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HIV Drug Resistance: An Overview



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ABSTRACT

HIV-1 belongs to the retrovirus family. Highly active antiretroviral therapy (HAART) is the current standard of care for HIV infection. The ability of HIV to mutate and reproduce itself in the presence of drugs is called “HIV drugs resistance” (WHO). Resistance of HIV to Antiretroviral Drugs (ARV) is one of the most common causes for therapeutic failure in people infected with HIV. The first report of HIV-1 drug resistance was to zidovudine (ZDV) in 1989. If the viral load rises above 200 copies/ml, it might be sign of emergence of drug resistance mutations. Viral load monitoring is still not available for everyone in limited resource settings. There are several resistance testing tests: genotype, phenotype, virtual phenotype and integrase inhibitor resistance sequencing tests. Genotype, phenotype and virtual phenotyping testing's used for NRTI, NNRTI and PI resistance mutations. ARV drug resistance in HIV is growing global concern. We should apply latest and current findings related to ARV drug resistance mechanisms. We should use resources wisely and in an effective manner.

INTRODUCTION

HIV-1 belongs to the retrovirus family and the etiologic agent of the AIDS that targets the human immune system (1). Resistance of HIV to Antiretroviral Drugs (ARV) is one of the most common causes for therapeutic failure in people infected with HIV (2). The first report of HIV-1 drug resistance was to zidovudine (ZDV) in 1989 (3). Highly active antiretroviral therapy (HAART) is the current standard of care for HIV infections. The introduction of ARV therapy exerts selective pressure over viral subpopulations to develop resistance (4). In 2012, an estimated 35.3 million people lived with HIV while 1.6 million AIDS-related deaths worldwide (1). ARV resistance impairs the response to therapy in patients with transmitted resistance, unsuccessful initial ARV therapy and multiple virological failures (5). The most recent ARV drugs have become superior against the administered drugs (1). Understanding the basic principles of HIV drug resistance is helpful in guiding individual clinical decisions and the development of effective regimen (5).

Currently available US FDA ARV drugs are as follows:

Table No. 1(6):

Categories	NRTIs	NNRTIs	PIs	Entry Inhibitors (CCR5 and Fusion inhibitors)	Integrase Inhibitors
Treatment naïve patients	Zidovudine (AZT) Stavudine (d4T) Lamivudine (3TC) Didanosine (ddI) Zalcitabine (ddC) Abacavir (ABC)	Nevirapine (NVP) Delviradine (DLV) Efavirenz (EFV) Rilpivirine ()	Ritonavir (RTV) Indinavir (IDV) Saquinavir (SQV) Nelfinavir (NFV) Amprenavir (APV) Fosamprenavir (FPV) Atazanavir (ATV)	-	-
Treatment experienced patients	Tenofovir (TDF)* Emtricitabine (FTC)*	Etravirine (ETV)	Tipranavir (TPV) Darunavir* (DRV)	Maraviroc* Enfuvirtide	Raltegravir*
Prevention of vertical transmission (WHO guidelines)	Zidovudine Lamivudine Tenofovir Emtricitabine	Nevirapine Efavirenz	-	-	-
Children	Zidovudine Stavudine Lamivudine Didanosine Emtricitabine Abacavir	Nevirapine	Ritonavir Nelfinavir Darunavir	Enfuvirtide	-
For post exposure prophylaxis	Zidovudine (AZT) Stavudine (d4T) Lamivudine (3TC) Didanosine (ddI) Emtricitabine (FTC) Tenofovir (TDF)	Efavirenz	Indinavir Nelfinavir Ritonavir Saquinavir Fosamprenavir Atazanavir Ritonavir/Lopinavir	Enfuvirtide	-
For pre exposure prophylaxis	Tenofovir and Emtricitabine in MSM				

*Approved for both treatment naïve and treatment experienced patients MSM – Men who have sex with men
NRTI = Nucleoside Reverse Transcriptase Inhibitors; NNRTI = Non Nucleoside Reverse Transcriptase Inhibitors; PI = Protease Inhibitors

DEFINITION

The ability of HIV to mutate and reproduce itself in the presence of drugs is called “HIV drugs resistance” (WHO) (7). Drug resistance in HIV is defined as a reduced susceptibility to a specific ARV drug (4). It has been clearly documented since the introduction of ARV therapy (4).

ARV drug resistance in HIV

The most common resistance associated mutations are within the non-nucleoside reverse transcriptase inhibitors (NNRTIs) drug class, followed by the nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) (4). The prevalence of resistance to integrase inhibitors in naive HIV positive patients is infrequent (4). The challenge of treating patients with multidrug-resistant HIV-1 has largely been addressed by the advent of newer PIs, second-generation non-nucleoside reverse transcriptase inhibitors and drug in novel class, including integrase inhibitors and CCR5 antagonist (8). The use of drug histories, genotype, phenotype, virtual phenotype, integrase inhibitor by sequencing, Trophile ES and Trophile DNA assays along with Gene sequencing Databases and expert consultation provide the tool needed to construct effective regimens (4).

Table No. 2 (9):

Table 2 - Resistance profile to antiretrovirals according to the Stanford HIV Drug Resistance Database, in the experienced group (n = 23)

Antiretroviral	Resistance (%)	Intermediate resistance (%)	Susceptible (%)
Abacavir	8.6	86.9	4.3
Didanosine	17.4	65.2	17.4
Emtricitabine	56.5	13	30.5
Estavudine	43.5	30.5	26
Lamivudine	52.1	17.4	30.5
Tenofovir	0	74	26
Zidovudine	48	26	26
Delavirdine	56.5	4.3	39.1
Efavirenz	56.5	4.3	39.1
Etravirine	4.3	56.5	39.1
Nevirapine	65.2	0	39.1
Atazanavir	4.3	43.4	52.1
Darunavir	0	30.4	69.5
Fosamprenavir	4.3	39.1	56.5
Indinavir	13	30.4	56.5
Lopinavir	0	43.4	56.5
Nelfinavir	30.4	17.4	52.1
Ritonavir	13	30.3	56.5
Saquinavir	8.6	39.1	52.1
Tipranavir	0	34.8	65.2

Types of resistance

Resistance is of two types:

- I. **Primary resistance or transmitted resistance:** When an individual is infected by strain of HIV-1 already resistant to one or more ARV drugs.
- II. **Secondary resistance or acquired resistance:** Development or resistance while on antiretroviral therapy (7).

Resistance may be intrinsic, such as the lack of activity of certain NNRTIs against HIV 2 virions (4).

Resistance in newer agents can emerge, however, resulting in the appearance of novel drug resistance mutations in the HIV- polymerase, integrase and envelope genes (8) .

Causes of resistance

Causes of resistance to ARV drugs are quite similar with the antibiotic resistance (10).

The World Health Organisation (WHO) has listed some conditions as factors that raise the development of drug resistance (4):

- ❖ Treatment with < 3 drugs
- ❖ Adding one drug to a failing regimen
- ❖ Interruption of treatment
- ❖ Prolonging the use of a failing regimen
- ❖ Inappropriate selection of drug (4).
- ❖ The extent to which virus replication continues during drug therapy, genetic barrier, and the effect of drug-resistance mutations on drug susceptibility and virus fitness (7).
- ❖ Influenced by natural genetic variability evolves with pharmacologic pressure from starting or stopping ARVs (4).

Changing patterns of HIV drug resistance

Changing ARV regimens and introduction of new ARV drugs have altered drug resistance patterns in resistance human immunodeficiency virus type 1 (HIV-1) (8). The last decade has seen a significant shift in the patterns of HIV-1 drug resistance. The widespread use of thymidine analogs such as zidovudine (ZDV) and stavudine (d4T) led to the common appearance of thymidine analog resistance mutations (TAMs). The accumulation of TAMs selected by ZDV and d4T resulted in cross-resistance to all members of NRTI class (8).

Resistance to Newer Drugs

1. Tipranavir

Of some 20 mutations at 16 protease codons identified as contributing to tipranavir resistance, many (e.g. at codons 13,35,43,58,74,and 83) have not been associated with resistance to other PIs (11).

2. Elvitegravir

Elvitegravir is an investigational Integrase inhibitor currently being in phase 3 clinical trials. Primary resistance mutations are based on results of *in vitro* selection experiments includes the Q143R, E92Q and T66I mutations (8).

3. Raltegravir

Raltegravir is the first INSTI approved for the treatment of HIV- infection. Data from clinical trials show that RAL resistance involves IN mutations Y143C(R), Q148H(R)(K) or NH, together associated secondary mutations that results in higher levels of resistance (8).

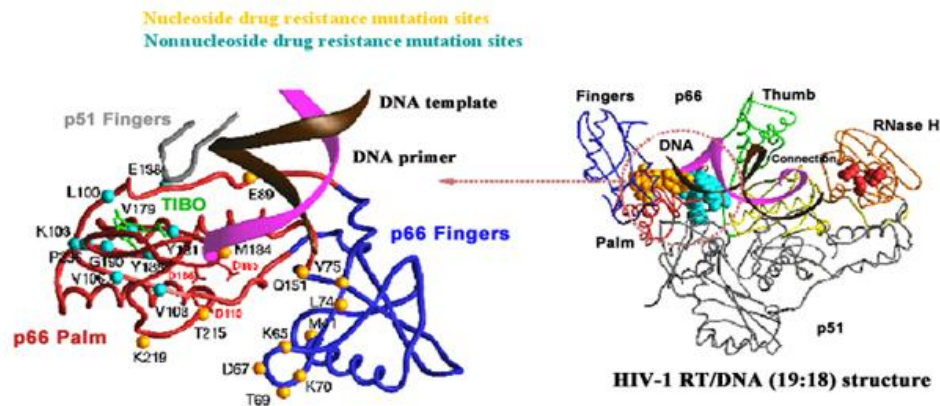
Mechanism of resistance

The HIV virion has two properties that increase its ability to develop ARV resistance: error prone copying and high rates of viral replication. HIV is non selective during copying and creates 1 error per each round of copying which may be base substitutions, insertions or deletions

.The combination of high rate of viral replication and error prone copying leads to multiple variants of virus known as quasi species (4).

Figure No. 1 (12):

Locations of Drug Resistance Mutation Sites in HIV-1 RT/DNA Structure



Proposed Mechanism of resistance

1} Drug Resistance by Antiretroviral (ARV) classes

1. Nucleoside reverse Transcriptase Inhibitors (NRTIs)
2. Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs)
3. Protease Inhibitors (PIs)
4. Integrase Inhibitors (INIs)
5. CC Chemokine Receptor 5 (CCR) Antagonists
6. Fusion Inhibitors (5)

2} Transmitted Drug Resistance

3} The basic mechanism of resistance includes

- A modulatory effect at the drug binding site.
- An enzyme activity that can remove the drug from its binding site.

- A size change in the drug binding site causing inability to compete for the enzyme (4).

Testing Methods for Antiretroviral (ARV) Resistance

There are several resistance testing tests: genotype, phenotype, virtual phenotype and integrase inhibitor resistance sequencing tests. Genotype, phenotype and virtual phenotyping testing's used for NRTI, NNRTI and PI resistance mutations (4). Neither genotype nor phenotypic testing overcome the barrier of detecting viral minority species (i.e. if viral species is present in less than <20% of the virion population (4,13).

1. Phenotypic Assay

Phenotype testing takes the virus and attempts to grow the virus in the presence of single drug (4,14). Phenotyping testing may be useful when attempting to identify resistance or sensitivity of virus to individual PIs and highly treatment-experienced patients (11). The most direct method measures the virus phenotypic susceptibility to drugs directly by culturing virus in the presence of increasing concentrations of the drug of interest. The conc. of drug required to inhibit replication by either 50% (IC_{50}) or 90% (IC_{90}) relative to a control virus is taken then taken as a measure of resistance. It commonly reports results as fold changes in the IC_{50} (7). Two phenotypic assays are available: Phenosense™ (Monogram Biosciences) and Anti-virogram™ (Virco) (5).

Advantages of phenotyping drug testing

- The amount of drug needed to inhibit the virus (at least in lab) is specified (11).
- It measures the cumulative effects of multiple drug mutations (7).
- Phenotypic testing also detects resistance that arises from previously unidentified resistance mutations (11).
- It provides quantitative assessment of susceptibility & interpretation is straight forward with good reproducibility (7).

Disadvantages of the assay

- High cost and lengthy turnaround time, (taking upto 4 weeks) (7) and the fact that values in susceptible range may be reported when there are resistant minority variants present (11).
- A more theoretical drawback is possibility of outgrowth of a minor highly resistant strain by minor resistant strain during the initial culture (7).
- It may not add new information to the genotype result or it may only give confusing results by only detailing single drug resistance when in actually a combination of drugs may yield hypersensitivity (i.e. lamivudine resistance can cause increased sensitivity to zidovudine) (4,15).

2. Genotypic assay

Genotypic assay looks at the point of mutation of the virus (4,14). Genotypic testing produces a nucleotide sequence usually encompassing the complete 297 nucleotides (or 99 amino acids) of HIV-Genotypic protease, and the 5' polymerase coding region of HIV-testing is reverse transcriptase, usually encompassing amino acids positions 40-240, the part of RTs containing the vast majority of NRTI- and NNRTI- resistance mutations (5). It is recommended in acute HIV infection (4). It uses PCR/DNA sequencing techniques to make many copies of HIV genetic material and then detect genetic sequence of particular viral enzyme, RTs and protease (7). There are two commercially available kits for genotypic resistance testing: TRUGENE[®]HIV-1 Genotypic Assay (Siemens, USA) and the Celera ViroSeq[®] HIV- Genotyping System (5).

Advantages of genotypic assay

- It has short turnaround time.
- It is less expensive and more sensitive to detect emerging resistance (7).
- Genotypic testing also permits determination of viral subtype and ruling out of contamination or sample mix-up by phylogenetic analysis (11).

Disadvantages of assay

- Sensitivity to detect minority species is low (7).
- A variant that constitutes less than 20-25% of total virus quasi species population in patient is generally not detected.

- Mutations conferring resistant to ARV drugs at positions is not previously characterized (7).
- A disadvantage is its reliance on algorithms for interpretation, given the fact that algorithm rely on expert opinion and thus emerge and are updated slowly (11).

3. Virtual Phenotypic Assay

It is the combination of first two methods. These are genotypes that are linked to known databases. These databases predicts phenotypic resistance and are helpful in interpreting genotypes of experienced patients (4).

Indian Scenario

In one study from South India where Stavudine or Zidovudine, Lamivudine & Nevirapine or Efavirenz was used as 1st line regimen like the ART programme of India, 88% of 138 first-line treatment failure patients, had ≥ 2 major non-nucleoside reverse-transcriptase and NRTI mutations. Of the NRTI resistance mutations, M184v was the most common (79%) Thymidine analogue mutations (TAMS) were found in 60% of patients. 25% patients had ≥ 3 NNRTI mutations (7, 16).

Monitoring Antiretroviral (ARV) Drug Resistance

Routine viral load monitoring and viral genotyping are the two things which help the clinicians to find better drug choices and combinations with minimum risk of drug resistance. Co-receptor tropism testing is also available to detect the resistance associated mutations. Viral load monitoring is important to identify individuals who are not adherent to their treatment and to switch treatment options according to the changes in viral load over time. If the viral load rises above 200 copies/ml, it might be sign of emergence of drug resistance mutations. Viral load monitoring is still not available for everyone in limited resource settings. For HIV-1 genotyping, there are several commercial or lab-developed tests are available to detect the resistance associated mutations. These tests mainly utilize PCR amplification and nucleotide sequencing of viral genes such as reverse transcriptase, protease, and integrase (1).

The promising new inhibitors: (but is no means of a comprehensive survey)

1- β -D-2,6-Diaminopurine (DAPD) is a dioxolane guanosine nucleoside analogue that is deaminated to 1- β -D-dioxolane guanosine (DXG). In the triphosphate form, DXG is an inhibitor of HIV- RT. This inhibitor has stimulated considerable interest as it appears to inhibit many strains of nucleoside analogue resistant HIV-1 (3, 17). The development of new generation NNRTI is focused on inhibitors that are able to suppress HIV-1 variants containing the common NNRTI mutations such as K103N. TMC120, a dianilinopyrimidine derivative, showed a high degree of activity against viruses harbouring single NNRTI mutations such as K103N, G190A and Y181C (3, 18).

Future Challenges

It is clear from currently available reports that among the untreated HIV-1 patients, the prevalence of known drug-resistance mutations is very low, when compared to alert cut-off (5%), which has been defined by the ad hoc working group of the WHO (19). Resistance has been reported to be limited in low and middle-income countries to 3.7% when compared with the 10-20% rates in Europe and US (4,20). HIV drug resistance field continues to move at a rapid pace. Each, new mutations are discovered. These mutations are due to subjected ARV therapy. Now, viruses becomes more resistant to ARV therapy due to consisting of potent inhibitory mechanisms. We obviously need to apply latest and current findings related to ARV drug resistance mechanisms. These understandings are important because they are directly related to resistance testing. In this way, we will become sure for therapy. We can also ensure that therapy is optimized and keeps pace with the relentless evolution of virus.

CONCLUSION

By referencing the sources mentioned below and reviewing papers, we come across to the fact that ARV drug- resistance in HIV is still increasing at high rate and treatment of HIV become complex due to introduction of new ARV drugs and classes of drugs in market and respected changes in virus's genetics. ARV drug resistance in HIV is growing global concern. We should apply latest and current findings related to ARV drug resistance mechanisms. These understandings are important as they are directly related to resistance testing. By combination of complex biochemistry and elegant structural studies one can reveal more details about the mutations involved. We should use resources wisely and in an effective manner.

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