding for the enzyme target of ARVs, i.e. RT, protease and envelope glycoprotein. The characteristic genetic changes indicating specific mutations predict the subsequent resistance phenotype.
   Many mutations are observed. It has become customary to label them as:

Primary or major: They reduce the drug sensitivity by themselves. Whereas *secondary or minor* mutations are those which reduce the drug susceptibility in combination with primary mutations or improve the replicative fitness of the virus isolates with major mutations.

Rules based algorithms: The online Stanford HIV database alogorithm and the *Agence* Nationale de Recherche Sur Le SIDA (*aNRS*) algorithms are updated regularly and are used as reference for interpreting levels of resistance to 17 FDA approved RT and protease inhibitors.

How to beat the emergence of resistance?

Golden Rule: Less virus in the body, it is less likely to replicate and mutate!

The goal of effective ART is viral suppression below the detectable limits (< 50 copies/ml) for the longest period of time. In practice, this may not be achieved in every case due to factors which are related to the patient as well as doctor.

Patient Related Factors

1. *Poor absorption* Poor absorption due to any reason results into lower serum concentration of the drug. Patients with advanced disease suffer from severe OIs like oesophageal candidiasis, CMV oesophagititis which make swallowing a painful experience. Vomiting due to any reason can expel the drug from GI tract. Diarrhoea due to OIs, HIV enteropathy, GI malignancies may all affect the absorption and thereby serum concentration.
2. *Pill burden* Larger the pill burden, higher the non-compliance! Apart from antiretroviral drugs, an AIDS patient may also be receiving primary/secondary chemoprophylaxis against OIs along with other drugs for various disorders.
3. *Drug* Drug interactions—Drugs used for treatment of various HIV related conditions may interact with ARVs affecting the serum concentration, e.g. Rifampicin with NNRTI and PIs.
4. *Non-adherence* Anti-HIV drugs need to be taken strictly in accordance to the prescription. The time intervals, relation to food etc have to be scrupulously observed. There are several reports which indicate that 70% adherence suppresses the viral load only in about 20% cases while 95% adherence suppresses the viral load in more than 80% of cases.[10] 95% adherence means missing less than 1 dose a month.
5. *Varying pharmaco-kinetic* Individual variability in pharmaco-kinetic of different drug may affect the serum concentration.
6. *Doctor related factors* Selection of appropriate combination of drugs, proper dosages, definite duration of the therapy are the responsibilities of the doctor. However, in many developing countries, including India, the experience is contrary to the expectations. All these factors ultimately result into inadequate viral suppression accelerating viral replication and resistant mutations.

Clinical Implications

Emergence of drug resistance resulting into the virological failure is one of the major factors in treatment failure. It is therefore imperative to select the first or the primary regimen of therapy which is most effective in suppressing the viral load below 50 copies and maintaining the suppression over several years. Careful and proper selection of both, the motivated and the adherent patient and the most effective first regimen of triple combination of ARVs is crucial.

DHHS guidelines given in Table 31.1 define the optimum parameters for initiation of HAART.

The first line of HAART therapy is selected with due consideration to its efficacy, toxicity profile, ease of administration and cost. The NRTI backbone is selected first from group B, such as: (a) ZDV + 3TC, (b) TDF + 3TC, (c) ddI + 3TC, (d) ABC + 3TC. (ZDV + Stav, Stav + ddI, 3Tc + FTC, these are irrational combinations, never to be used). To this backbone, the drug from the group A is added, e.g. 2NRTI + 1NNRTI or 2NRTI + 1PI.

The virological, immunological and clinical response need to be assessed regularly. An effective first line regimen will show the decline in viral load by six weeks and will successfully suppress the virus to undetectable limits by 24 weeks. This response is appropriately reflected in rise in CD4 cell count and clinical improvement.

The non response to HAART can be defined as:

1. Reduction in the viral load which is less than 1.5 to 2.5 logs
2. Rising viral load after initial suppression and
3. Falling CD4 cell count, despite adherence.

The second line therapy needs a careful selection after considering various factors. At this stage, if the genotypic assays are available they guide the second line therapy based on the spectrum of resistance, cross resistance, viral susceptibility, etc. Patients adherence and the affordability may limit the choice of drugs in developing countries.

While switching therapy to second line, general principles to be observed are:

1. One active drug should not be added to a failing regimen.
2. Include at least two fully active agents in new regimen preferably with new mechanism of action.
3. If two active agents are not available, consider rPI + optimized background of drugs that provide partial activity.

The epidemic is maturing; larger population is becoming symptomatic requiring HAART. Many countries are presently offering the first line therapy either free or at a minimal cost. Extraordinary patient effort is required to comply with the drug regimen, particularly if they are expensive and inconvenient. Incomplete viral suppression under these circumstances predispose to emergence of drug resistant mutants. In the United States, as many as 50 percent of patients receiving ARVs are infected with the viruses that express resistance to atleast one of the available ARVs. There is a growing concern about the transmission of drug resistant strains (Fig. 31.5).[11]

Most of the data available on drug resistance is with reference to HIV-1 subtype B, which is the strain in the US and western Europe. Molecular characterization of HIV-1 isolates from Mumbai[12] showed a predominance of HIV-1 subtype C. Compared to subtype B, the drug naïve cases of subtype C exhibited important polymorphisms, substitution reaching to above 90 percent. However, only 2 out of 128 isolates showed M184V substitution in RT indicative of 3TC resistance. With the Govt. of India's ambitious programme of free ART, one needs to be cautions regarding emergence of drug resistant mutants in our populations. Multiple factors such as irrational combinations, inadequate dosages, non-adherence, advanced disease at the time of detection, etc. contribute to the rising emergence of viral resistance in India. The first line therapy in the Govt. program includes ZDV or Stav, both of which are associated with development of TAMs, making the selection of drugs for the second line therapy difficult. 136 drug experienced patients suspected to be resistant on the clinical parameters revealed that 129 had multiple drug resistant mutations.[13,14] It is well-known that clinical failure becomes apparent much after the virological failure.

Future Developments

With the rising challenge of drug resistant mutant strains, newer drugs need to be added to the armamentarium. Table 31.4 shows the drugs in the pepeline.

The newer targets of antiretroviral drugs are not only viral enzymes but also the chemokine receptors which facilitate the viral entry (Table 31.5).

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