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# Problems and Proceedings to Antiretroviral Therapy for the Treatment of AIDS

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**Abstract**

Long term use of antiretroviral therapy suppresses HIV infection in real. But due to mutation there is chance of drug resistance, drug toxicity, drug penetration, adherence to therapy, viral replication in cellular reservoirs and augmentation of host immune responses. Viral replication

causes the accumulation of drug resistance, mutations, increase in viral loads and disease progression. The optimization of antiviral therapy by the understanding of the pathogenesis of drug resistant HIV-1 is essential. In the present review outlines various problems to HIV therapy and discussions about the success of antiviral therapy.

***Keywords:*** Replication, drug resistance, antiviral therapy, progression.

## Introduction

Acquired immune deficiency syndrome (AIDS) is considered one of the most dangerous and a pandemic [1] disease which is present over a large demographic area of the world. The disease may cause Kaposi's sarcoma, pneumocytis carini pneumonia [2] and serious opportunistic infections. AIDS is the most serious infectious [3] disease and actively spreading worldwide among society. Human immunodeficiency virus infection/acquired immune deficiency syndrome (HIV/AIDS) is a disease of human immune system caused by infection with human immune deficiency virus. AIDS is called when a person infected with HIV has a CD4+ count of less than 200cells/μL or has an AIDS defining condition. While antiretroviral treatment [4,5] reduces the risk of death and complications from the disease these medications are expensive and may be associated with side effects. Antiviral agent has made HIV/AIDS a more manageable disease in some industrialized nations and several vaccines [6] are about to enter phase III clinical trials.HIV will doubtless continue to impose a terrible burden of morbidity and mortality. More importantly establishing the ground rules that underpin the evolution of HIV will lead to better vaccines and antiviral agents. The present review gives idea about targets of HIV by antiretroviral drugs, problems and proceedings to antiretroviral therapy and tests for HIV-1 drug resistance.

## Current targets of HIV antiretroviral drugs

The drug resistance is due to monotherapy.Hence therapy with potent combinations of three or more antiretroviral [7] drugs show that rapidly reduce circulating levels of plasma HIV to below detectable levels for periods of several years or more(Table-1).

**Table (1): Different classes of HIV-1 antiretroviral drugs**

| Class | Drugs | Viral target |
|---|---|---|
| Nucleoside reverse transcriptase inhibitors | Zidovudine, diadanosine, zalcitabine. stavudine,lamivudine,abacavir,tenofovir | Reverse transcriptase |
| Non-nucleoside reverse transcriptase inhibitors | Nevirapine,delavirdine,efavirenz | Reverse transcriptase |
| Protease inhibitors | Saquinavir,indinavir,ritonavir,nelfinavir, Amprenavir,lopinavir,atazavir | Protease |
| Fusion inhibitors | Enfuvirtide | Envelope gp41 |

**Drug targets by Polymerase protein (Pol)**

The polymerase protein (Pol) of HIV-1 is produced as a Gag pols ($Pr^{160\ Gag\text{-}pol}$) fusion polyprotein [8]. The pol gene precursor is divided to produce three viral enzymes such as protease, reverse transcriptase and integrase.The HIV-1 protease enzyme plays a vital role acting to specifically cleave Gag and Pol precursor polypeptides into functionally active proteins.HIV protease is an aspartic proteinase and is responsible for break of the Gag (p55) and Gag-Pol (p160) polyprotein products yielding the functional core proteins (p17,p24,p7,p6) and essential enzymes (reverse transcriptase, integrase, protease) required to produce mature HIV [9].When HIV protease is chemically blocked the formation of these core proteins is disrupted and assembled to form virions.These are immature and non infectious. The HIV-1 reverse transcriptase (RT) enzyme is a RNA-dependent DNA polymerase enzyme which is responsible for replicating the RNA genome [10].The RT enzyme changes the single stranded virion into doubled stranded DNA for subsequent integration into the host cell genome. RT is obtained from a Gag-Pol precursor that is processed by protease to yield a heterodimeric enzyme composed of a 66kDa protein (p66) and a 51 kDa protein (p51) [11].The p66 kDa protein (p66) is degraded to p51 and p15.Polymerase activity resides within the p51 fragment and RNAase activity is linked

with the presence of p15. Integrase is a 31kDa protein derived from the C terminal portion after the processing of $Pr^{160\ Gag\text{-}Pol}$ and is required for integration of a double stranded DNA copy of the viral RNA genome into the host chromosome.

**Drug targets by envelope protein**

HIV fusion and entry occur via the interaction of trimeric envelope gp160 spike (gp120 and gp41) with receptor molecules on the surface of target cells [11].The fusion process is a vital step in the viral replicative cycle making it an attractive target for antiretroviral drugs. Fusion inhibitor such as enfuvirtide (T-20) target is the crucial fusion step of the viral life cycle.Enfuvirtide is administered parenterally and inhibits fusion of the viral and cell membranes by binding to a portion of thegp41 molecule[12].

**Drug resistance and antiretroviral therapy**

**Protease inhibitors (pls) and drug resistance**

The HIV protease enzyme is a dimeric aspartyl protease essential for the posttranslational cleavage of precursor Gag-Pol polyproteins during virion maturation to generate building blocks required for assembly of new virus particles [13,14].The activity of this protein is required for virus infectivity rendering it a major drug target. Resistance mutations in the protease gene result from amino acid substitutions at or near the active site interfering with binding of the inhibitor because of conformational perturbations or to amino acids lying outside the active region. The cleavage site mutations do not produce drug resistance themselves, but compensate for changes in protease that result from primary and secondary mutations. Resistance to PIs emerges rapidly when these inhibitors are administered at inadequate doses or as part of suboptimal regimens [15].Generally, high-level resistance to PIs results from the sequential accumulation of amino acid substitution in the Pr gene along pathways that usually vary between different PI drugs [16].The first approved PIs were saquinavir (SQV) and indinavir (IDV) [17].These drugs are potent and require the emergence of multiple mutations before high level resistance. The resistance to SQV often confers resistance to IDV and vice versa [18].When viral suppression to

below the limit of detection is not achieved in a dual NRTI plus PI regimen, early mutations often occur to lamivudine followed by mutations associated with resistance/cross resistance to other NRTIs.Nelfinavir (NFV) has a lower genetic barrier to resistance than SQV and IDV.It is susceptible to the single D30N and L90M mutations which develops quickly in PI native patients. The presence of two or more mutations including D30N, G48V, 150V, V82A/F/T/S, 194V and L90M generally confers cross resistance to all the three of these PIS [19] whereas a single mutation at codon 30 does not infer cross resistance to other PIs.Amprenavir (APV) when used as a first-line PI is inclined to select for the 150V mutation conferring cross-resistance to lopinavir (LPV). LPV has a high genetic barrier to resistance [20], but a recent study has suggested that APV-selected resistance may confer cross-resistance to LPV44.Atazanavir (ATV) is susceptible to the 150L mutation. The patients who have taken PI-based therapy before commencing with ATV a number of both primary and secondary mutations may be associated with ATV resistance [21].

**Nucleoside reverse-transcriptase inhibitors (NRTIs)**

NRTIs inhibit the reverse transcriptase enzyme by competing with the endogenous nucleosides for the incorporation into the DNA chain generated by reverse transcription of HIV RNA. All nucleoside analogues must be triphosphorylated within the cell resulting inhibition of reverse transcriptase activity and premature chain termination. Resistance to NRTIs develops from nucleotide changes with the RT gene and the subsequent generation of amino acid substitutions in the RT enzyme [22].NRTI induces a predictable set of genetic changes generally with primary mutations arriving first and secondary mutations developing during continued therapy. The resistance to zidovudine develops with the sequential selection of specific mutations in the RT gene including codons [23] 41(M41L), 67(D67N),70(K70R),210(L210W),215(T215Y) and 219(K219Q).These mutations may result resistance to didanosine,zalcitabine,tenofovir and abacavir.Mutations linked with didanosine resistance involve codons 65,74 and 184.Two sets of mutations such as Q151Mcomplex and the T69S insertion mutations confer resistance to all the currently available NRTIs.The primary

codon change in Q151M complex [24] is linked with secondary mutations at codons 62(A62V),75(V75I),77(F77L) and 116(F116Y) which reduces the sensitivity to NRTIs.The T69S-S-S or T69S-S-A insertion mutations occur after prolonged treatment with multiple nucleosides which resistance to all NRTIs.

**Non-nucleoside reverse transcriptase inhibitors (NNRTIs)**

NNRTIs are non competitive inhibitors of HIV-1 RT which bind to a hydrophobic cavity near the active site of reverse transcriptase, causing a conformational change in the enzyme.NNRTI binding [25] sites are restricted to beta sheets consisting codons 100-110 and 180-190.The common mutations [26] in HIV selected by NNRTIs are L100I, K103N, V106A, V108I, Y181C/I, Y188C/L, G190A/E/S, P225H and P236L3. Y181C and K103N are linked with significant cross-resistance between nevirapine,delavirdine and efavirenz. The Y181C mutation is resistant to nevirapine and delaviridin, but not to efavirenz [27]. Y181C reduces NNRTI binding affinity leading drug resistance while the K103N mutation acts by preventing the formation of the binding pocket [28].

**Resistance to the fusion inhibitor T-20**

Resistance mutation toT-20 appears mainly in the Env gp 41 region.Substitutions occurred near the N-terminus of the HR-1 region in the highly conserve GIVQQQ sequence known to be critical for fusion [29].

**Factors of HIV-1 drug resistance**

**HIV genetic heterogeneity**

HIV-1 infection is characterized by a high degree of genetic variability within infected persons with the population present at a certain time point within an infected person consisting of a complex mixture of heterogeneous strains [30] termed quasispecies. Quasispecies differ in their antigenic and phenotypic properties and compete among themselves for survival [31] and propagation. The subsequent overgrowth or dominance of a certain viral strain over another is

largely determined by its relative adaption to a given intra host environment a factor particularly relevant for the emergence of drug resistance variants.

**Error in reverse transcriptase enzyme**

HIV-1 mutation rate is nearly one hundred times higher than those of DNA viruses, bacteria or other eukaryotes [32].The rate of nucleoside substitutions introduced by reverse transcriptase is approximately $10^{-4}$ per nucleotide per cycle of replication which is equal to one nucleotide substitution per genome [33] during a single replication cycle.Insertions,deletions and duplications contribute to the genetic heterogeneity [34] of HIV-1.HIV-1 turnover is rapid and it is estimated that approximately $10^{9}$ virions per day are generated in an infected individual. The lifespan of plasma virus and virus producing cells is very short with a half-life of approximately two days and an almost complete replacement of wild type strains by drug resistant virus occurs in plasma within 2-4 week. During antiretroviral treatment rapid viral turnover in combination with a high mutation rate is a primary factor behind the emergences of HIV variants with antiretroviral drug resistance.

**Genetic recombination**

Each retrovirus particles contains a dimer RNA genome and a reverse transcriptase enzyme which can switch templates during proviral synthesis. Drug resistant mutations in HIV-1, leading to increased resistance to a particular drug by recombination or the generation of multi drug resistant variants. Recombination may cause to the acquisition of mutations that compensate for a loss in viral fitness or replicative capacity due to previous acquisition of resistance mutations. The recombination creates multiple drug resistant virus out of two single drug resistant strains, it is generally believed that the capacity of the virus to recombine facilitates the evolution of drug [35,36] resistance.

**Selective pressures imposed by antiretroviral drugs**

Drug resistant HIV strains are generally reduced fitness [37] compared to wild-type counterparts in the absence of drugs. The decrease in viral fitness is accompanied by the

emergence of primary mutations. Continued drug selective pressure then allows the virus to select secondary mutations which compensate for the primary mutations allowing restoration of wild type enzymatic activity of the enzyme (Pr or RT) targeted by the drug.Viriants exist within the population that are naturally resistant to some extent to a particular drug before the commencement of HIV antiretroviral therapy. The presence of an antiviral drug changes the selective pressure on the viral population. When drugs that only partially inhibit HIV-1 replication are administers the resulting evolutionary pressure selects for resistant strains.Resistence emerges at a rate that is proportional to the frequency of pre existing variants and their relative growth advantage in the presence of drug [38].

**Drug pharmacokinetics**

The pharmacological activity of antiretroviral drugs is dependent on unbound drug entering cells that harbor HIV and multi drug combination therapy. The pharmacokinetics of orally administered antiretroviral involves absorption, first-pass metabolism in the intestine and liver, systemic distribution, metabolism and removal (excretion).Many antiretroviral bind to plasma proteins [39], influencing uptake into cells, as only unbound drugs in plasma can pass across the cell membrane efficiently. Important pharmacokinetic [40] parameters to consider include the volume of distribution (concentration of drug in plasma for a given amount of drug in the body), rate of clearance (efficiency of drug excretion), drug half-life (determines the course of accumulation of the drug in the body in chronic dosing) and the degree of fluctuation within a dosing interval. These parameters can vary significantly between patients, resulting in differences in drug absorption, drug metabolic, excretory activity, drug distribution and the overall efficacy of drug regimens [41].

**Suboptimal drug penetration in CNS**

There is limit of drug penetration to central nervous system(CNS).The blood-brain barrier is located between the blood and brain tissue and the blood CSF barrier is formed by the choroid plexus. High plasma protein binding of protease inhibitors and their unidirectional efflux by P-

glycoprotein membrane proteins in the blood-brain barrier limit CNS penetration and absorption [42] of antiretrovirals.Thus the CNS represents a site where ongoing viral replication may occur in the absence of antiretroviral suppression.

**Patient adherence**

Adherence prevents the emergence of resistant strains, but incomplete patient adherence coupled with an array of other pharmacologic factors result in the presence of a heterogeneous population and the possibility of selecting for viral resistance. Poor access to healthcare providers and counseling and broader issues such as poverty and poor literacy add to difficulties in patient adherence [43] in these areas. Drug-use, high-risk behaviors and depression contribute to poor adherence. The incidence of adverse effects [44] associated with the administration of antiretroviral agents is dependent on a number of factors including the ethnic origin of the patient, use of additional medications and host factors. Adverse reactions to antiretroviral therapy may affect its clinical efficacy through adherence problems. Adverse effects linked with antiretroviral agents include mitochondrial toxicity, hypersensitivity, lipodystrophy, dyslipidaemia and type 2 diabetes. Other effects include cardiomyopathy, peripheral neuropathy and pancreatitis. All antiretroviral drugs are linked with liver dysfunction through either direct or indirect [45,46] mechanisms.

**Viral reservoirs**

The reservoirs of HIV occurs in various tissues e.g CNS,male genital tract etc.Some anatomical sites may be non-permissive to immune surveillance and effective drug penetration, thus serving as potential sites of persistent HIV replication e.g. the respiratory, gastrointestinal and reproductive tracts [47].

**Cellular reservoirs of HIV-1**

Cellular reservoirs of HIV-1 arise from the ability of HIV to infect a variety of immune cell types including monocytes, macrophages, NK cells and T lymphocytes in addition to other non-immune based cells. Cellular reservoirs of HIV include memory CD4+ T lymphocytes,

blood monocytes and macrophages /cells of macrophages [48] lineage.

**Tests for HIV-1 drug resistance**

**Genotypic testing**

Genotypic assays use nucleic acid sequencing methods to detect all mutations within the RT and Pr genes. It may use line probe or clip-based assays to identify mutations within the RT and Pr genes to be associated [49] with drug resistance. Genotypic assays depend upon amplification of the Pr and RT genes from viral RNA in plasma (by means of RTPCR).Genotypic testing is inexpensive and readily available in many laboratories, but there are limitations to this approach of assessing drug susceptibility which include variable reproducibility and increased potential for laboratory error. The interpretation of drug resistance profiles through sequence-based analysis is also an issue and inconsistencies in this area are evident between the various internet resources. In additions current assays only detect viruses representing 5-20 percent of the total population and resistance present in minor subpopulations is often missed [50].

**Phenotypic testing**

The functional characteristics and growth properties of a viral isolate are referred to as the viral phenotype.Phenotyping measures the susceptibility of the virus to inhibition by a particular drug. The commercial recombinant assays such as AntiVirogram (Virco) and Phenosense (Virologic) may be used for testing [51].These assays relay on the incorporation of plasma derived HIV-1 RT and Pr genes into the backbone of an HIV-1 reference strain.The recombinant is then tested ex vivo to measure the IC50 and to measure a fold-change in susceptibility compared to wild type virus. Limitations of these assays include interpretation problems, as biological or virologic cut-offs measured by the assays do not incorporate achievable drug levels and can thus over or under-estimate the likelihood of a clinical response to a given drug.

## Conclusion

AIDS is a pandemic disease which spreads globally in human society. Hence there is need for effective treatment for controlling HIV1 infection. There is control in the mortality and morbidity of HIV infected individuals by the implementation of antiviral therapy. Use of different combination therapies target HIV life cycle and facilitate the successful long term survival of patients.

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