AIDS: A Review of Targets and Approaches for Treatment

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Untreated HIV infection is characterized by a gradual deterioration of immune function. Most notably, crucial immune cells known as CD4 positive T lymphocytes are disabled and killed during the typical course of infection. These T lymphocyte cells play central role in the immune response. The study of HIV structure, genome and its life cycle has revealed many exciting target sites for acquired immunodeficiency syndrome treatment. Many conventional treatments for acquired immunodeficiency syndrome are merely capable of prolonging the patient's life but are unable to completely eradicate the virus as the virus mutates rapidly and develops resistance. So there are many recent novel approaches under consideration like integrase inhibitor, fusion inhibitors and ribozymes for the treatment of acquired immunodeficiency syndrome.

Acquired immunodeficiency syndrome (AIDS) is one of the most frightening syndrome worldwide. AIDS was first identified in 1983 in U.S.A. and studies revealed that the virus entered the U.S. population sometime in the late 1980, Centre for Disease Control and Prevention (CDCP) has defined AIDS as, all HIV-infected people with fewer than 200 CD4+ T cells per cubic millimeter of blood (normal is 1000-1200). There are now 40 million people living with AIDS worldwide and globally 24.8 million people have died of AIDS since the beginning of the epidemic and it is estimated that 68 million will die of AIDS by 2020. (http/www.avert.org/globalisation.htm). In 2003, United Nations Programme on HIV/AIDS (UNAIDS) published statistical report, which revealed that 38 million people were living with HIV/AIDS world wide and more than 20 million deaths have taken place. Worldwide 5 million new HIV infections occur each year. (http://health.yahoo.com/health/centers/hiv_aids/1.html). In India 1 11 608 AIDS cases were reported till 31st July, 2005 (http://www.nacoonline.org/facts_reportjuly.htm) and the estimated number of HIV infections were found to be about 5134 million (http://www.nacoonline.org/facts_hivestimates04.htm).

AIDS gradually reduces the immune power of the body and is associated with conditions like opportunistic infection by bacteria, fungi and viruses (opportunistic infections are infections that do not occur in healthy people but occur when a person's immune system is weakened) and development of certain cancers like cervical cancer and lymphomas, AIDS-related Kaposis Sarcoma (www.niaid.nih.gov/factsheets/howhiv.htm).

HIV, Human Immuno Deficiency Virus, is a RNA virus of retrovirus family and a lentivirus, which is the subfamily of retrovirus, HIV is the cause AIDS, other viruses in the subfamily such as oncovirinae cause tumor and cancer while members of the spumavirinae subfamily are non-pathogenic in nature. Retroviruses are 0.08 μm-0.1 mm in diameter and make a complementary DNA copy of their RNA, which is necessary for their replication. Reverse transcriptase enzyme helps in carrying out the process of reverse transcription. The complementary DNA formed gets incorporated into host cell genes as part of the infection cycle[1,2].

Structure of HIV (www.niaid.nih.gov/factsheets/howhiv.htm):

HIV has 0.1 μm in diameter and is spherical in shape. 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 special areas of the cell membrane (human) known as the Lipid Raft. These lipid rafts are rich in cholesterol and glycolipids. There are some proteins called env proteins which are embedded in the viral envelope. These proteins consist of a cap made

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up of three molecules called gp120 and a stem consisting of three gp41 molecule that anchors the structure in the viral envelope. Viral core (capsid) is bullet shaped core or capsid, made of 2000 copies of another viral protein called P24. The capsid surrounds two single strands of HIV RNA. Each RNA contains nine genes of HIV. The genes gag, pol, env which contain information necessary to produce structural proteins (gp120, gp41) and vif, tat, rev, nef, vpr, vpu which are regulatory genes which contains information necessary to produce proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. There are two types of human immuno deficiency virus i.e., HIV-1 and HIV-2, out of which HIV-1 is most common and HIV- 2 is present in West Africa which shares 50% homology in amino acid sequence with HIV-1[3,4].

REPLICATION CYCLE OF HIV

The replication cycle of HIV consist of six stages - Entry of HIV into cells, reverse transcription, integration, transcription, translation, assembly and budding.

Entry of HIV into cells[5]:
One or more of the virus gp120 molecules binds tightly to the CD4 molecule on the cell surface. The binding of gp120 on CD4 results in conformational change in the gp120 allowing it to bind a second molecule on the cell surface known as co-receptor. The envelope of the virus and the cell membrane of the host cell fuses, leading to entry of the virus into the cell. The gp41 of the envelope is critical for the fusion process which contributes as a target site for fusion inhibitors.

Reverse transcription[6]:
After entering into the cytoplasm, the uncoated HIV RNA serves as a template from which complementary DNA strands are transcribed. This is catalyzed by RNA dependent DNA polymerase enzyme (reverse transcriptase), which can act as another target site for anti HIV therapy.

Integration[6]:
After reverse transcription, the double stranded DNA circularizes and enters the nucleus for integration. A second essential enzyme called integrase mediates integration of these proviral DNA into the host chromosome.

Transcription[5,6]:
mRNA is synthesized from the integrated DNA with the help of host cell enzymes. This is regulated by viral cellular machinery for example tat gene which encodes a protein that accelerates transcription.

Translation[5,6]:
After HIV mRNA is produced in the host cell's nucleus, it is transported to the cytoplasm for structural proteins production. For example, a protein encoded by the rev gene allows mRNA encoding HIV structural proteins to be transferred from the nucleolus of the cytoplasm. Without the rev protein, structural proteins are not made. In the cytoplasm, the virus co-opts the cells protein making machinery including structures called ribosome to make long chains of viral protein and enzymes, using HIV mRNA as a template.

Assembly and budding (www.niaid.nih.gov/factsheets/howhiv.htm):
Newly made HIV core proteins, enzymes, and genomic RNA gather inside the cell and an immature viral particle formed, 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 life cycle, 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.

During budding, there is interaction between the HIV gag protein and molecule in the cell which directs the accumulation of HIV components in special intercellular sacks, called multivesicular bodies (MVB) that normally carry protein out of the cell. In this way, the HIV hijacks the normal cell machinery and mechanisms.

APPROACHES FOR AIDS TREATMENT[6-8] (www.aidsinfo.nih.gov/drugs)

The various approaches for AIDS treatment are based on the structure and replication cycle (life cycle) of HIV. The different target sites for the treatment are lipid raft, env proteins, vif gene, reverse transcriptase enzyme, protease enzyme, integrase enzyme, fusion of HIV T-cells and HIV RNA. The antiretroviral drugs approved for clinical use in the world have been enlisted in Table 1 and the combinations of these drugs approved for use in India are shown in Table 2.

Nucleoside reverse transcriptase inhibitors (NRTI):
The conversion of viral RNA to proviral DNA can be

TABLE 1: ANTIRETROVIRALS APPROVED FOR USE[17]

NRTI	NNRTI	PI	Entry Inhibitors
Zidovudine (ZDV)	Nevirapine (NVP)	Saquinavir (SQV)	Enfuvirtide* (T-20)
Stavudine (d4T)	Efavirenz (EFV)	Indinavir(IDV)	
Lamivudine (3TC)	Delavairidine* (DLV)	Ritonavir (RTV)	
Didanosine (ddI)		Nelfinavir (NFV)	
Zalcitabine* (ddC)		Lopinavir (LPV/r)	
Abacavir (ABC)		Atazanavir* (ATV)	
Emtricitabine *(FTC)		Amprenavir*(APV)	
Tenofovir (TDF)(Nucleotide RTI)		Fos-amprenavir* (FPV)	
		Tipranavir (TPV)*	

NRTI stands nucleoside reverse transcriptase inhibitors, NNRTI stands for non-nucleoside reverse transcriptase inhibitors and PI denotes protease inhibitors. *Drugs not available in India

TABLE 2: RECOMMENDED NNRTI–BASED REGIMENS FOR INDIA (1-NNRTI + 2-NRTIS)

NRTI	NNRTI
Preferred	Nevirapine
Zidovudine + Lamivudine	or
or	Efavirenz
Tenofovir + Lamivudine	
Alternative	
Stavudine* + Lamivudine	
or	
Abacavir + Lamivudine	
or	
Didanosine + Lamivudine	

NRTI stands nucleoside reverse transcriptase inhibitors and NNRTI stands for non-nucleoside reverse transcriptase inhibitors *Stavudine is considered as an alternative ARV because it is associated with a high incidence of adverse events including peripheral neuropathy, pancreatitis, hyperlactatemia and lipodystrophy and dyslipidemia that may not be reversible on treatment discontinuation[18]

prevented by inhibiting reverse transcriptase enzyme action. They act at an early and essential step in HIV replication, which prevent acute infection of susceptible cells but have little effect on cell already infected with HIV. They competitively block the active site of the enzyme. These drugs are first phosphorylated by host enzyme in the cytoplasm then they become active.

These drugs lacks 3'-OH group, when incorporated into DNA terminates chain elongation. USFDA approved nucleoside reverse transriptase inhibitor are abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, disoproxil fumerate, zalcitabine and zidovudine. The combinations approved by USFDA are abacavir/lamivudine/zidovudine, abacavir/lamivudine, emtricitabine/tenofovir disoproxil fumerate, lamivudine/zidovudine. Some new drugs under investigation are alovudine, amdoxovir, DPC817, elvucitabine and racivir.

Non-nucleoside reverse transcriptase inhibitors (NNRTI):

These drugs bind adjacent to the enzyme's active site non-competitively, inducing conformational changes in the active site of the enzyme. Non-nucleoside reverse transcriptase inhibitors do not undergo phosphorylation for its action. Non-nucleoside reverse transcriptase inhibitor approved by USFDA are delavirdine, efavirenz and neviparine. The drugs under investigation are calanonide A, capravirine and TMC 125.

Protease inhibitors (PI):

HIV-Protease is a non-competitive enzyme, which is a dimer consisting of 99 amino acid monomers. Each monomer has one aspartic acid in the active site for drug binding, which acts as the catalytic site. Human proteases like renin and cathepsin are monomers. This structural difference between HIV proteases and human proteases causes 1000 times more affinity of protease inhibitor for HIV protease than human proteases. All protease inhibitor binds reversibly to the active site of the HIV protease. This prevents protease from cleaving the viral polyprotein into active enzyme, which leads to immature and non-infectious viruses are formed. The drugs approved by USFDA are amprenavir, atazanavir, fosamprenavir, indinavir, nefinavir, ritonavir, saquinavir and tipranavir. USFDA also have approved one combination of drugs for inhibiting protease, which is lopinavir/ritonavir. TMC 114 is under investigation for this category.

Fusion inhibitors (FI):

This is a new class of antiHIV drugs, intended to protect cells from infection by HIV by preventing the virus from attaching to a new cell and breaking through the cell membrane. Researchers hope that these drugs can prevent infection of a cell by either free virus (in the blood) or by contact with an infected cell.

Enfuvirtide was approved drug by FDA on March 13, 2003, for the treatment of HIV-1 infection. Some other fusion inhibitors under investigation are AMD070 (AMD11070), BMS-488043 (043), GSK-873,

140 (AK602), Peptide T, PRO 542 (CD4-IgG2), SCH-C, SCH-D, TNX-355, UK-427, 857, FP21399, Schering C (SCH-C), T-20 (enfuvirtide, fuzeon) and T-1249.

Integrase inhibitors:
After HIV's genetic code is changed from a single strand by the reverse transcriptase enzyme, it gets inserted into the genetic code of the infected cell. Then the HIV genetic code gets "read", producing new viruses. Scientists hope that integration will be another point in the HIV life cycle that can targeted by drugs. L-000870810 is an investigational drug that is not yet approved by the FDA for use outside of clinical trials. It is being studied for the treatment of HIV infection. This medicine does not cure or prevent HIV infection or AIDS and does not reduce the risk of passing the virus to other people. L-000870810 has been generally well tolerated in early human clinical trials. Further development of L-000870810 has been halted due to liver and kidney toxicity seen with long-term dosing in dogs. S-1360 by Shionogi and GlaxoSmithKline is currently in Phase II clinical trials.

Zinc finger inhibitors:
The inner core of HIV is called the nucleocapsid. It is held together by structures called "zinc fingers". Zinc finger inhibitors (or zinc ejectors) are drugs that can break apart these structures and prevent the virus from functioning. Scientists believe that the nucleocapsid core cannot mutate very easily, so a drug that works against zinc fingers might be effective for a long time. Drugs that attack zinc fingers could have serious side effects. Azodicarbonamide (ADA) is one of the zinc finger inhibitors which have been tested in a Phase I/II clinical trials.

Antisense drugs:
These are a "minor images" of a part of the HIV genetic code. These drugs lock onto the virus from its function. One antisense drug HGTV43 by Enzo Therapeutics, is just entering Phase II clinical trials.

CD4 receptor antagonists:
Recently, a novel class of small molecule inhibitor, BMS-378806, developed by a team at Bristol-Myers Squibb showed excellent potency against many HIV-1 laboratory and clinical isolates. It blocks the binding of gp120 to CD4 receptor. More recent studies indicated that the inhibitors exhibit its anti-HIV activity by interrupting the essential CD4 induced conformational changes in gp120[9-11].

CCR5 antagonists:
The chemokine receptor CCR5 is a G-protein coupled receptor. It is the primary co-receptor with CD4 by which HIV-1 strains infect their host cells. Individuals homozygous have a defect in CCR5 expression have been identified as being highly resistant to HIV infection, while this defect does not cause a significant health problem. In addition, infected individuals heterozygous for the defective gene appears to exhibit delayed disease progression. Eradication of HIV-1 is impossible even by with combination chemotherapy using HIV-1 protease inhibitors and reverse transcriptase inhibitors, called highly active antireteroviral therapy (HAART). Moreover, there are several problems with HAART, such as the emergence of drug resistance, side effect profiles and difficult dosing regimens.

Therefore, the discovery of novel anti HIV-1 agent with new mechanism of action is still needed and CCR5 antagonist as HIV-1 entry inhibitors is considered to be a new and attractive target. The promising CCR5 antagonists, which have shown significant activity against HIV infected cell lines are, peptide mimics derived from first extracellular loop of CCR5 toward HIV-1, 5-oxopyrrolidine-3-carboxamide derivatives, 1-benzothipine-1,1-dioxide and 1-benzazepine derivatives containing a tertiary amine moiety[12-16].

RIBOZYME[4,6-9]

Ribozymes are attractive potential therapeutic agents due to the specificity of binding and cleavage potential for turnover and lack of immunogenicity. Ribozymes are enzymatic molecules that can cut specific sequences in HIV and destroy the virus. Ribozymes cut HIV at several stages of its life cycle and are active against strains that are resistant to conventional anti-viral therapy. Ribozyme gene therapy could be used as an adjunctive or stand-alone therapy, and is potentially cost-competitive with other antiviral therapies. The target site selection is generally based on three criteria, biological significance of the target RNA and the presence of an appropriate target triplet sequence and accessibility of this sequence to ribozyme action. For hammerhad ribozyme cleavage, the target site is generally GUX, although in certain cases NUX may be used (where N represents any nucleotide and X represents adenine, cytocine, and uracil). To date, therapies for the treatment of HIV-1 infection have focused on the reduction of viral load using drugs that

interfere with replication of the virus with only a modest effect on the restoration of T-cell counts. Triple combination therapy (TCT), generally involving two reverse transcriptase inhibitors and one protease inhibitor, is the most recent and relatively successful development in the management of HIV infection. As to the appropriate target cells for antiHIV-1 ribozyme gene therapeutic, hematopoietic CD34+ stem cells would appear to be the ideal choice since they have the potential to produce several generations of protected cells in multiple linkages. Another approach for the treatment of HIV-infected individual is the infusion of gene manipulated CD4+ T-lymphocytes. Ribozymes constructs can protect CD4+ T-cells from HIV-1 infection in patients.

Clinical trials are currently being conducted by several groups utilizing the LNL6 vector and the recombinant RRz2. Currently, an anti-HIV-1 ribozyme is being tested in two separate phase I clinical trials. The next step for these studies are moving to testing in phase II clinical trials. Ribozyme therapy offers several potential advantages over conventional therapies in that it can potentially impact on both viral load and restoration of the immune system. Ribozymes offer a possible new gene therapy-based anti-HIV approach. Their potential advantages include specificity, multiple turnover, stable expression and lack of immunogenicity. Studies up-to-date indicate that ribozymes are effective in suppressing HIV-1 replication in cell culture systems.

Vaccines:

AIDS vaccines are tested in various stages over several years, as with most other vaccines. Initial laboratory work is followed by animal studies and then human clinical trials. Many of the modern licensed vaccines we are using today have taken several decades before they were cleared and they may have complicated stages while the development. Experts believe a safe and effective AIDS vaccine may be found within the decade, but most of the experts feel it may take much longer time than that. In keeping with international regulatory requirements, three phases of vaccine clinical trial are performed and the details are given in Table 3.

A phase I clinical trial was initiated on 7th Feb 2005 in India under an AIDS vaccine programme, conducted by the National AIDS Research Institute (NARI) in Pune, Maharashtra. This program is governed by a Memorandum of Understanding (MOU) between the Government of India and the International AIDS Vaccine Initiative (IAVI). The Government of India is represented by the National AIDS Control Organization (NACO) in the Ministry of Health and Family Welfare and the Indian Council of Medical Research (ICMR). All approvals for the trial have been obtained from the office of the Drugs Controller General of India (www.medicinalnewstoday.com). The vaccine under trial is tgAACO9, which consists of a portion of genetic material (gag i progenes and a portion of reverse transcriptase enzyme coding gene) inserted in to the adeno-associated virus that does not cause disease in humans (www.nari-icmr.res.in).

The challenges in developing AIDS vaccines are that HIV continually mutates and recombines. This mean, a vaccine would need to protect the person from many strains of the virus. Vaccines against other viruses have only had to protect the person against one or a limited number of strains. The NIAID funds scientists to analyze the genes of the different strains of HIV through the HIV Variation Project and the HIV Sequence Database

TABLE 3: REGULATORY REQUIREMENTS FOR HIV VACCINE CLINICAL TRIALS

	Phase I	Phase II	Phase III (Efficacy trial)
Key questions asked in each Phase	Does the vaccine cause side-effects in humans?	Does the vaccine cause side-effects in humans?	Does the vaccine cause side-effects in humans?
	Does the human immune system respond to it?	Does the human immune system respond to it?	Are the vaccine effective meaning-does it prevent HIV infection or does it delay progression to disease?
	What is the ideal dose of the vaccine?	What is the refined dosage and the vaccination schedule?	-
Number of volunteers required	Between 20-100 adults	Between 100-250 adults	Between 2500-20,000 adults
Profile of volunteers	Healthy HIV uninfected people who are unlikely to be exposed to HIV, in other words they practice low risk behavior	Healthy HIV uninfected people	HIV uninfected people who have a higher chance of exposure to HIV, i.e., people who are at risk
Duration of clinical trial	Between one to two years	Approximately two years	Between three to five years

and Analysis Unit.

HIV infects helper T cells, the immune cells that orchestrate the immune response. It is very difficult to design a vaccine that, to be effective, needs to activate every cells that are infected by the virus. HIV can be transmitted as both free virus and from infected cells. This may mean that both arms of the immune system may need to be stimulated. Researchers do not know what constitutes an effective immune response to HIV. It might be antibodies, activated immune cells, a third immune response, or a combination of immune responses.

There are many reasons to be optimistic for developing AIDS vaccine. Perhaps most compelling is the fact that the human immune system can control HIV under certain circumstances. For example, in most individuals with acute HIV infection, the immune system is successful in dramatically down modulating the burst of viremia seen in the weeks following infection. In addition, a small subset of HIV-infected individuals show little or no immune system deterioration and low levels of viral replication even after 15 or more years of infection in the absence of antiretroviral therapy. Other individuals, including sex workers, have multiple exposures to HIV but remain uninfected. Studies of acute infection, "long-term non-progressors," and multiple exposed/uninfected people continue to provide clued regarding the immune responses one would want to elicit with a vaccine.

Experimental vaccines have proven protective in animal models of AIDS and in phase I and phase II human trials. The candidate HIV vaccines have been well tolerated and immunogenic. In human studies, cross-clade CD8+CTL response to candidate HIV vaccines have been observed, as antibodies that can neutralize a broad spectrum of HIV subtypes. These findings indicate that the problem of viral diversity and multiple clades may not be insurmountable.

In addition, epidemiological studies indicate that mucosal transmission is relatively inefficient in the absence of other sexually diseases. This suggests that moderate immune response at the mucosa may be protective finally. Recent studies indicate that HIV vaccine efficacy trials among high-risk volunteers are feasible. In this regard, considerable progress has been made in establishing the domestic and international infrastructure for the assessment of HIV vaccines and other prevention efforts.

New approaches; herbals:

Recently an antiHIV activity was confirmed in oleander. In preliminary screening, the ethanol extract of leaves showed high antiHIV-1 activity (IC_{50}= 1.56 µg/ml). AntiHIV activity of oleander is contributed by the active flavanone glucosides namely querceitin 3-O-[(6-O-sinapoyl)-(ß-D-glucopyranosyl-(1→2)-ß-D-glactopyranoside, kaempferol 3-O-{(ß-D)-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)-]-D-galactopyanoside}, querceitin 3-O-[(6-O-feruloyl)-(ß-D-glucopyranosyl-(1→2)-ß-D-galactopyranoside and querceitin. Recently, dipyranocoumarin derivatives, (+)-calonolide A, (-) and soulattrolide has shown significant reverse transcriptase inhibitory activity for HIV-1[13-15]. The above-mentioned plants and the compounds isolated from them can be further studied to find an effective cure of AIDS.

RECENT FINDINGS

Scientists in Australia's tropical north are collecting blood from crocodiles in the hope of developing powerful antimicrobial drugs for human, after tests showed that the reptile's immune system kills the HIV. The crocodile's immune system is much more powerful than the human immune system which prevents life threatening infections after savage territorial fights that often leave the animals with gaping wounds and missing limbs. Initial studies of the crocodile immune system in 1998 found that several antibodies in the reptile's blood killed bacteria resistant to penicillin, such as *Staphylococcus aureus*. (The Times of India, Delhi Edition, 18th August, 2005).

CONCLUSIONS

AIDS has enormous therapeutic challenge due to complexity of HIV pathogenesis, multiple levels of control of proviral expression and the ability of the virus to rapidly mutate. Fusion inhibitor like enfuvirtide is USFDA approved. Integrase inhibitor and ribozymes are under clinical trials and expected sooner for clinical use. These recent advances are quiet promising and less susceptible to resistance for therapeutic failure due to resistance development.

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