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ABC of AIDS and Antiretroviral Therapy: Perspectives and Obstacles

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Abstract

Human immunodeficiency virus type 1 (HIV-1) is undoubtedly the primary cause of the acquired immunodeficiency syndrome (AIDS), which is a slow, progressive and degenerative disease of the human immune system. The pathogenesis of HIV-1 is complex and characterized by the interplay of both viral and host factors. Practically every stage in the viral life cycle and every viral gene product is a potential target. Although HAART has made long-term suppression of HIV a reality, drug resistance, drug toxicity, drug penetration, adherence to therapy, low levels of continued viral replication in cellular reservoirs and augmentation of host immune responses are some of the most important challenges that remain to be sorted out. Novel targets for the management of HIV infection have become increasingly relevant in view of extensive drug resistance, side effects and high pill burden of some of the conventional anti-retroviral agents. These agents include chemokine receptor antagonists, integrase inhibitors, maturation inhibitors, zinc finger inhibitors, pharmacological CDK inhibitors, Tate-TAR interaction inhibitors, anti-CD4 monoclonal antibodies and antisense oligonucleotides. In this review we gave a basic overview of the virology of HIV-1 including the functions of the different HIV-1 proteins required for effective viral replication, various obstacles to HIV therapy, perspectives related to the issues that are critical in determining the success or failure of HAART, current methods for detecting HIV-1 drug resistance and various novel targets for the management of HIV infection.

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Introduction

Acquired immune deficiency syndrome (AIDS) is a collection of symptoms and infections which reduces the immune power of the human body by the HIV. Centre for disease control and prevention (CDCP)

has defined AIDS as, all HIV- infected people with fewer than 200 CD_4+ T-cells per cubic millimeter of blood (normal range is 1000-1200). Over 40 million people are currently infected with the human immunodeficiency virus (HIV), and over 25 million deaths have been attributed to the virus since the beginning of the epidemic. It has been theorized that the virus originated from monkeys during the development of an oral polio vaccine in Africa in the 1950's [1]. HIV is a part of lenti virus family and is a sexually transmitted pathogenic retrovirus which can be divided into two types i.e., HIV-1 and HIV-2, out of which HIV-1 is wide spread among humans, showing symptoms within five years of infection and HIV-2 is localized in West Africa and it takes longer time for symptoms to appear. AIDS, since having no cure resulting in its invariably fatal nature[2,3,4]. Opportunistic infections like tuberculosis, Pneumocystis carinii pneumonia, toxoplasmosis, and Kaposi sarcoma along with the HIV leads to decreased immune system of the body. Various opportunistic infections with AIDS are represented in fig.1.

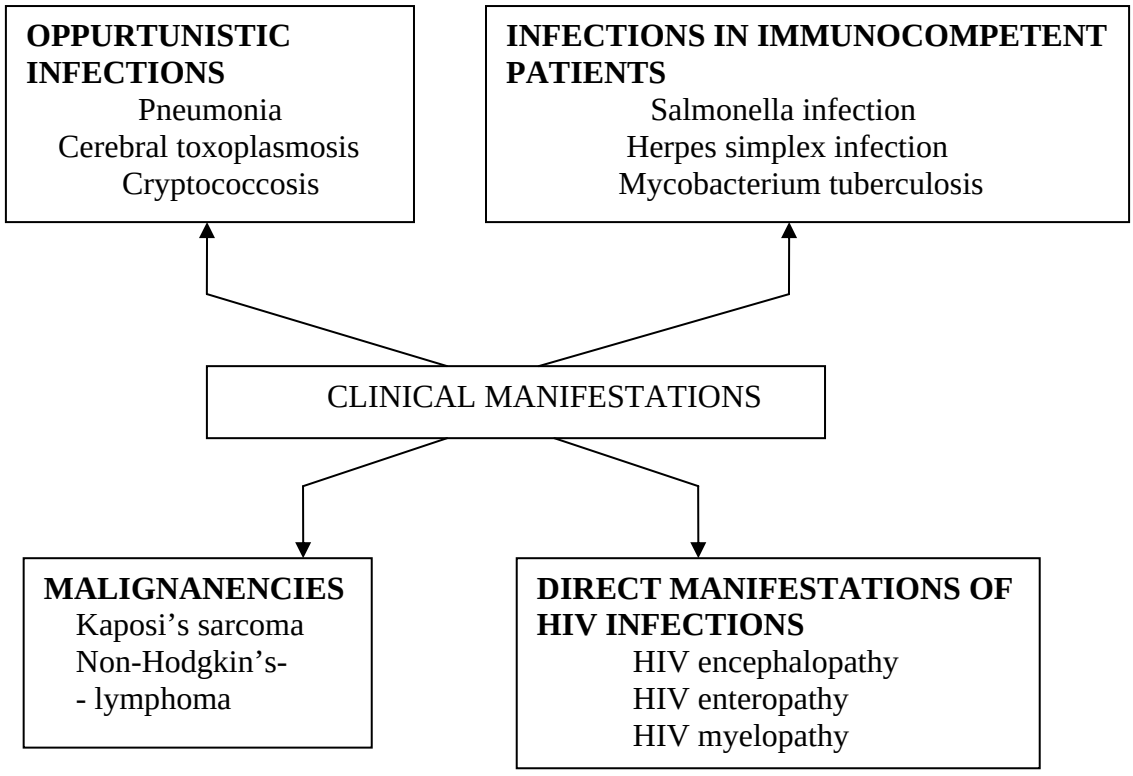

Fig 1. Various opportunistic infections with AIDS.

TRANSMISSION OF HIV

Trasmission of HIV essentially requires body fluids (Semen, vaginal secretions, blood, milk) containing the virus or virus infected cells. The distribution of risk factors for HIV transmission is as follows[5,6,7] (statistics are approximate values):

- Homosexuality- 60%
- Heterosexuality-15%
- Intravenous drug abusers- 15%
- Transmission of blood and blood products- 6%
- Others (shaving razors, used blades, organ and tissue transplantation) - 4%

MOLECULAR VIROLOGY OF AIDS

❖ **Structure of HIV:**

HIV is spherical in shape with diameter 0.1 µm. The outer coat called viral envelope is composed of two layers of fatty molecules called lipids. HIV may enter and exit the host cells through lipid rafts which are rich in cholesterol and glycol lipids. There are envelop proteins which are embedded in viral envelope. These glycoproteins (one gp_{120} and three gp_{41} molecules) constituting cap and a stem mediates the viral entry into host cells . The former's outermost part contains a variable region known as V_3 loop, and is responsible for inducing a strong immune response[8]. A p_9 nucleocapsid protein interacts non-covalently with the viral genome, a p_{17} matrix protein anchors the internal face of the viral envelope and a p_{24} capsid protein encloses the genome. Viral core (capsid) is bullet shaped core made of 2000 copies of p_{24}. The capsid surrounds two single strands of RNA (diploid genome). Each RNA contains nine genes of HIV. The genes gag, pol, env contains information to produce structural proteins (gp_{120}, gp_{41}) and vif, tat, rev, vpr, vpu which are regulatory genes, contains information to produce proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. HIV has three enzymes- Reverse transcriptase, Protease and Integrase.

- **Enzymes in the HIV life cycle**
- **Reverse transcriptase**

Structure:

Reverse transcriptase is a unique DNA polymerase found in retrovirus. It is a heterodimer protein (composed of two different polypeptides) that has a 66 KDA subunit (P_{66}) and a 51 KDA subunit (P_{51}). The P_{66} subunit contains five sub domains such as "fingers", "thumb", "palm", "connector" and "RNase H". The P_{51} subunit has four subunits of P_{66}, but it lacks RNase sub domain. P_{66} subunit consists of 3 catalytic residues (ASP 110, ASP 185 and ASP 186) at its active site[9].

Function:

Aspartic acid residues (110, 185 and 186) at the polymerase active site ligate two metal ions (possibly magnesium) which interacts with the phosphates of DNA primer and incorporated nucleotides. The P_{51} subunit serves as a binding site for the anti-codon stem and loop of tRNA Lys been built from the RNA template, the RNA is cleaved into pieces at the RNase H nuclease site[10]. Once the RNA is cleaved from the complex, a second DNA strand is made, complimentary to the first DNA strand, to form the final double helix DNA molecule. The role of P_{51} and P_{66} is represented in fig. 2.

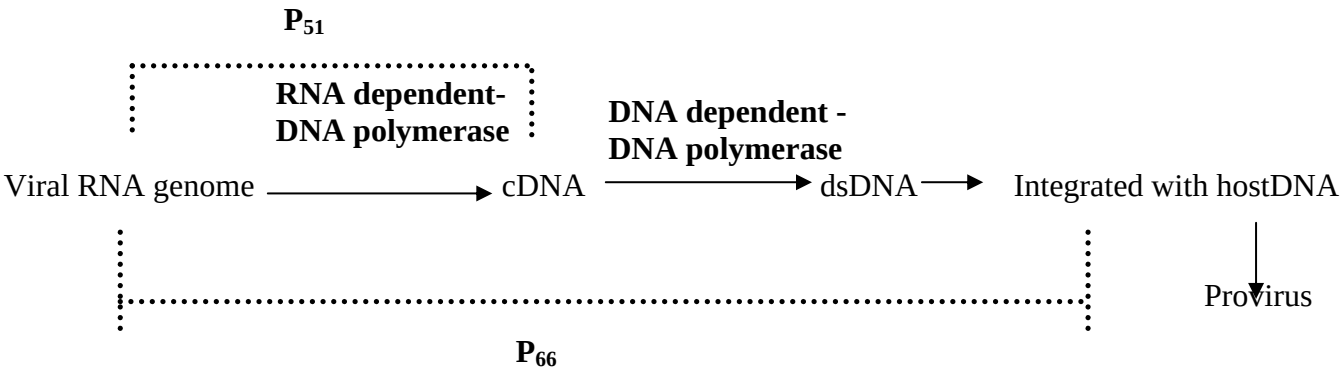

Fig 2. Role of P_{51} and P_{66} in HIV lifecycle.

- **Protease**

Structure:

Protease is an aspartyl protease enzyme showing unique activity for HIV proteins. It has two strands of proteins which are identical in their amino acid content and sequencing holded together by hydrophobic effects. It is comprised of 99 amino acids with two aspartic acid residues at active site.

Function:

HIV protease cleaves the natural poly proteins (gaG protein) into essential functional protein products during the maturation process of the virion. If the poly proteins are not cleaved, the virus fails to mature and is incapable of infecting a new cell[11].

- **Integrase**

Structure:

HIV-1 integrase (288 amino acids) has three domains: the catalytic core, C-terminal and N-terminal domains. Catalytic core domain consists of active site responsible for catalysis of all reactions of integration or disintegration. The C- terminal domain confers the capacity to bind both viral and host DNA.

Catalytic core domain (CCD):

CCD of HIV-1 integrase consists of five stranded β-sheet with six surrounding helices. The amino acids that are conserved at the active site are Asp64, Asp116, and Glu-152. Studies have indicated the HIV-1 integrase to be multimeric proteins with no certainty in monomer number. Examination of crystal structure shows two contacts between CCD monomers suggesting a dimeric model, with a large solvent excluded surface (1300A° per monomer). Dimer is stabilized by salt bridges and hydrogen bonds involving β- strand and α –helices-1, 3, 5 and 6. Here the putative active sites are separated by ~35A° [12]

DNA binding domain:

Each monomer is constructed of five β-strands that are arranged in an antiparallel orientation to form five stranded β- barrel. A hydrophobic interaction occurs between β-sheets (Tyr 243, Ala 248 and Val 259) from one, with Leu 242, Val 250, Ile 257 and Val 259 of other. Further stabilization occurs by hydrogen bonding between carboxylate of Glu 246 of one and carboxamide of Gln 252 of other.

DNA binding domain with large saddle shaped groove bounded by loops connecting β-strands 1 and 2 in each monomer, has dimension (12A°).It was observed that Lys 264 from each monomer interacts with sugar-po_4 backbone of DNA. Despite of structural dimer evidence, there are spatial problems to be considered. The five nucleotide spacing between the phosphates on two ends of each half of target DNA requires an active site of about ~15A°. But here the active site space is ~35A°.

Function:

HIV integrase catalyzes integration of HIV DNA into host chromosome.

❖ **Life cycle of HIV (Replication of HIV)**

HIV multiplies inside living cells by using the synthesizing machinery of cell. This growth cycle involves following stages (Fig. 3).

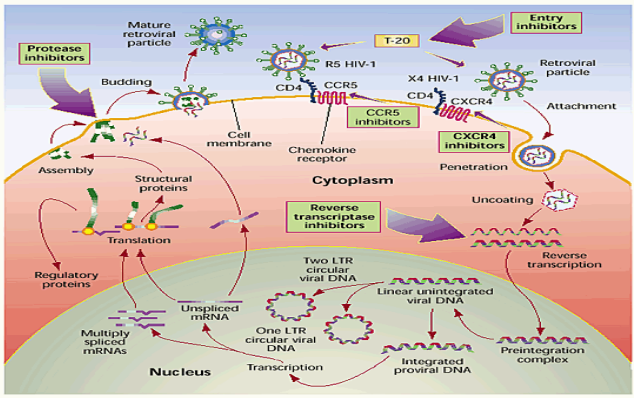

Fig 3: Schematic diagram of HIV lifecycle showing the anti-retro viral drug targets.

- **Attachment of virion to the surface of the host cell:**

HIV-1 infection is initiated by binding of the virion gp_{120} to the CD_4 receptor. Up on binding, a conformational change within gp_{120} is induced which exposes the co-receptor binding sites in gp_{120}[13]. The major co-receptors required for the entry of HIV-1 are host chemokine receptor- either CCR_5 or $CXCR_4$. Up on interaction between gp_{120} and the host chemokine receptor, gp_{41} binds to a host heparin sulfate, triggering fusion of the host and viral membranes, and the entry of the capsid into the cytoplasm. A protein called Tat increases transcription rate. So, gp_{41} of the envelope contributes as a target site for fusion inhibitors.

- **Replication of virus (Reverse transcription):**

After entering into the cytoplasm, viral reverse transcriptase (RTase) transcribes the viral genome into complimentary DNA (cDNA), using a cellular lysine tRNA molecule as a primer. So, RTase act as a target for anti HIV therapy.

- **Integration:**

The formed DS DNA circularizes and enters the nucleus for integration, mediated by V_{pr} and V_{if} accessory proteins. Viral integrase enzyme insert the viral genome into the host's chromosomal DNA, which is called as provirus. So, integrase is another target site for anti HIV therapy.

- **Transcription:**

After integration, cellular factors mediate transcription of viral transcription factors located on the pol or env genes. In the transcription, mRNA is synthesized from the integrated DNA with the help of host cell enzymes. This is regulated by a protein called Tat which accelerates the transcription rate.

- **Translation:**

After HIV mRNA is produced in the host cell's nucleus, it is transported to the cytoplasm for structural proteins production. Viral structural components are located on the gag gene and are initially transcribed and translated (in the cytoplasm) into a pre-protein, upon maturation of virus. A gag-pol derived pre-protein is translated and yields RTase, Integrase and Protease[14,15]. The mRNA of the env gene, which codes for viral surface glycoproteins, is translated in the ER to yield a gp_{160} pre-protein. various steps of regulation after translation is depicted in fig. 4.

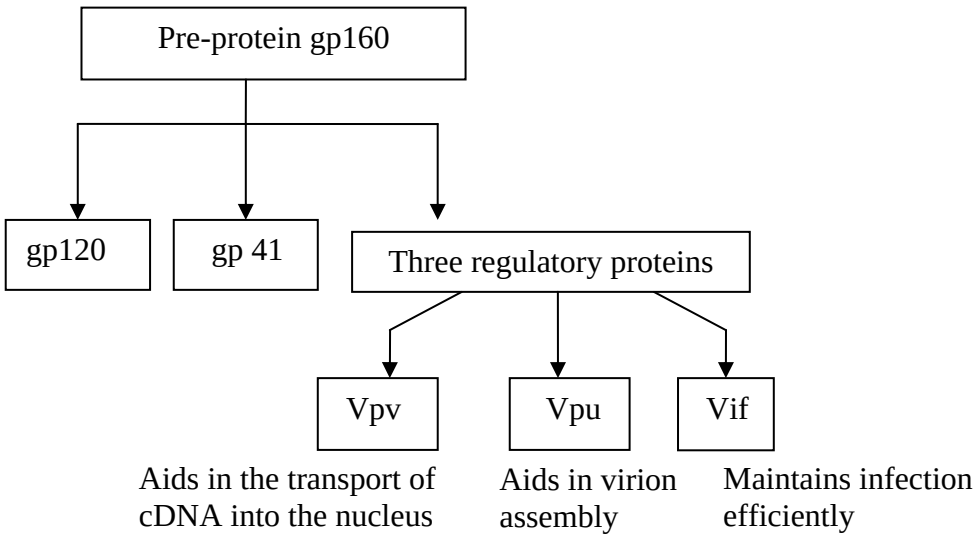

Fig 4. Glycoproteins which mediates the viral entry into host cells.

- **Assembly and budding:**

Newly made HIV core proteins, enzymes and genomic RNA at different parts of the host cell assemble inside the cell to form a virion. This immature viral particle buds off from the cell, acquiring an envelope that includes both cellular and HIV proteins from the cell membrane. During this part of the viral lifecycle, the core of the virus is immature and the virus is not yet infectious. The long chains of proteins and enzymes that make up the immature viral core now cut into smaller pieces by a viral enzyme called Protease. HIV Protease cleaves the natural polyproteins (gaG) into essential functional protein products during the maturation of the virion. During budding, HIV components accumulate in multivesicular bodies (MVB) that normally carry protein out of the cell. In this way, the HIV hijacks the normal cell machinery and mechanisms. So Protease also contributes as a target site for anti HIV therapy.

"CONVENTIONAL OR OLD" TARGETS FOR ANTI-RETROVIRAL THERAPY

Anti-retroviral (ARV) agents target different stages in the life cycle of HIV, viz. binding (virus attachment to the CD4+ cells), fusion (viral and cellular membrane fusion), reverse transcription of viral RNA to DNA, integration of viral DNA into cellular DNA, transcription of proviral DNA into viral RNA, protein synthesis from the HIV RNA, post translational modification of proteins through proteolytic cleavage, virus assembly and budding from cells .

❖ **Reverse transcriptase inhibitors (RTI'S)**

Strategies against reverse transcriptase involves the utilization of nucleosides and non- nucleosides.

- **Nucleoside Reverse transcriptase inhibitors (NRTI'S):**

Nucleoside analogues (Nucleoside reverse transcriptase inhibitors) lack the 3^{1}- hydroxyl group and get phosphorylated first by host enzymes in the cytoplasm to become active. It includes Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir[16]. NRTI'S inhibits RTase by binding the active site and prevents the continued polymerization of DNA chain (fig. 5).

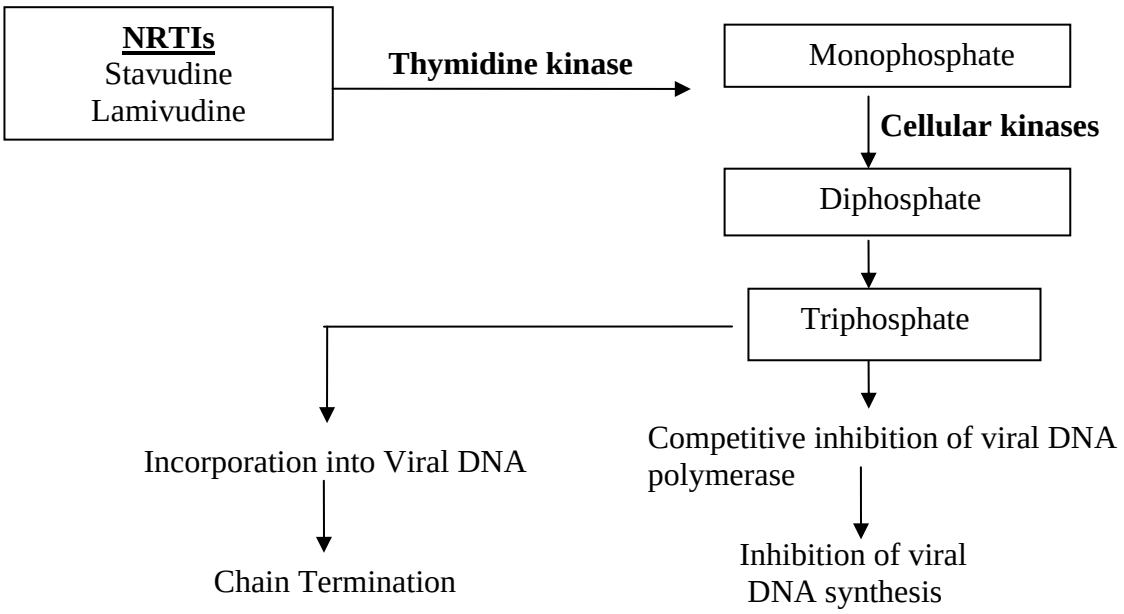

Fig 5. Mechanism of inhibition of viral DNA synthesis by Nucleoside Reverse Transcriptase inhibitors.

- **Non-nucleoside reverse transcriptase inhibitors (NNRTI'S):**

Non-nucleoside analogues (non-nucleoside reverse transcriptase inhibitors) do not undergo phosphorylation to become active. NNRTI'S bind RTase non-competitively at a site that is distal from the active site, induces a detrimental conformational change within the enzyme which leads to loss of enzyme activity. They include Efavirenz, Nevirapine, and Delaviridine[17].

- **Protease inhibitors:**

A molecule (protease inhibitor) that mimics the functionality and shape of the gaG protein cleavage region, which cannot be cleaved by HIV protease, are excellent inhibitors of the enzyme. They include Saquinavir, Ritonavir, Indinavir, Lopinavir, and Nelfinavir[18]

- **Integrase inhibitors:**

Integration of cDNA derived from the retroviral RNA into the host cell DNA occurs through the PIC (preintegration complex) in three steps: 3^{o}end processing, strand transfer and gap repairs. The first two steps are mediated through the viral integrase whereas the third step is mediated by cellular proteins[19]. Integrase inhibitors such as raltegravir (previously known as MK-0518) inhibit the strand transfer process. Raltegravir is a hydroxypyrimidinone carboxamide with a serum half-life (t ½ of approximately 12 h) and is metabolised by glucoronidation with an IC_{95} of 30 nM[19] .There is also no functional equivalent of this enzyme in humans, making it less likely for the development of toxicity. Raltegravir is already in clinical use and received FDA approval in October 2007. Other agent in this class include GS 9137 (Elvitegravir from Gilead Sciences) which was in the process of enrolling patients in the phase III clinical trials[20].

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

HAART is a combination therapy which typically includes a minimum of two nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitor (PI) and/or a non-nucleoside reverse transcriptase inhibitor (NNRTI).

❖ **Objective of HAART:**

To reduce plasma viral load levels below the level of detectability of the current ultrasensitive viral load assays (<50 copies/ml).

❖ **Impact of HAART on opportunistic infections:**

- Decreased incidence of oppurtunistic infections.
- Successful treatment of oppurtunistic infections.

❖ **Antiretroviral regimens:**

- Two nucleoside reverse transcriptase inhibitors (Zidovudine or Stavudine + Lamivudine or Didanosine) plus either one non-nucleoside reverse transcriptase inhibitor (Nevirapine or Efavirenz) or one protease inhibitor (Indinavir, Nelfinavir or Saquinavir) or two protease inhibitors (Ritonavir + Saquinavir).

- Three nucleoside reverse transcriptase inhibitors (Zidovudine + Lamivudine + Abacavir).

OBSTACLES TO SUCCESSFUL ANTIRETROVIRAL THERAPY

❖ **Emergence of drug resistance during HAART:**

- **RT inhibitors and drug resistance:**

There are currently ten approved RTIs, including six NRTIs and four NNRTIs. Despite the success of RTIs and their pivotal role in HAART, specific aminoacid mutations are associated with resistance to several different RTIs and mutational complexes conferring broad cross-resistance with in this class have also been observed.

1.Nucleoside reverse transcriptase inhibitors:

NRTIs were the first class of effective antiretroviral compounds, and Zidovudine (AZT) was the first drug to reach clinical practice[21] . All nucleoside analogues must be triphosphorylated with in the cell enabling inhibition of RT activity and premature chain termination. Resistance to NRTIs develops from nucleotide changes within the RT gene and the subsequent generation of aminoacid substitutions in the RT enzyme [22,23] . Each NRTI induces a predictable set of genetic alterations, generally with primary mutations arriving first, and secondary mutations developing during continued therapy[24]. In case of Zidovudine, resistance develops with the sequential selection of specific mutations in the RT gene including codons 41, 67, 70, 210, 215 & 219[25, 26]. These mutations are known as Thymidine analogue mutations (TAMs) or Nucleoside analogue mutations (NAMs). Mutations associated with didanosine resistance involve codons 65, 74, and 184, while resistance mutations for zalcitabine include those at codons 65, 69, 74, and 184[27].

The number of mutations required to induce resistance and the cross-resistance also varies among the agents.

2. Non-nucleoside reverse transcriptase inhibitors:

NNRTI binding sites are largely restricted to beta-sheets comprising codons 100-110 and 180-190[28]. In contrast to many of the NRTIs, a single mutation can cause high level resistance to NNRTIs. The most common mutations in HIV selected by NNRTIs are at codons 100, 103, 106, 108, 181, 188, 190, 225 & 23 [29]. Mutation at codon181 confers a high level of resistance to Nevirapine and Delavirdine, but not to Efavirenz[30,31]. codon181 mutation reduces NNRTI binding affinity leading to drug resistance, while codon103 mutation acts by preventing the formation of the hydrophobic binding pocket, thus reducing binding affinity indirectly. codon181, also been reported to reverse AZT resistance when introduced to isolates carrying major AZT resistance mutations[32]. The rapid induction of resistance has been observed most strongly during NNRTI monotherapy, and rapidly emerging resistant virus may often completely replace wild type strains within 2 to 4 weeks[33].

- **Protease inhibitors and drug resistance:**

The dimeric aspartyl protease of HIV, is required for the post-translational cleavage of precursor Gag-Pol polyproteins during virion maturation generating building blocks for assembly of new virus particles [34-39] and presenting its essentiality for viral infectivity. Mutations in the protease gene result from aminoacid substitutions at or near the active site interfering with binding of the inhibitor because of conformational perturbations to aminoacids lying outside active region resulting in resistance. The latter is frequently compensated for the deleterious effects of primary mutations[40-42] involving mutations in the cleavage sites in the Gag-Pol polyprotein precursor, lying outside the protease gene domain[43]. The most common mutations in HIV selected by PIs are at codons 30, 48, 82,90, 150 and 184. Nelfinavir has a lower genetic barrier to resistance than Saquinavir and Indinavir, and is susceptible to codon 30, 90 mutations. The selective advantages conferred by PIs resistance mutations depend upon the nature of the drug, its local concentration and the impact of the mutation of infectivity. Resistance to PIs emerges rapidly when

these inhibitors are administered at inadequate doses or as part of suboptimal regimens[44] Generally, high-level resistance to PIs results from the sequential accumulation of amino acid substitutions in the Pr gene, along pathways that usually vary between different PI drugs[45,46]. First FDA approved PIs - Saquinavir (SQV), Indinavir (IDV) are intrinsically potent and requires multiple mutations emergence but resistance on one confers resistance on other[47,48]. Interpretation of Protease mutants is complicated by the extensive polymorphisms found in the Pr gene of HIV-1 isolates from untreated patients. In one study, variation was noted in nearly 48 per cent of PR codons compared with the consensus (wild-type) sequence[49].

❖ **Causative factors of HIV-1drug resistance and failure of HAART:**

Even though sustained opportunistic infections and other malignancies make the HIV a long term chronic infection, the decrease in morbidity and mortality associated with HAART regimens, and the significant increase in the life expectancy of treated HIV-infected individuals, the eventual failure of therapy and progression to AIDS is still almost inevitable (fig. 6).

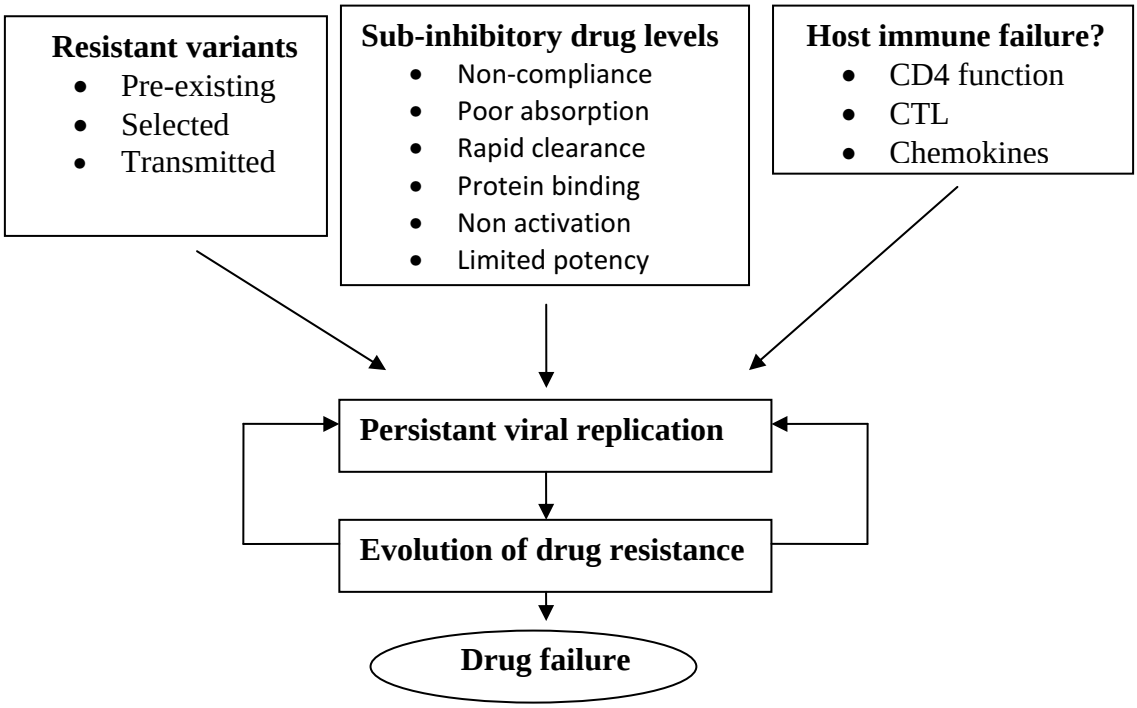

Fig 6: Evolution of HIV drug resistance and pathways leading to the failure of therapy.
CTL, cytotoxic T lymphocyte.

CURRENT METHODS FOR DETECTING HIV-I DRUG RESISTANCE

Although HIV-1 drug resistance is usually acquired during anti-HIV drug therapy, drug resistance can also be transmitted between individuals and recent studies also suggest that transmitted HIV-1 drug resistance is gradually increasing[50]. Resistance to current antiretroviral drugs is determined by mutations in the genes that encode the Pr and RT enzymes. Primary mutations are those that alter binding of the drug to its target and result in an increasing the amount of the drug necessary to inhibit the enzyme. Secondary mutations increase the level of resistance by improving the fitness of viruses carrying primary mutations.

Anti-retroviral resistance is determined by showing reduced susceptibility of HIV-1 to the given drug. Standardized assays are now available allowing quick

assessment of genotypic or phenotypic drug resistance in plasma HIV-1. The two methods are as follows:

❖ **Genotypic testing:**

Genotypic assays use either nucleic acid sequencing methods to detect all mutations within the RT and Pr genes, or novel methods such as line-probe or chip-based assays to identify select nucleotide changes known to be associated with drug resistance[51].All such assays depends upon amplification of the Pr and RT genes from viral RNA in plasma (by means of RT- PCR) or from proviral DNA derived from peripheral blood mononuclear cells (PBMCs). Automated sequencing provides the most comprehensive data as the entire Pr and RT genes are accounted for, however this may be more than is required in many clinical situations. The major limitations now like interpretation of drug resistance profiles through sequence-based analysis is an issue, virus detection representing only 5-20 percent of the total population, and resistance present in minor sub populations is often missed resulting in development of more sensitive methods to detect minority resistance strains[52].

❖ **Phenotypic testing:**

Phenotype of a virus refers to functional characteristics and growth properties of a viral isolate. It is now performed in a few laboratories taking longer to report results and is considerably costlier than genotypic testing. In the context of drug resistance, it measures the susceptibility of the virus to inhibition by a particular drug. Direct testing of clinical isolates in PBMC from seronegative donors has been used, but is time consuming. Moreover, PBMC from different donors vary in their ability to support the growth of HIV-1, leading to significant inter-assay variation. Many of these problems have been overcome with the availability of commercial recombinant assays such as AntiVirogram (Virco) and PhenoSense (ViroLogic)[53].

NEW AGENTS AMONG EXISTING CLASS: NEXT GENERATION NRTI, NNRTI AND PI

❖ **New Reverse Transcriptase inhibitors:**

Several new RT inhibitors are under investigation for future use in antiretroviral regimens. Some of them like TMC 125, Amdoxavir, DPC 083, Emtricitabine etc. TMC125, a new analogue is found to show good results in clinical trials. Amdoxavir, a guanosine analogue[54], is deaminated to active dioxolane guanine in vivo, which has shown activity against some nucleoside- resistant viruses in vitro including codon 69 insertion for multinucleoside resistance[55]. TMC125 is a NNRTI which has shown promising results in studies of both NNRTI native and NNRTI experienced. DPC 083, a derivative of Efavirenz, has shown increased activity and the resistance to DPC 083 requires more than one substitution[56].

❖ **New Protease inhibitors:**

Some of the current new investigative protease inhibitors include TMC 114, Tipranavir. Tipranavir, a potent anti-HIV agent in vitro inhibits isolates with phenotypic resistance to currently available PIs. TMC 114, a nonpeptidic PI, is an analogue of TM 126, has in vitro activity against isolates with high- level drug resistance to current PIs. Atazanavir, a protease inhibitor approved recently has been shown to have in vitro activity against some variants with PI resistance mutations[57].

❖ **New Integrase inhibitors:**

A number of potential viral integrase inhibitors have been identified, including a diketo analogue S-1360, diketo acid L-708, 906 and a novel integrase inhibitor V-165[58]. S-1360 has shown strong antiviral activity in vitro. The development of antiviral resistance to L-708,906 was recently studied by

growing HIV-1 strains in the presence of increasing concentrations of the compound resulting in the emergence of the resistance mutations T66I, L74M, and S230R.

NOVEL TARGETS AND AGENTS UNDERGOING PRE-CLINICAL OR EARLY CLINICAL STUDIES

The problems associated with the use of conventional antiretroviral (ARV) agents, viz., extensive drug resistance, difficulty in maintaining adherence, short and long-term side effects, have made the need of newer agents. Novel targets that are being reviewed include those recently approved for HIV treatment like chemokine receptor antagonists, integrase and maturation inhibitors, as well as those which are in various phases of clinical or pre-clinical development like the zinc finger inhibitors, pharmacological CDK inhibitors, Tate-TAR interaction inhibitors, antisense oligonucleotides, oxidizers of HIV envelope, and agents acting on the HIV proviral DNA.

❖ **Zinc finger inhibitors (ZFIs) or zinc ejectors:**

The HIV-1 nucleocapsid protein (NCp7), which has a role in the early and late stages of the viral life cycle has two highly conserved CCHC (Cyse-CyseHis-Cys) retroviral zinc fingers. Mutations in these regions lead to the formation of non-infectious virions. ZFIs based on SAMT (S-acyl 2-mercaptobenzamide thioester) targets NCp7[59].

❖ **Chemokine (CCR5 and CXCR4) receptor antagonist:**

HIV can bind either with CCR5 or CXCR4 molecules, which are co-receptors needed for viral entry in addition to the CD4+ molecule. X4-tropic or T-tropic viruses (CXCR4 receptors are found mostly in lymphocytes) are syncytium inducing and are found predominantly in later stages of HIV infection, whereas R5-tropic or M tropic (CCR5 receptors are found in macrophages and lymphocytes) are nonsyncytium inducing and found in early infection. A strain of HIV might also have mixed tropism. In natural HIV infection prevalence of X4 viruses increase over time. X4 strains are more pathogenic and their appearance is associated with CD4 decline and rapid disease progression. Chemokine receptor antagonists (maraviroc, vicriviroc, aplaviroc, AMD070) are a novel class of HIV-1 entry inhibitors that disrupt binding of HIV-1 to either CCR5 or CXCR4 co-receptors[60]. Since these agents target a cellular receptor instead of a viral enzyme or protein, emergence of viral resistance may occur more slowly than with other antiretrovirals.

❖ **Maturation inhibitors:**

Maturation inhibitors work in the later stages of HIV life cycle. They act by blocking the proteolytic cleavage of CA (mature capsid protein) from SP1 (precursor spacer protein) by binding to the CA-SP1 cleavage site and thereby blocking the conversion of p25 to p24 antigen. As a result non-infectious viral particles are produced. Bevirimat (PA-457) is the prototype (produced by Panacos Pharmaceuticals, Inc.). Pharmacologically it is a dimethyl succinyl betulinic acid with a t ½ of 2.5-3 days. The drug was found to be active in nanomolar range.

❖ **Pharmacological cyclin dependent kinase inhibitors (PCIs):**

Cyclin dependent kinases are cellular as well as viral enzymes. Natural inhibitors of CDKs such as p21/Waf1 are found in cells with latent HIV-1[61].The cellular enzymes regulate cell cycle and HIV-1 transcription and are the targets for pharmacological CDK inhibitors such as roscovitine, CYC202 and flavopiridol. Roscovitine (a purine derivative) reversibly competes for ATP binding site in CDKs. The PCIs also induced apoptosis in latent and activated HIV-infected cells without the release of infectious virions. Since the PCIs act on cellular targets they are likely to be useful in the prevention of drug resistant
HIV[62].

❖ **Tat-TAR interaction inhibitor:**

These agents, which are in the pre-clinical stage of development, prevent the interaction between transactivator of transcription (Tat protein) and the transactivator response region (TAR) of HIV RNA. This interaction is primarily between the ARM (arginine rich motif) of Tat and the three base bulge region of TAR RNA. The compounds within this category can be classified between those that act on the 3 base bulge region and those that act on the TAT protein. They include agents such as WM5 (6-aminoquinolone derivative), which is a substituted purine having side chains with amino or guanidyl group, and trehalose derivatives with guanidine groups [63].

❖ **Anti-sense oligonucleotides:**

Anti-sense oligonucleotides are short RNAs, which can be designed to target various functionally important sites of HIV-1 RNA. The oligonucleotides act on targets such as TAR (transactivator response region) of HIV-1 genome, Psi domain responsible for dimerization of HIV-1 RNA, or HIV-1 envelope gene. These agents act by neutralisating HIV-1 expression. The LNA (locked nucleic acid) modified antisense oligonucleotide was found to be superior to DNAzymes in inhibiting HIV expression. One antisense drug, HGTV43 by Enzo Therapeutics, is starting Phase II trials [64].

❖ **Anti-CD4 monoclonal antibody:**

TNX-355, being developed by Tanox, Inc. is a humanized anti-CD4 IgG4 monoclonal antibody, which acts on domain 2 of the CD4 receptor. The agent is not immunosuppressive and does on interfere with the immunological functions of CD4 cells like antigen presentation. It has synergistic effect with the fusion inhibitor enfuvirtide.

❖ **Viral decay accelerator:**

These agents encourage mutations in HIV to the point that the virus is no longer functional. The prototype agent is KP-1461[65].

CONCLUSION

As the global pandemic of HIV continues to spread, the need for effective treatment has become increasingly pressing. Despite large scale efforts and dedication of resources for development of novel drugs worldwide, the production of an agent with the ability to prevent HIV infection still seems a long way off. In contrast, dramatic improvements in the mortality and the morbidity of HIV infected individuals have been achieved with the implementation of HAART. Continued advances in currently prescribed RT and PR inhibitors along with the inclusion of new antiretroviral agents in combination therapies targeting additional elements of HIV lifecycle, could facilitate successful long-term survival and management of HIV infected individuals well beyond current limits. Despite optimism about the future of HIV antiretroviral therapies, the issues of drug resistance, cross-resistance, pharmacokinetics and patient adherence, drug toxicity, and the augmentation of host immune responses remain challenging issues in the management of HIV infection. Finally the evidence reported in this review suggests that there is hope that “HUMANITY WILL EVENTUALLY CONQUER HIV”.

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