CHAPTER 2

REVIEW OF LITERATURE

Ganser-Pornillos et al described the HIV-1 genome. HIV-1 is an enveloped positive strand RNA virus belonging to the lentivirus family of retroviruses. It contains two linked copies of a single-stranded RNA (each ~ 9 kb long), each of which contains the genetic information necessary to encode all 15 viral proteins.

Briggs and Krausslich reported genetic organization of HIV-1. During the replication, double-stranded DNA is formed by reverse transcription of viral RNA. The dsDNA is then integrated into the host cell genome where it is referred to as the proviral DNA” containing 9 ORFs. The three large ORFs env, gag, and pol are present in all retroviruses. The gag gene encodes the gag precursor polyprotein, pr55. Proteolytic cleavage of pr55 produces the structural proteins - p6 capsid (p24), matrix (p17), nucleocapsid (p7). env (gp160) is cleaved to form gp120 surface and gp41 transmembrane glycoproteins.

The pol encoded region undergoes proteolytic cleavage to liberate the viral enzymes - protease, Reverse Transcriptase and integrase; which are essential proteins for viral replication. Nef, Vif, Vpu and Vpr are the accessory proteins. Tat and Rev are the regulatory proteins (Briggs & Krausslich, 2011).

Lu et al. described HIV-1 LTR and its function. The HIV-1 long terminal repeat (LTR) is nearly 640 bp in length and is comprised of U3, R, U5 region. It is present at both ends of viral DNA. The U3 region is further subdivided based on sites of transcription factors, present in LTR and the impact these factorsexert on LTR mediated gene expression.

The ends of the LTRs play a role during integration of dsDNA into cellular DNA. Once the integration occurs, the 5' LTR act as the promoter for viral genome, while the 3' LTR provides for the nascent viral RNA polyadenylation and encodes the
accessory protein, Nef. Regulatory viral and cellular proteins modulate LTR-mediated expression of HIV-1 proviral genome. Integrated 5’LTR is always precisely organized into two distinct nucleosomes termed Nuc-0 and Nuc-1.

Arhel4 reviewed HIV-1 uncoating. The envelope protein (Env) of HIV-1 binds to CD4 first, leading to structural conformational changes and then binds to chemokine receptors CCR5 or CXCR4 resulting in fusion of the virion and plasma membranes allowing entry of the virus into the cell. Uncoating of virus takes place in the cytoplasm, and involves partial dissociation of capsid in order for reverse transcription to take place to form ds cDNA. Transportation and integration of this DNA occurs with the help of Vpr and integrase protein of virus.

Seale5 explained the pathogenesis of HIV-1. During viral expression, this DNA of provirus is copied out to produce new viral genomes and spliced and unspliced mRNAs, which are in turn translated to produce various viral proteins. Components of the virion are then shifted to the plasma membrane where assembly takes place. After budding and release from plasma membrane, these virion particles mature into fully infectious virions. Gradual reduction in number of CD4 T-cells occurs as a result of death of infected CD4 T-cells during HIV infection.

Sepkowitz6 reviewed several HIV inhibitors. Use of HAART comprising NRTIs and NNRTIs and protease inhibitors has effectively reduced HIV virus load in infected individuals.

Bradbury and Samaras7 studied the effects of HAART on life of patients. Regular use of HAART for several years has improved health of patients. Duration of life has increased by several years. Identification and development of new drugs and their inclusion in HAART regimen would provide additional clinical benefits.

Letvin8 discussed present HAART therapy, availability of drugs in developing countries, toxicity etc. In recent years an Integrase Inhibitor raltegravir and one entry inhibitor Maraviroc which blocks CCR5 have been approved by FDA for clinical use in patients infected with HIV. These two drugs are included in HAART. Though HAART has been successful, prolong treatment leads to some undesirable side effects like hepatotoxicity.
There are issues associated with treatment regimens. Most of RT inhibitors are lipophilic in nature. Poor bioavailability, pharmacology and tissue distribution are concerns of HAART. In the developing world high cost of drugs make the treatment unaffordable for patients. Sub-Saharan Africa and Southeast Asia has been reported to be the centre of pandemic. The rate of transmission and infection is very high in heterosexual women.

Griffith reviewed the perspectives of antiviral drug resistance. The presently used therapy i.e., Highly Active Antiretroviral Therapy aims at reducing the viral load and thereby bringing CD4 counts to normal level. NRTIs, NNRTIs, Integrase Inhibitor and protease inhibitors and a fusion inhibitor constitute HAART.

Jennifer et al. studied the quality and impact of drug toxicities on South African communities. The HAART therapy controlled the virus but failed to remove virus totally from body. Their toxicity, high cost, deleterious side effects and drug resistance make their use limited. This necessitates the development and generation of new anti-HIV agents with different mechanisms of action.

Vigouroux et al. reported the toxic effects of HAART. The presently used therapy i.e., Highly Active Antiretroviral Therapy aims at reducing the viral load and thereby bringing CD4 counts to normal level. However patients receiving HAART for prolong period reported hepatotoxicity, headache, nausea, weakness, pain etc.

Lafeuillade shed light on importance of public awareness. In these areas of awfully high rate of transmission, it is believed that education and behavioral awareness among individuals, reducing stigma would definitely help in reducing or controlling the spread of virus. The development of successful prophylaxis strategies, including use of chemical agents for prevention will lead to reduction in transmission and infection of HIV.

Nachega et al. described the effects of HAART and ways to prevent toxic effects of treatment. The HAART therapy controls the virus but fails to remove virus from body completely. Their toxicity, high cost, unpleasant effects and drug resistance make the use use of these drugs limited. Hence, new agents with different mechanism of action needs to be developed at the earliest.
Fultz et al14 and Lehner et al15 described transmission of HIV-1 by sexual route. The major route of HIV infection is through sexual transmission. In genital tract and rectal submucosa, CD4 T-cells, dendritic cells, and macrophages get infected with virus. The envelope protein of HIV-1 binds to CD4 receptor and one of the chemokine receptors CCR5 or CXCR4 which is then followed by fusion of virion and plasma membrane allowing entry of virus into the cell.

In the cytoplasm, dsDNA is formed upon transcription of viral RNA. The DNA is translocated into the nucleus where it is integrated into cellular DNA with the help of integrase enzyme of the virus. Viral gene is transcribed to produce various viral proteins. Virion components are brought to the plasma membrane where virion assembly occurs. After budding and release from plasma membrane these virion particles mature into fully infectious virus.

Omar and Bergeron16 talked about development of microbicides. Throughout the world, sexual route of transmission is the most common mode of HIV infection. One of the important strategies of preventing sexual transmission is use of microbicide during sexual intercourse. Females are mostly affected. Microbicide formulation is a female controlled, safe and cost effective method.

Obeiro et al17 discussed microbicide development. It has been realized that controlling transmission of virus in the developing countries would help to curb transmission rate of HIV worldwide. A microbicide with moderate efficacy (50-60%) could prevent millions of new cases of HIV infection each year throughout the world.

Vogt and Hirsch18 reviewed the strategies to prevent sexual transmission of HIV. Since this is the major route of virus transmission and women are more likely to acquire HIV-1 from infected seminal fluid from heterosexual partners, development of effective microbicide products is getting immense importance in the present fight against HIV transmission and prevention.

Kelly and Shattock19 reviewed microbicide candidates in development. Presently none of the molecule has proven successful in clinical development. Entry inhibitors acting at mucosal site are being evaluated in preclinical and clinical phases of
development. These compounds exert effect on virus crossing mucosa or attaching mucosal cells or submucosal cells (LCsDCs, macrophages, and CD4+ T-cells). The entry inhibitors can also prevent transmission of virus from one cell to another. Development of safe and effective microbicide involves huge investment.

Mertenskoetter and Kaptur discussed about specificity of candidate microbicides. Microbicides can be categorized as nonspecific compounds and specific molecules. The nonspecific microbicides are active against most of the viruses. N-9 nonoxynol is a prototype of this class of microbicide.

Specific microbicide acts or targets viral or cellular proteins and inhibits viral entry, transcription, integration, maturation. Eleven agents are being evaluated in various phases of clinical study. More than 50 are presently being evaluated as microbicide candidate at preclinical development stage.

Buckheit, R.W et al discussed development in the area of topical microbicides. Sexual transmission is a major route of HIV transmission and infection in the world. One of the important strategies to prevent sexual transmission and infection is use of microbicide. HIV is spreading at an alarming rate. Women are mostly affected and hence a female controlled method for prevention of transmission is required. Microbicide provides a safe, cheap and woman controlled method.

Balzarini, J. et al reviewed microbicide drug candidates and their classification. Microbicide is a formulation of one or more anti HIV agents that can prevent infection either by killing virus or preventing its entry or attachment to cells thereby preventing its infection. Microbides have been classified based on their mode of action.

Van Damme, L., et al and Fichorova R N et al studied the cytotoxicity and efficacy of nonoxynol 9. It is an anionic surfactant developed as spermicide. N9 disrupts viral envelope and thereby kills virus. Recent studies have indicated that presence of N9 actually enhances release of inflammatory cytokines and facilitates viral entry into the cells.
Vanpouille et al25 discussed HIV-1 microbicides, mechanism of action of microbicides in their reviews. Microbicide is a formulation of one or more anti-HIV agents that can prevent infection either by killing virus or preventing its entry or attachment to cells. Looking at the intensity of AIDS pandemic and availability of therapeutic molecules with considerable side effects and problem of drug resistance, it becomes imperative to identify novel molecules which could be potential lead molecules for developing a microbicide formulation to prevent HIV-1 infection.

Poynten et al26 evaluated safety and efficacy of N9. N-9 has shown very good results in preclinical studies. Later on it was found that N-9 instead of preventing, facilitated the HIV-1 transmission. This was due to increase in secretion of proinflammatory cytokines upon N-9 application. This caused mucosapermeable to virus and thus there was increase in infection reported.

Rusconi27 reported mechanism of action of Pro2000. It is a polymer of naphthalene sulfate. Pro2000 has high affinity for CD4 binding.

Huskens, D et al28 found out mechanism of action of Pro2000. It blocks virus attachment to cell surface and thereby prevent infection.

Keller et al29 studied the safety of pro2000. PRO2000 was successful in safety and efficacy studies. It is now being evaluated as microbicide candidate.

Cohen30 reported that PRO 2000 could achieve an overall protection of 30% during 3 year follow-up. The results from this study were quite encouraging and motivating for scientific community working on development of microbicide.

Williams et al31 reported ACIDFORM. It is a pH-modifying compound. It is presently undergoing phase II trial. ACIDFORM has been found safe for microbicide application in phase 1 trial.

Mbopi-Keou et al32 evaluated invisible condom for safety microbicide candidate. Invisible Condom is made up of polyoxy ethylene–polyoxy propylene copolymer. It is being investigated in phase II clinical trial. This microbicide, forms a layer over the mucosa that prevents pathogen entry. A 14-day intravaginal application study showed that invisible condom is safe.
Skoler-Karpoff et al. evaluated carrageenan and cellulosesulfate as microbicide candidates. These inhibitors of HIV entry failed to reduce transmission of virus. Development of these two compounds as microbicides is stopped.

Rupp et al. evaluated safety and efficacy of Vivagel. Vivagel (SPL7013) is a dendrimer. It has shown potent activity towards HIV and HSV. It has been proven to be effective in preclinical studies. Safety assessment in human indicated Viva gel as safe for human use.

Ramjee reviewed several compounds at various stages of development. In preclinical phase of developed more than 50 compounds are being evaluated. These compounds target HIV entry, fusion and or reverse transcription process.

Gibson and Arts talked about properties of candidates for use as microbicide. A microbicide for HIV is expected to block virus at very early events of HIV life cycle like entry, fusion, transcription etc. The microbicides can be stable, lipophilic in nature. They should exert the effect locally. Microbicides are not supposed to enter systemic circulation.

Vermeire et al. reported anti-HIV activity of CADA (cyclotriazadisulfonylamide). It is a new class of compounds that down regulate cell surface expression of CD4 receptor in human cells. A gel formulation of CADA exhibited synergistic effect when used along with celluloseacetate phthalate.

Wiesner and Vilcinskas reviewed entry inhibitors. Monoclonal antibodies mAbs (PRO-140), small molecules (SCH-C, SCH-D, and UK-427857), modified chemokine (NNY-RANTES and PSC-RANTES), inhibit entry of the virus by blocking CCR5 or CXCR4 receptors. Treatment with modified chemokine agents results in inhibition of infection and this effect is particularly mediated by down regulation of chemokine receptors. However there are some practical issues of stabilitytherapeutic efficacy.

Forthal and Moog studied agents that bind to HIV envelope. HIV gp120 trimer is bound to trimeric gp41 protein. This association is noncovalent. mAbs acting on env protein have broad neutralizing activity against several HIV isolates.
Combination of mAbs b122G12, and 2F5 exerted synergistic effect of neutralization. This combination has been shown to successfully protect macaques challenged with HIV gp120.

Pugach40 evaluated neutralizing antibodies and anti-HIV drugs sensitivities of HIV-1 isolates. BMS-806 inhibitor, has been shown to target CD4 interacting domain on gp120.

This compound is being evaluated for microbicidal activity.

Balzarini41 reviewed Carbohydrate-binding agents (CBA). HIV gp120 consists of glycans. Almost all HIV clades have envelop proteins made up of glycans. 50% of the glycans present are high-mannose type glycans. The glycans act like shield and protect HIV from recognition by immune cells. Cyanovirin-N (CV-N) and plant lectins can bind to the glycans present on env proteins and prevent attachment/binding of virus to the cell and infection of the cell.

Boyd et al42 reported anti-HIV activity of Cyanovirin. Cyanovirin has been shown to successfully block entry of virus.

Huskens et al43 showed that intravaginal or rectal application of Cyanovirin prevented SHIV infection of monkeys.

Gengiah et al44 reported microbicidal activity of tenofovir gel. The NtRTI tenofovir, is approved by FDA for systemic use. The tenofovir gel is being assessed in phase II clinical study. Tenofovir is known to develop resistance in patients infected with HIV. Phase II study involving application 3% tenofovir in women indicated that the drug enters systemic circulation. Low levels of tenofovir were detected in serum/plasma samples of patients.

Lewi et al45 reviewed Reverse Transcriptase Inhibitors. NNRTIs unlike NRTIs show very specific activity against HIV RT. They can act directly and do not require modification by cellular enzymes to become active. TMC-120 and UC-781 are well ahead of other candidates in clinical trials. These compounds are lipophilic in nature. Both the compounds efficiently blocked infection in monocyte-derived dendritic cells and T-cells co-culture. These compounds showed high
therapeutic index in primary cultures. UC-781 has very high affinity for HIV RT. In cervical tissue explants it has been shown to inhibit local infection and transmission of HIV. TMC 120 has also evaluated in such studies where it exerted similar effects as that of UC 781.

Nel et al. 46, 47 in 2009 and 2010 reported microbicidal activity of TMC 120 and its pharmacokinetics. TMC-120 was incorporated in silicon and formulated as a vaginal ring. In hu-SCID mice the TMC 120 ring formulation has effectively inhibited infection. Since this compound is lipophilic in nature, its bioavailability upon oral administration is less. Very low levels of drug were detected in blood after application of TMC120 gel.

Charlene S. Dezzutti et al. 48 have evaluated several potential microbicide candidates like CAP, Carraguard, PRO 2000, and cellulose sulphate along with their placebos for toxicity in epithelial and cervical cell lines. These compounds seemed to be relatively non toxic and efficacious against HIV infection in PBMCs and macrophages.

Kilmarx, P.H., et al. 49 reported safety and efficacy of carraguard in phase III clinical trials.

El Sadr, W.M et al. 50 Halpern, V et al. 51 evaluated safety and efficacy of cellulose sulphate as a candidate microbicide. None of these agents showed promising results in the trials.

Patricia Fletcher et al. 52 evaluated UC781, NNRTI as a microbicide. It has low molecular weight and inhibit HIV in low micromolar range. In human explant culture, UC781 inhibited infection of vaginal tissue and prevented spreading of HIV by migratory cells.

Lanier, E.R. et al. 53 reported tenofovir a Reverse Transcriptase inhibitor, that had shown promising HIV-1 microbicidal activity.

Mayer, K.H., et al. 54 evaluated tenofovir for safety and efficacy in phase II clinical trials and its 1% gel is presently being evaluated in phase III trials.
Donglei et al. have discussed in detail anti-HIV agents from natural resources. Some of agents belong to terpenoids, coumarins. Some compounds are alkaloids, polyphenols. Compounds from class of tannins and flavonoids are reported to have anti-HIV activity. These drugs show specific mechanism of action based on their physicochemical nature.

Asres et al. reviewed anti-HIV-1 natural products. The natural products can be synthesized fully or obtained from microorganisms. Many such products have been assessed in clinical studies for generation/development of new, safe and cheaper alternatives for treatment of HIV infection.

Sakalian et al. and Temesgen and Feinberg reported HIV inhibition by bevirimat and its mechanism of action. Bevirimat is derived from the plant metabolite betulinic acid. Bevirimat has been shown to act at the late stage of HIV life cycle by blocking maturation of virions. It is presently investigated in phase IIb study in HIV-infected patients.

Smith et al. reported activity of bevirimat. Bevirimat was semi-synthesized by modifying betulinic acid at C-3 position by 3,3-dimethylsuccinyl chloride. In lymphocyte culture infected with HIV-1 it showed an EC50 value of < 0.35 nM.

Stoddart et al. proved that bevirimat inhibits maturation of virions. Bevirimat did not affect early steps of viral life cycle instead it acted at a late stage in HIV replication. Studies to understand mechanism of action revealed that bevirimat inhibited the processing of the viral gag polyprotein at a specific step. It interferes in the formation of mature capsid protein p24 from the capsid precursor p25 in a dose-dependent manner. This inhibition led to the generation of immature viral particles lacking infectivity. This gave rise to maturation inhibitors as a new class of anti-HIV drugs.

Singh and Bodhiwala reviewed several natural compounds with anti-HIV activity. Michellamine B, harmine, macrocarpals, Batzelladines, calanolide A and B, calceolarioside B, mallotojaponin, are known to possess anti-HIV properties.
Kashman et al. reported anti-HIV-1 activity of calanolide. Calanolide was the most active anti-HIV-1 compound isolated in the NCI led discovery program from rainforest tree Calophyllum lanigerum. (+)-Calanolide inhibited various laboratory strains of HIV-1 with EC50 of 0.10 to 0.17 µM.

Currens et al. showed that calanolide inhibits HIV-1 Reverse Transcriptase. It has been demonstrated to have selective activity towards HIV-1 RT. It did not interact with HIV-1 RT. Calanolide has no effect on cellular DNA polymerase. The mechanism of action revealed that calanolide interferes with dNTP binding site present in HIV RT. In combination with nevirapine it showed synergistic effect, suggesting that it was different than the general class of nucleoside RT inhibitors.

Arisawa et al. reported isolation of Methylene-bis-(diacetyl phloroglucinol) from the culture of Pseudomonas aurantiaca. It belongs to the group of antibiotics and possesses antibacterial activity against gram-positive bacteria at concentrations of 0.1-1.0 µg/ml. It also possessed antimalarial activity.

Arisawa in 2003 showed presence of Dimeric phloroglucinol in Hypericum japonicum and these include sarothralens B-D and japonicine A. These compounds possess antimicrobial and antimalarial activity. Dimeric phloroglucinol mallotojaponin isolated from Mallotus japonicus showed reduction in reverse transcription by more than 90% at concentration of 25 µg/mL.

Nankane H. reported anti-HIV activity of Dimeric phloroglucinol derivatives. Mallotojaponin, mallotophenonemallotochromene and mallotolerin were demonstrated to have HIV inhibitory activity. 26, 38, 67 and 75% reduction in RT activity was observed in the presence of 10 µg/ml of mallotophenonemallotolerin, mallotojaponin and mallotochromene, respectively. A dosedependent response was seen in case of all the compounds. at a concentration of 25 µg/ml mallotojaponin showed more than 90% reduction, Mallotojaponin and mallotochromene were found to be more potent than mallotophenone and mallotolerin. A moderate inhibition of enzymatic activity was seen in in vitro assay employing MS-2 phage RNA as the template primer.
Daikonya et al. reported cytotoxic activity of mallotojaponin and its analogues. Mallotojaponin and several of its analogues viz. butyryl mallotojaponin, isobutyryl mallotojaponin, mallotolerin, butyryl mallotolerin, isobutyrylmallotochrome, butyrylmallotochrome, isobutyrylmallotochrome, mallotojaponol and mallotochrome isolated from pericarps of Mallotus japonicus exhibited cytotoxicity in HeLa cells with CC50s ranging from 285 ng to 49.1 µg/ml and anti-herpetic activity ranging from 88 ng to 48 µg/ml.

Hong et al. The derivatives of mallotojaponin, mallotophilippen A and mallotophilippen B were isolated from the hexane extract of Mallotus philippensis. These compounds have an effect on nitric oxide production. These compounds also inhibit secretion of histamine from peritoneal mast cells of rat. These results suggest antiallergic and anti-inflammatory activity.

McCormick, J. L. et al. reported isolation of buchhapine and anti-HIV activity. It is a quinolone alkaloid purified from methanolic extract of the epigeal part of Euodia roxburghiana. Buchapine (EC50 0.94 µM) exhibited anti-HIV activity in CEMSS cells infected with HIV-1.

Sun et al. isolated a new lignin longipedunin A from the roots and stems of Kadsura longipedunculata. Longipedunin A showed an IC50 of 50 µg/mL against HIV-1 PR. Higher activity of longipedunin A than the other dibenzocyclooctadiene lignans indicated that a trans-cinnamic acid ester group might be an important functional group for anti-HIV PR activity (Sun, Chen et al., 2006)[146].

Reutrakul V et al. showed presence of flavonoids in methanolic extract of Ochna integerrima. Four new flavonoidglycosides were isolated. In syncytium formation assay, the compounds showed anti-HIV-1 activity with an EC50 of 29.1, 14.0, 102.4 and 45.8 µg/mL, respectively.

Lee et al. have recently reviewed plant derived anti-HIV triterpenes. Plants of the genus Schisandra are known to contain dibenzocyclooctadiene lignans, as well as lanostane and cycloartane triterpenes (Lee, Miyashiro et al., 2008).
Mu et al. studied several species of Schisandra genus. This led to the discovery of a series of new norcycloartane triterpenoids, nor triterpenoids and bisnor triterpenoids that possessed anti-HIV-1 activity.

Luo et al. reported isolation of Rubriflordilactones A and B from the leaves and stems of Schisandra rubriflora. They are highly unsaturated rearranged bisnor triterpenoids. Rubriflordilactone B showed anti-HIV activity, with an EC50 of 21.1 µM and TI of 12.39.

Xiao et al. isolated Lancifodilactone H, a trinorcycloartane triterpenoid with a biosynthetically modified sevenmembered lactonering, and lancifoic acid A, a new A ring seco cycloartanetriferpenoid, from the S. lancifolia. They exhibited anti-HIV activity, with EC50 values of 37.4 and 33.2 µM, respectively.

Kleinwachter et al. reported isolation of four new lanostane triterpenes, colossolactones V - VIII, from the mushroom Ganoderma colossum widely present in Vietnam. The colossolactone V and VI with seco A and E rings differ structurally from previously isolated colossolactones, that possessed a seven member lactone A ring and six membered lactone E ring.

Kleinwachter et al. evaluated lupanes for anti-HIV-1 activity. Two new lupanes isolated from the EtOAc extract of the resins of Garcinia hanburyi showed HIV inhibition activity with EC50 of 5.6 and 6.0 µg/mL, respectively in the syncytium formation assay. They displayed HIV-1 RT inhibitory activity with IC50 of 116.9 to 16.3 µg/mL respectively.

Dine et al. assessed protease inhibition by lactones. Colossolactone VII has a lactone E ring and seco A ring while colossolactone VIII has both A and E lactone rings. Colosolactone VIII showed anti-HIV PR activity with an EC50 of 14.6 µM. Colosolactone VIII (with a double bond at C-8/C-9) exhibited higher activity than the related colossolactones VI with two double bonds at C-7/C-8 and C-9/C-11. The unsaturation pattern would alter the 3D structures and, as a result, the spacing of pharmacophore in the compounds would be altered, which have a significant influence on activity (Colossolactones VI was inactive).
Chen et al. isolated two cucurbitacins, hemslecin A and B, from the tubers of Hemsleya jinfushanensis. These compounds were early isolated from many plants of this genus that are used to cure inflammatory diseases such as enteritis, diarrhea, and bronchitis, etc.

Both the compounds inhibited syncytia formation with EC50 values of 3.09 and 2.53 µg/mL, and p24 antigen production with EC50 values of 3.97 and 18.90 µg/mL, respectively. Fusion of infected H9 cells and C8166 cells were blocked by compounds with EC50 of 1.8 and 11.9 µg/mL. Although these compounds showed slight interaction with HIV-1 IN, they did not inhibit HIV-1 RT PR, and NCp7 zinc ejection. It was concluded that these compounds can block or compete the binding site of HIV-1 or modulate molecular expression which is necessary for HIV-1 binding to reduce the entry of the virus.

Zhang et al. showed that the acetyl group can enhance the anti-HIV-1 activities of Sesquiterpenes. Sesquiterpenesoxypheyllendiol B and 1,2,3,4-tetrahydro-2,5-dimethyl-8-(1-methylethyl)-1,2-naphthalenediol were isolated from Litsea verticillata. These compounds showed reduction in replication of HIV in the HOG.R5 cells, with IC50 values of 54.6 and 91.0 µM respectively.

Mori Tal discovered Griffithsin (GRFT), a novel anti-HIV protein with 121 amino acids and mol wt. of ~13kDa from a hydrophilic extract of the red alga Griffithsia sp collected from the waters of New Zealand.

Ziolkowska and Wlodawer showed that the GRFT has wide spectrum of activity against various laboratory isolates including R5 and X4 tropic strains. It showed EC50 ranging from 0.043 to 0.63 nM. GFRT has been shown to interfere fusion of virus.

Fang et al. reported isolation and anti-HIV-1 activity of dimeric 64 kDa meliobiose-binding lectin, from the seeds of Bauhinia variegata. The plant belongs to the subfamily Caesalpiniaceae of the Leguminosae family. This lectin showed inhibition of RT with an IC50 of 1.02 µM.
Swanson et al84, discovered BanLec, a jacalin-related lectin isolated from Musa acuminata. It has molecular mass of ~30 kDa and exists as dimer. BanLec has been demonstrated to bind glycan present on envelop and block the entry of virus. It is active at low nanomolar to picomolar range of concentration.

Chauthe et al85 recently reported anti-HIV-1 activity of dimeric phloroglucinol derivatives. Seven dimeric phloroglucinolshad shown moderate to potent anti-HIV-1 activity in vitro, however only two analogs inhibited HIV-1 transcription by inhibiting Reverse Transcriptase enzyme in vitro. For otherfive compounds, modifying side chains resulted in altered mechanism of action.

Al-Rehaily et al86 isolated a quinoline alkaloid buchapine from Euodia roxburghiana. Ahmed et al87 evaluated HIV-1 inhibition by several derivatives of buchapine in CEMGFP cells. SAR study demonstrated that the ring B which is nonsubstituted and free 2-OH are important for effectively inhibiting HIV replication.

Cummins et al and Badley et al studied autophagy and apoptosis in HIV infected cells. AIDS is marked by the massive reduction in the number of CD4+ T cells. This massive reduction is the outcome of their apoptosis, autophagy and activation induced cell death (AICD). The T cell death seen in HIV-1 disease is a multifactorial event with many participants.

Li et al and Campbell et al showed that Tat plays major role in the apoptosis of the uninfected bystander cells.

Chenet et al, has shown that Tat is released by the infected cells and taken up by bystander uninfected cells. This extracellular Tat mediates apoptosis of uninfected cells by both intrinsic and extrinsic pathway. The tool used for the intrinsic pathway is Bim, a BCl2 relative that is pro-apoptotic.

Campbell et al studied interaction of Tat with cytoskeletal proteins. Tat interacts with αβ-tubulin/microtubules, stimulates microtubule assembly and prevents its depolymerisation, thus resulting in exceptionally stablemicrotubules. This perturbation of microtubule dynamics liberates Bim (sequestered in
microtubules) which then translocates to mitochondria resulting in release of cytochrome c, thus initiating apoptosis.

Westendorp et al and Dianzani et al studied apoptotic pathways and role of Tat in HIV infected cells. Tool used by Tat for activation of the extrinsic pathway is Fas Ligand (or CD178). Tat upregulates the expression of FasL.

Several other researchers like Dabrowska et al and Kim et al studied role of Tat in bystander cells. Apart from the role of Tatin apoptosis of uninfected bystander cells, few reports indicate a role of Tat in killing of infected cells also.

Gee et al in 2006 studied Tat mediated immune responses suppression. They observed that TAT mediated immune suppression is an outcome of other activities of Tat such as modulation of gene expression of various cytokines and Tat mediated apoptosis. Tat upregulates IL10 levels and this IL10 suppresses gp120 specific T cell response.

Carrollet et al showed that Tat represses MHC-I promoter to lower the cell surface expression of the viral antigens and avoid immune surveillance.

Schuster and Kriegtein studied effect of Tat on various protein levels. Tat also plays with the expression levels of various proteins inducing apoptosis like TGFβ. It upregulates the expression of TGFβ that potentially induces apoptosis of immune cells leading to induction of immunosuppression.

Ott et al studied post translation modification of Tat. Multiple modifications of Tat at various positions regulate its activity in the infected cell. Some modifications turn Tat ON while other turn it OFF. Tat acetylated at lysine 50 and 51 by HAT p300 is important for transcriptional activation of the LTR promoter.

Kiernan et al and Denget et al studied acetylation of Tat. Acetylation at K50 liberates Tat from Tat-TAR complex. Ac50-Tat binds to the elongating RNA polymerase II recruit P/CAF to the elongating polymerase. Tat is also acetylated by P/CAF at lysine 28, thus enhancing its binding to p-TEFb complex. Thus, acetylation of Tat at
two different sites K50 and K28 manifests different functions both aiming at successful transcription.

Pagans et al showed that Tat is deacetylated by SIRT1 or sirtuin 1, a class II NAD+ dependent HDAC. SIRT1 thus functions as Tat deacetylase and hence regulator of its activity.

Kwon et al. studied relation between TAT and SIRt1. SIRT1 has other substrates like NFκB-p65, p53PCAF etc. Tat inhibits deacetylase domain of SIRT1, thereby blocking its deacetylation activity on p65, to keep p65 in active acetylated form for its participation in HIV-1 transcription. Since Tat itself is a substrate of SIRT1, how inhibition of SIRT1 effects the deacetylation of Tat is not yet explored.

Boulanger et al. and Xie et al. studied methylation of Tat. Tat is methylated at arginines 5253 and lysines 50 and 51. Arginines 52 and 53 are substrates of proteinarginine methyltransferase 6 (PRMT6). Methylation of Tat decreases its interactions with TAR and hence negatively affect Tat-TAR-CyclinT1 ternary complex formation.

Sivakumar et al. studied post translational events of Tat. Compromising on its key function of LTR transactivation, Tat on getting methylated at R52 and R53 becomes more stable and these modifications curtail its degradation.

Van Duyne et al. and Endo-Munoz et al., studied activity of methylated Tat. Lysines 50 and 51 of Tat are substrates of lysine protein methyltransferases, SETDB1 and SETDB2, to which Tat interacts. Methylation at K51 of Tat is inhibitory to Tat’s activity on LTR. However the same residue of Tat when mono-methylated by Set7/9 (KMT7) enhances Tat mediated LTR transactivation. This exemplify the complex regulation by the interplay of post translational modifications of Tat protein, where the modifiers and site modified both add to the intricacies of this regulation.

McMillan et al. and Brand et al. studied ubiquitination of Tat. Tat is ubiquitinated at lysine 71 by proto-oncoprotein Hdm2 and this ubiquitination enhances Tat mediated LTR transactivation (Breset al., 2003). Phosphorylation: PKR
(interferon induced, double stranded RNA dependent serine threonine kinase) as part of the antiviral defense phosphorylates eIF2 rendering it inactive to participate in translation. Tat competes with eIF2 for binding to PKR which results in the phosphorylation of Tat at Ser62 Thr64 and Ser68. Phosphorylation of Tat by PKR enhances its binding with TAR.

Homes et al. and Ammosova et al. studied phosphorylation of TAT. Protein kinase C phosphorylated Tat at Ser46 in vitro. Tat is also reported to be phosphorylated at serines 16 and 46 by CDK2/Cyclin E. This phosphorylation is important for LTR activation. Each modification of Tat works to fine-tune the functions of Tat. But any of the functions ascribed to Tat cannot be completed without the help of the host proteins. Tat modulates a number of host proteins at transcriptional, post-transcriptional, translational, and protein activity levels for virus proliferation. It works at nearly each step of the viral life cycle for its successful completion.

Garcia et al. and Anderson et al. worked on modulation of cell surface receptor by Nef. Although Nef has been assigned various functions, but the best characterized effect of Nef is its ability to downregulate cell surface CD4 molecule.

Mariani et al. found that nef modulates expression of CD4 by lysosomal transport and degradation of CD4.

Benson et al. studied CD4 down regulation. CD4 down modulation has been shown to benefit the virus by two ways. It prevents super-infection of host cell, which may lead to premature death of infected cell as shown by Benson and colleagues, where expression of SIV Nef in T cell lines rendered them resistant to HIV-1 infection.

Lama et al. and Ross et al. studied the effect of Nef on CD4 expression. It helps to overcome high cellular CD4 expression that can otherwise inhibit progeny release and viral infectivity by sequestration of viral env. The molecular mechanism of Nef mediated CD4 down modulation is now relatively well described.

Kirchhausen et al. studied endocytosis of CD4. The cytoplasmic tail of CD4 contains a dileucine motif that has been implicated in endocytotic transport of CD4.
Marsh et al studied Ef and its interaction with CD4. In T cells, CD4 is maintained at cell surface by Src-family Tyrosine kinase Lck, which by binding to the dileucine motif of CD4 prevents its recognition by endocytotic machinery.

Aiken et al studied PKC and Nef interaction. Protein kinase C (PKC) mediated phosphorylation of serines, proximal to this dileucine motif, induces both dissociation of Lck and interaction with AP-2 clathrin coated adapter complex. This interaction results in CD4 molecule internalization and followed by its degradation in the lysosome.

Bandres et al and Salghetti et al showed that Nef binds with the dileucine motif of CD4 cytoplasmic tail and thereby disrupts Lck/CD4 interaction to expose internalization signal in CD4.

Greenberg et al and Gall et al studied Nef interaction with clathrin complexes. Nef has been also shown to interact with medium (μ) subunit of clathrin adapter complexes. Thus, Nef serves as a link between CD4 and endocytotic machinery to target CD4 for endocytosis followed by its degradation.

Piguet et al have shown that Nef sequentially interacts with adapter complex and β-COP (β-subunit of COP I coatamer protein) to target CD4 to early endosome to mediate its lysosomal degradation.

Schwartz et al studied MHC1 and Nef interaction. Nef has been also shown to attenuate the surface expression of MHC-1 by its endocytosis from plasma membrane to the trans golgi network (TGN) (Greenberg et al., 1998; LeGall et al., 2000).

Greenberg et al and LeGall et al studied effect of Nef on MHC-1. By down modulating MHC I expression HIV-1 avoids cytotoxic T lymphocytes (CTL) mediated killing of infected cells and maintain the persistent infection in host cell.

Crum et al studied PACS-1 and Nef interaction. PACS-1 (Phospho acidic clusters sorting protein-1) is shown as another key player in Nef induced MHC-1 down regulation by binding to the acidic cluster of Nef (62EEEE65).
Blagoveshchenskay et al has shown the role of ARF 6 (ADPribosylation factor 6) in conjunction with PACS-1, in Nef induced MHC-1 downregulation.

Bell et al and Swigut et al reported downmodulation of CD3 and CD28 receptors by Nef. Nef also inhibits the MHCII restricted peptide presentation by reducing the surface level of mature MHC II while increasing the level of immature MHCII, which are functionally incompetent.

Xu et al studied role of Nef in apoptosis. Apoptosis is recognized as the major line of cellular defence against virus infections mediated via CTL, which can recognize and induce apoptosis in virus-infected cells. Nef induces FasL expression on infected cells which leads to induction of cell death in bystander cells including CTL.

Yoon et al studied Fas ligand and Nef interaction. Nef also enhances Fas induced apoptosis as well as apoptosis via mitochondria dependent pathway. In contrary to this, a recent report has shown that Nef protects infected cells during Fas mediated apoptosis.

Wolf et al reported that Nef also blocks apoptosis in infected cells by inducing Bad phosphorylation by PI3K and PAK dependent pathway. The role of Nef in apoptosis is always debatable as there are contradictory reports regarding its role in apoptosis but it seems that it enhances apoptosis in bystander cells and inhibits apoptosis in infected cells.

Klotman et al studied expression of Nef in HIV infection. Nef is an early viral protein. Appearance of Nef transcripts has been observed immediately following infection.

Simmon et al observed that Nef is expressed at early stage in viral replication at a period before Tat and rev come into the picture. Also in a microarray analysis, Jurkat T cells overexpressing Nef have shown increased HIV-1 LTR activity by upregulation of a number of gene products, known to activate LTR. Nef is also known to upregulate the expression of various host factors required for Tat function such as CDK9, TAR binding protein (TARBP), Tat-Sf1 and Purα.
Joseph et al. studied the effect of Nef on transcription factors. Activation of various transcription factors like AP1, NF-κB and JNK by recombinant Nef has been reported in U937 cell line, leading to stimulation of viral replication in chronically infected U937 cells. Nef is also implicated in the inactivation of STATs like STAT1 and STAT3 in human monocytes/macrophages. Our lab has also shown earlier involvement of Nef in LTR driven gene expression. Nef interacts as well as co-localizes with Tat in both cytoplasm and nucleus to enhance viral gene expression and virus production.

Kumar et al. reported that Nef also enhances viral gene expression through HSP40. It not only interacts with HSP40 but induces its expression in HIV-1 infected cells. This interaction mediates the translocation of HSP40 into the nucleus which in turn facilitates viral gene expression by becoming part of PTEF-b complex.

Another report by Witte et al. also suggests a positive role of Nef in Tat mediated transcription. Here, Nef mediates recruitment of Eed (embryonic ectodermal development) protein from nucleus to cytoplasm. As Eed is known to negatively regulate HIV-1 LTR via its interaction with HDAC1 and 2 and transcription factor YY1, its translocation alleviates transcriptional repression.

Chowers et al. and Miller et al. studied the role of Nef in modulation of cell surface receptors. Besides the well-established role of Nef in modulation of cell surface receptors and viral replication, it is also known to enhance viral infectivity.

Schaffer et al. have shown that Nef increases infectivity of the virion by enhancing the cytoplasmic delivery of virus particle (Schaffer et al., 2001) whereas around the same time it was reported that Nef modifies the viral envelope to increase viral infectivity.

Xu et al. showed that Nef modulates cellular signaling in both T cells and macrophages.

Arold et al. and Saksel et al. have tried to understand the mechanism of this modulation. The most common approach involves the identification of Nef interacting players of cellular signal transduction pathways. Mutational analysis
suggests that most of the signalling molecules that bind to Nef interact with its core domain, often via the Pro-rich sequence. Nef binds to the SH3 domain of Src kinases through the P72xxP75 motif of Pro-rich sequence.

Grzesiek et al showed that Nef displays a high (nanomolar) affinity for the SH3 domains of Hck and Lyn, and a rather modest (micromolar) affinity for those of Lck, Fyn and Src. Mutational studies have further revealed the importance of core domain residues for interaction with a PAK kinase 23 and TCRζ. Induction of Fas ligand expression by HIV-1 involves the interaction of Nef with the Tcell receptor zetakain.

In a report by Iafrate et al, Nef has been found to inhibit TCR signaling by downregulating CD3, CD4 and CD28, the key players of antigen induced Tcell stimulation machinery.

Schragger et al and Wanget et al showed Nef dependent increase in TCR signalling leading to increased IL2 production upon αCD3 and αCD28 stimulation.

Baur et al studied TCR signalling. Intracellular localization of HIV-1 Nef was found to be important for activation and inhibition of early events in TCR signalling.

Heguy et al studied Rev mediated transport of RNA transcripts. Rev mediated nuclear export of unspliced and singly spliced viral RNA transcripts by interacting with Rev Responsive Elements (RRE) can be inhibited by Rev transdominant negative proteins, RRERNA decoys, anti-sense Rev/RRERNA s, siRNA/Fvs, ribozymes, aptamers, schimeric proteins and small inhibiting molecules.

Pollard et al studied Rev and host cell factors interaction. Rev M10 protein contains aminoacid substitutions at NES region and binding of its cellular partners is inhibited thereby blocks the export of viral RNA transcripts. Rev 38 protein has deletions in NES and is accumulated in the cytoplasm thereby forming inactive oligomers that delay the transport of Rev into the nucleus. NS1RM-Rev chimeric protein, a fusion protein of NS1 protein from Influenza virus form mixed oligomers with the Rev protein and as it is dominant over the wildtype Rev protein it inhibits the export of viral RNA transcripts.
Heguy et al showed that NES specific sFvs recruit the Rev in cytoplasm and accelerate the degradation of Rev protein. Small molecule inhibitors of Rev activity such as Leptomycin B, which interacts with CRM1 blocks the NES of Rev protein. It has been also shown to bind to the NES of cellular proteins.

Bogerd et al reported that Pyronin Y and diphenylfuran block the formation of Rev/RRE complex. As these compounds also intercalate the DNA, treatment with these compounds resulted into elevated levels of cell death. Anti-sense RNAs directed at sequences located in 5'-UTR and hairpin ribozymes that target U5 region inhibit translation of viral mRNA transcripts.