Current Trends in **Pharmaceutical Research** 2018, 5(1):44-65
ISSN 2310-4820
 www.dibru.ac.in/ctpr

Anti-HIV small interfering RNA (*si*RNAs) therapeutics in the management of Neuro-AIDS

Trinayan Deka* and Malay K Das

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004 INDIA

Abstract

At early period of characterization of HIV, it was considered that the virus infections were limited to only immune system. But it turns wrong when in 1985 HIV was recovered also from other tissues including brain. HIV induces several motor and cognitive disorders causing behavioral changes like short-term memory, reduced mental concentration, weakness, slowness of hand and leg movement, personality disorder, lethargy and social withdrawal which can be termed collectively as neuro-AIDS. After the discovery of small interfering RNA (siRNA) in 1990s, there has been a successful progress on improving its application for treatment of various diseases starting from cancer to viral infection. It has showed promising effect against HIV infection. Various steps like viral entry, reverse transcription, and integration the HIV replication cycle have been targeted, to inhibit the early stages of infection. Recent advances in the field of nanotechnology offer an unprecedented opportunity to enhance the power of siRNA mediated gene therapy by providing both an efficient delivery system as well as target specificity. Here an attempt is being made through this review to highlight progress of siRNA therapeutics against neuro-AIDS.

Keywords: *si*RNA, neuroAIDS, antiHIV therapeutics, nanomedicine.

Introduction:

Since the discovery of HIV (Human Immunodeficiency Virus) and AIDS (Acquired Immunodeficiency Syndrome), various advances in the field of understanding its pathology, treatment and prevention has been achieved. But neither the effective cure for AIDS not yet exists nor complete eradication of HIV achieved. As per UN AIDS report on 2016, approximately 36.7 million people have HIV worldwide with the number of new infections that year being about 1.8 million. This is down from 3.1 million new infections in 2001. Slightly over half the infected population are

*E-mail: trinayandk@gmail.com

women and 2.1 million are children [1]. After primary infection in the human body HIV virus can be remained dormant for up to ten years; during that period no symptoms are caused by the virus. Using host cell resources the dormant viral DNA may be transcribed, producing new RNA genomes and viral proteins, which are packaged and released from the cell as new virus particles that will begin the replication cycle anew. Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV (Lymphadenopathy Associated Virus) and HTLV-III (Human T cell Lymphotropic Virus III). HIV-1 is more virulent and more infective than HIV-2 [2] and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Due to its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa [3]. The drugs used for management of HIV infection are known as antiretroviral drug (ARV). There are several classes of antiretroviral agents that act on different stages of the HIV life-cycle. The use of combined regime of multiple drugs that affect the viral pathogenicity in different ways is known as highly active antiretroviral therapy (HAART). HAART is better than ART in decreasing the patient's total burden of HIV, maintaining function of the immune system, and preventing opportunistic infections that often lead to death [4].

HIV when enters the body, it not only confines to a particular organ but may affect different organs. HIV-1 causes multisystem disorder which includes the central nervous system too [5]. The neurological complications associated with HIV/AIDS are known as NeuroAIDS. The estimated overall prevalence of neuroAIDS among patients receiving highly active antiretroviral therapy but also requiring neurological care is over 25% [6]. The direct intrusion of the virus in brain leads to HIV associated dementia (HAD) characterized by neurological, motor and cognitive impairments resulting in progressive neurodegenration, also known as HIV encephalopathy (HIV-E) and AIDS dementia complex (ADC) [7]. However, with the use of HAART a more subtle form of CNS dysfunction known as HIV associated neurocognitive disorder (HAND) has become increasingly common in HIV patients [8]. HIV may enter into CNS either directly or as “Trojan passenger” via trafficking of infected monocytes, macrophages, and/or T-cells across the tightly junctioned brain microvascular endothelial cells (BMECs) of blood-brain barrier (BBB) [9].

Drug delivery to brain is being always a challenge to the scientist. Nanoparticle drug delivery has a significant success to reach the brain and treat various CNS disorders. The potential advantages of using nanomedicine over conventional HIV therapies include the capacity to incorporate, encapsulate, or conjugate a variety of drugs to target specific cell populations and to offer tunable and site-specific drug release. A wide range of nanomaterials have been used in biomedicine, these encompass dendrimers, liposomes, micelles, nanoemulsions, nanocapsules, nanocrystals, nanotubes, and nanoparticles. There are many limitations in all existing nanocarrier based drug delivery systems that affect the target specificity, delivery efficacy, release kinetics and bioavailability of desired amount of drugs at the target site. So, from a drug delivery point of view, a targeted brain specific delivery and drug release (sustained or on demand release) from the nanocarrier is very much needed to eradicate HIV reservoirs [10].

In recent years *si*RNA (small interfering RNA) based therapeutics have emerged with significant prospects. Since the discovery of RNA interference (RNAi) in mammalian cells, harnessing this pathway for the treatment of disease has become great interest. Craig Mello and Andrew Fire in 1998 were first to identify the RNAi mechanism, which involves the pairing of a short miRNA sequence to an endogenous mRNA target. RNA-silencing approaches have been designed to block specific steps within the viral replication cycle to control viral infection [11, 12]. It has been crucial to select of targets for RNAi-based therapies against HIV-1, so that a long-term therapeutic effect can be obtained without the emergence of resistant strains. Various steps like viral entry, reverse transcription, and integration the HIV-1 replication cycle have been targeted, to inhibit the early stages of infection [13,14]. RNAi also has a promising powerful strategy for intracellular therapy against HIV-1. RNAi can be used as an antiHIV-1 approach through stable expression of precursors, such as short hairpin RNAs (shRNAs), which are processed into siRNAs that can elicit degradation of HIV-1 RNAs. At the beginning of 2008, the first clinical trial using a lentivirus with an RNA-based gene therapy against HIV-1 was initiated [15].

Because of the limitations and adverse effects associated with current ART/HAART regimens, it is necessary to develop alternative therapeutic strategies that are safer with more efficacies and more resistant to viral escape. Anti HIV *si*RNA can be used to overcome various contradiction of HAART. The *si*RNA itself would act as drug of the HIV instead of therapeutic drugs. Delivery of such anti

HIV *si*RNA using nanotechnology may increase its bioavailability and specificity on the target. This review mainly focuses on various aspects of *si*RNA therapeutics towards neuroAIDS and their future prospects.

Anti-HIV drugs:

There are more than 25 anti-retroviral drugs with six mechanistic classes have been approved for use in HIV-infected individuals [16, 17]:

- **Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs):** They act by competitive inhibition of HIV reverse transcriptase enzyme and termination of the DNA chain; they interrupt the HIV replication cycle. e.g., Tenofovir, Zidovudine, Lamivudine etc.
- **Non-nucleoside reverse transcriptase inhibitors (NNRTIs):** All NNRTIs exhibit the same mechanism of action as NRTIs. They show potent activity against HIV-1 and are part of preferred initial regimens. e.g., Delavirdine, efavirenz, nevirapine etc.
- **Protease inhibitors (PIs):** They act as competitive inhibitors that directly bind to HIV protease, preventing the subsequent cleavage of polypeptides. e.g., Atazanavir, indinavir, squainavir etc.
- **Integrase strand transfer inhibitors:** They competitively inhibit the strand transfer reaction by binding metallic ions in the active site. e.g., Dolutegravir, elviegravir.
- **Fusion inhibitors (FIs):** They are the first class of antiretroviral medications to target the HIV replication cycle extracellularly to prevent the fusion of HIV to the CD4 or other target cell and received accelerated FDA approval in 2003. Enfuvirtide is the only product marketed in this class, at present.
- **Chemokine Receptor5 (CCR5) antagonists:** They selectively and reversibly bind the CCR5 coreceptor, blocking the V3 loop interaction and inhibit the fusion of the cellular membranes. e.g., Maraviroc.

Limitations of ARV drugs

Basically, combinations of three or more class of antiretroviral drugs are formulated for HAART (Highly Active Anti-Retroviral Treatment) regimens. With the proper HAART treatment, plasma viral load can decline below the detection limit and median life expectancy of AIDS patients may also rise by tenfold [18]. A concomitant rise in the other form of CNS dysfunction such as minor cognitive impairments/motor disorders has widely been noticed in the patients on HAART

regimes. Despite significant advances in the treatment of human immunodeficiency virus (HIV), there remain challenges. HIV is a chronic disease and patient adherence to treatment is critical over a lifetime. Poor therapy adherence increases the likelihood of virological failure and emergence of resistant strains of HIV [19]. Poor aqueous drug solubility is a major limitation, negatively impacting oral bioavailability for many antiretroviral drugs [20]. Complete eradication resulting in cure has long been a focus of research efforts but the existence of cellular and anatomical regions where the virus can continue to replicate in sub-therapeutic drug concentrations creates sanctuary sites [21], which reseed the blood when therapy is withdrawn [22]. Moreover, the antiretroviral drugs have several adverse effects like lactic acidosis, pancreatitis, peripheral neuropathy, hepatic steatosis, lipoatrophy, rash, diarrhea, nausea, vomiting, dizziness etc [16].

Neuro-AIDS:

At early period of characterization of HIV, it was considered that the virus infections were limited to only immune system. But it turns wrong when in 1985 HIV was recovered also from other tissues including brain. Mechanism of HIV infection causing neuronal injury and apoptosis in the host are not distinctly known. Through infected monocytes HIV enters the Central Nervous System (CNS) and activates macrophages and microglia and further toxin release which activate different pathways of neuronal dysfunction [5]. HIV induces several motor and cognitive disorders causing behavioral changes like short-term memory, reduced mental concentration, weakness, slowness of hand and leg movement, personality disorder, lethargy and social withdrawal. Minor cognitive motor disorder (MCMD) is a suitable form of CNS dysfunction common in HIV patients. HAART is not successful to control HIV associated dementia (HAD), HIV-1 infection becomes chronic and even rise in disease has been reported. MRI reports say that HIV infection is associated with progressive cortical atrophy which might be caused by neuronal loss and demyelination worsening in certain cognitive functions [9]. To understand the mechanisms of HIV-1 entry into the CNS through BBB several *in vitro* experimentations have been done so far. The amount of HIV DNA circulating in PBMCs was reported as the key factor for severity of HIV-1 associated neuropathogenesis [23]. It was hypothesized that HIV-1 enters the CNS, in disguise as a commuter in cells trafficking to the brain. The HIV-1 infected CD4+ cells, like T cells and monocytes, which are circulated in blood, crossed the Blood Brain Barrier (BBB) and introduced the infection into CNS [24]. Other proposed hypothesis for the entry of HIV-1 to brain is the migration between/ transcytosis of

endothelial cells. All types of the CNS cells like astrocytes, oligodendrocytes, neurons, macrophage and microglia, are easily infected by HIV-1 as they have receptors and co-receptors for HIV-1 entry, but only macrophage and microglia get infected most commonly which are the resident immunocompetent cells of the brain [25, 26]. BBB plays a central role in neuropathogenesis as it serves as the channel through which free virus and infected immune cells enter the brain. Progressive HIV infection and immune compromisation affect the BBB functions, leading to easy entry of viral fragment into the brain. It has been reported that HIV-1 gp120 protein and also Tat protein are behind BBB deregulation. PKC signaling pathways and receptor-mediated $Ca2^{+}$ release are the involved pathways resulting into cytotoxicity of the brain endothelial cells (Kanmogne et al., 2005) leading to downregulation and rupture of tight junction proteins (TJPs) of HBMECs, by the induction of proteasome by HIV-1 [27]. It has been studied that circulating virus or envelope proteins may also cause BBB dysfunction during primary infection. CNS infection of HIV is detected by viral RNA load in CSF (Woods et al., 2009; Morgan et al., 2011). Chemokines like monocyte chemo attractant protein (MCP)-1 control PBMCs relocation through BBB. Cellular migration engages adhesion molecules and differential regulation of inflammatory cytokines, leading to BBB disintegration and finally immune 112 Current Perspectives in HIV Infection dysregulation by letting sufficient entry of infected or activated immune cells into the brain causing neuronal injury [28, 29].

Nanotechnology approach for treatment of neuro-AIDS:

It is difficult for most of the ARTs to diminish infection from tissue reservoir other than blood like brain. HIV nanotherapeutics has advantages of controlled and sustained release of the drug during transportation and at the site of localization, altering the organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects, toxicity, and adverse drug reactions. Site-specific targeting via receptor-mediated endocytosis can be achieved by attaching targeting ligands to the surface of the nanocarriers matching to a specific cell receptor or through the use of magnetic guidance. The nanocarriers loaded with specific P-gp efflux inhibitors can result in increased ARV drug concentration in the CNS. The system can be used for various routes of administration, including oral, nasal, and parenteral [30]. The reduced first-pass hepatic metabolism and increased blood circulation time of nanoparticles make them suitable for the purpose of passive targeting. The accumulation of higher drug concentration at the BBB may enhance the drug permeability via passive diffusion.

Nanocarriers for the treatment of neuroAIDS are small sized (1−100 nm) particles derived from various materials like polymer, lipid, metal, dendrimer, etc. They can be transported across the BBB without any damage to the BBB [31]. The enhanced transport mechanism may due to the following:

- NPs open the tight junctions between endothelial cells and enable the drug to penetrate the BBB.
- NPs are transcytosed through the endothelial cell layer.
- NPs are endocytosed by endothelial cells and release the drug inside the cell.
- Coating agents for NPs such as polysorbates inhibit the transmembrane efflux systems (i.e., P-gp) and increase BMVEC membrane fluidization and facilitated endocytosis [32]. The hydrophilic coating with polysorbates also improves the solubility of poorly soluble drugs.

The efficacy of nanoformulations is further improved due to higher drug loading in nanocarriers with increased specific surface area. It results in initial burst release of drug leading to its active pharmacological level followed by a constant slow release for a prolonged time. Thus, the dose size and dosing frequency are minimized avoiding dose-related toxicities of ARV drugs.

Nanotechnology opens the door to overcome cellular and anatomical barrier like BBB which inhibits transport of ARTs to brain by the presence of tight endothelial cell junctions and efflux transporters such as P-glycoprotein (P-gp), a multidrug-resistant protein. Nanocarriers bypass multi drug resistant transporters such as P-gp that may efflux drugs entering freely through the plasma membrane. The Pluronic® (BASF - The Chemical Company, Florham Park, NJ) is block copolymers based on ethylene oxide and propylene oxide that enhance the *in vivo* efficacy of antiretroviral. Pluronic P85 has shown to inhibit the interaction of P-gp with nelfinavir and saquinavir [33]. An elegant study using a nanosuspension of indinavir showed that macrophages loaded with this nanosuspension could bring about a measurable reduction in antiviral load in the brains of an HIV-infected rodent model [34]. Magnetic nanoformulations have been used for targeting active nucleotide analog reverse transcriptase inhibitors to the brain by application of an external magnetic force and thereby eliminating the brain HIV reservoirs [35]. Conjugation with transferrin (Tf), allows antiretroviral drug-loaded nanoformulations to permeate across biological barriers such BBB via a receptor-mediated transport mechanism. The antiviral efficacy and the transversing ability of the QR-Tf-saquinavir

nanoformulation (Tf-conjugated quantum rods) across an *in vitro* model of BBB were evaluated with stable incorporation of saquinavir within Tf-conjugated quantum rods [36]. Kuo and Chen (2006) reported that the use of PBCA (polybutylcyanoacrylate) nanoparticles enhance the *in vitro* BBB permeability of ARV drugs zidovudine and lamivudine by 8–20 and 10–18 fold, respectively. In the same study, application of other acrylic polymer nanoparticle, methylmethacrylatesulfopropylmethacrylate (MMSPM), showed 100% rise in the BBB permeability of zidovudine and lamivudine [37]. Destache et al. demonstrated that nanoformulations of ritonavir, lopinavir, and efavirenz with PLGA can maintain a sustaining peak of about 28 days in the mouse brain, which is limited to only days with free drugs [38]. Similarly, Rao et al. demonstrated that at 2 weeks postadministration, PLA nanoparticles in conjugation with Tat peptides could result in 800-fold higher level of ritonavir in a mouse brain in comparison with drugs delivered in solution [39]. An *in vitro* study was done by Sumit and coworkers on colloidal gold-loaded, poly(D,L-lactic-co-glycolicacid)-based nanoparticles containing stavudine. To minimize the systemic toxicity of stavudine, providing reduced required drug dose and improved drug delivery over an extended period (63 days), macrophage targeted nanosystems were developed [40]. A nanoformulation consisting polybutylcyanoacrylate (PBCA) and methyl methacrylate sulfopropyl methacrylate (MMA-SPM) nanoparticles was investigated by Kuo and co-workers on several antiretroviral agents specifically for brain targeting [41].

Dendrimers have been used as carriers of antiretroviral peptides and genes for HIV inhibition, and more surprisingly, many recent studies showed that they themselves can be used as antiretroviral agents [42, 43]. Dutta and co-workers loaded lamivudine into mannose-capped polypropyleneimine dendrimers and obtained an increase in antiretroviral activity, cellular uptake, and reduced cytotoxicity with respect to the free drug [44]. Efavirenz-loaded tuftsin conjugated 5th-generation polypropyleneimine dendrimers (TuPPI) were also been prepared. Tuftsin is a natural macrophage activator tetrapeptide (Thr-Lys-Pro-Arg) able to bind specifically to mononuclear phagocytic cells enhancing their phagocytic activity. The authors reported that the dendrimer system is able to prolong the *in vitro* drug release up to 144 h with respect to 24 h of the PPI polymer. Moreover, a 34.5 times higher cellular uptake and reduced viral load by 99 % at a concentration of 0.625 ng/mL was reported; this activity was more significant in HIV infected macrophages than uninfected cells [45]. Silver complexes with anionic linear globular dendrimer showed very potent antiretroviral activity with non-severe toxic effect [46]. Various

combinations of anionic carbosilane dendrimers with sulfated (G3-S16) and naphthyl sulfonated (G2-NF16) ended groups with different ARVs against HIV-1 infection were developed Vacas-Córdoba and co-workers. The G3-S16 and G2-NF16 dendrimers showed a synergistic or additive activity profile with zidovudine, efavirenz, and tenofovir in the majority of the combinations tested against the X4 and R5 tropic HIV-1 in cell lines, as well as in human primary cells [47].

Kim et al. investigated multi-vesicular liposomes for the delivery of the drug into the cerebrospinal fluid in a Sprague Dawley rat model. It was demonstrated that the half-life of lipo-zalcitabine in the brain of Sprague Dawley rats can be prolonged to 23 h as compared with 1.1 h for non-encapsulated drug [48]. Further, the superiority of the CNS-targeting ability of liposomes loaded with AZT-myristate (prodrug of AZT) was studied by Jin et al. It was shown that, with about 98 % encapsulation efficiency and longer half-life, a higher concentration of AZT was found in the brain and other organs of rats [49]. Researchers envisage the development of a stealth antiCD4-conjugated immunoliposome containing two antiretroviral drugs (nevirapine and saquinavir) that can selectively home into HIV-infected cells through the CD4 receptor. The drugs delivered via anti-CD4-conjugated immunoliposomes inhibited viral proliferation at a significantly lower concentration as compared to free drugs. Both drugs were found to localize in different regions of the liposome. The release of the reverse transcriptase inhibitor was dominant during the early phases of the release while in the later phases, the protease inhibitor is the major constituent released [50].

Solid lipid nanoparticles (SLN) caused less non-specific cell toxicity even compared to nanoparticles made of PLGA, which has long been the standard for biocompatible materials [51]. Using a human brain microvessel endothelial cell line (hCMEC/D3) representative of the BBB, a significantly improved accumulation of [3H]-atazanavir was obtained when the drug was delivered by SLN. Cytotoxicity experiments indicate that SLN exhibits no toxicity in hCMEC/D3 cells up to a concentration corresponding to 200 nM of atazanavir [69]. Similarly, higher cellular accumulation of rhodamine- 123, a substrate of efflux transporter P-gp, was also shown in this study. Thus, it was predicted that SLN may either mask or bypass the efflux pump [52]. Zidovudine palmitate-loaded SLNs prepared by Heiati et al. are the first reported antiretroviral SLNs. Trilaurin was used as the lipid core in these systems. Dipalmitoyl phosphatidylcholine alone or in combination with dimyristoylphosphatidylglycerol was used as a coating. The resultant SLNs were

either neutral or negatively charged. Drug loading was dependent on the outer phospholipid coat, with higher phospholipid content resulting in greater drug incorporation [53]. Researchers prepared SLNs loaded with stavudine, delavirdine, and saquinavir independently and evaluated their ability to cross the BBB *in vitro* using human BMVECs. The entrapment efficiency of the drugs followed their lipophilicity, with the more lipophilic saquinavir having the maximum entrapment efficiency, indicating the better suitability of SLNs to more lipophilic drugs. The permeability of the drugs was improved 4–11-fold when incorporated into SLNs [54]. The *in vitro* efficacy of various lipids nano-ART must be authenticated by *in vivo* studies before their application for human use.

Several classes of cyclodextrins, including β-cyclodextrin, methyl-β-cyclodextrin, and 2-hydroxypropyl-β-cyclodextrin, were studied for their ability to improve the solubility of the hydrophobic antiretroviral agents, efavirenz and UC781 [55, 56]. Recently, carbon nanotubes have shown promise as antiHIV-1 therapeutic. These carbon nanomaterials offer potential advantages over the more widely studied nanoparticle systems including their ability to cross cellular membranes and shuttle drugs, biomolecules including DNA, proteins, into various types of cells such as cancer cells and T cells [57-60].

Recent advances in the field of nanotechnology offer an unprecedented opportunity to enhance the power of *si*RNA mediated gene therapy by providing both an efficient delivery system as well as target specificity [61]. The *si*RNA technology is a novel method to achieve complete and persistent knockdown of gene expression. Challenges to efficient *si*RNA delivery and activity include: (1) design of effective *si*RNA sequences; (2) bioconjugation with a safe and efficient delivery system such as nanoplexes; (3) formulation of a nanoplex with favorable pharmacokinetics; (4) stabilization of the *si*RNA in biological systems; (5) efficient delivery and entry of the *si*RNA nanoplex into target cells; and (6) prevention of endosomal escape of *si*RNA nanoplexes from the intracellular milieu. Additionally, the *si*RNA nanoplex should have minimal effects on nontarget genes and should avoid inadvertent stimulation of the immune system [62, 63].

Progress on *si*RNA therapeutics for treatment of Neuro-AIDS:

Since the discovery of RNA interference (RNAi) in mammalian cells, there has been great interest in harnessing this pathway for the treatment of disease. RNAi is an endogenous pathway for post-transcriptional silencing of gene expression that is

triggered by double-stranded RNA (dsRNA), including endogenous microRNA (miRNA) and synthetic short interfering RNA (*si*RNA). By activating this pathway, *si*RNAs can silence the expression of virtually any gene with high efficiency and specificity, including targets traditionally considered to be 'undruggable' [64, 65]. *si*RNA based therapeutics are under development for the treatment of diseases ranging from viral infections to hereditary disorders and cancers [66]. The goal of RNAi-based therapy is to activate selective mRNA cleavage for efficient gene silencing. It is possible to harness the endogenous pathway in one of two ways: either by using a viral vector to express short hairpin RNA (shRNA) that resembles microRNA precursors, or by introducing *si*RNAs that mimic the Dicer cleavage product into the cytoplasm. Synthetic *si*RNAs harness the naturally occurring RNAi pathway in a manner that is consistent and predictable, thus making them particularly attractive as therapeutics. Moreover, as they enter the RNAi pathway later, *si*RNAs are less likely to interfere with gene regulation by endogenous microRNAs [67].

The initial steps to identify potent lead *si*RNA candidates start with bioinformatics design and involve *in vitro* studies to determine silencing efficacy, verify that unwanted off-target effects are absent and introduce chemical modifications, if needed, to improve stability and specificity. Turning siRNA into drug begins with in silico design and *in vitro* screening of target *si*RNAs, is followed by incorporating stabilizing chemical modifications on lead *si*RNAs as required, and ends with the selection and *in vivo* evaluation of delivery technologies that are appropriate for the target cell type/organ and the disease setting [68]

Once inside a cell, one strand of an *si*RNA binds to the endogenous cytoplasmic RNA induced silencing complex (RISC), which captures an mRNA bearing a complementary sequence, and then the RISC Argonaute RNase cuts the target mRNA to initiate its degradation. The active (antisense) strand of the *si*RNA is stable within the RISC for weeks, but it is diluted with every cell division [69]. Intracellular delivery of *si*RNAs is foiled by their large size (~12 kDa) and negative charge. Although they are too large to cross cell membranes, *si*RNAs are small enough to be filtered by the kidney. As a consequence, unless they are conjugated to other molecules or incorporated into complexes, intravenously injected *si*RNAs are rapidly excreted [70]. In order to have a concrete impact on the health of patients, promising strategies should be proposed to translate therapeutics *si*RNAs into the clinical setting. Naked *si*RNAs are unstable in human plasma and too large and

negatively charged to cross the cellular membranes. Small interfering RNA (*si*RNA) therapeutics has facing multiple barriers along the pathway from administration to delivery to the intracellular target site. The major barriers for both nanoparticle formulated and targeting ligand conjugated *si*RNAs and possible strategies to overcome are given in the Table 1.

Table 1: Barriers to *si*RNA therapeutics and strategies to overcome them

Barriers	Approaches	Description
Entering circulation or target tissue	Parenteral Administration	IV administration used for liposome nanoparticles and other nanoparticles
	Subcutaneous Injection	GaINAc conjugates are administered subcutaneously and presumably reach the target tissue via lymphatics
Excretion	PEGylation	Increases the molecular weight of siRNA or delivery vehicle to avoid renal excretion
	Cholesterol conjugation	Cholesterol-conjugated siRNAs bind to circulating lipoprotein particles
	Nanoparticle formulation	Nanoparticles are above the renal filtration cut-off
Immune recognition	Nucleic acid backbone Modifications	2′-O-methyl and 2′-fluoro modifications block innate immune stimulation
	PEGylation	Surface charge minimizes binding to phagocytic cellsand other cells
Extravasation	Target tissues with leaky Vessels	The liver and spleen have a fenestrated endothelium. Tumours can have leaky vessels.
	Endothelial transcytosis	Theoretically attractive approach to gain access to any tissue
Cellular uptake	Targeting ligand	Conjugate siRNA or delivery vehicle to receptor-targeting moiety (ligand, aptamer or antibody fragment) for cell-specific uptake.

(Contd.)

(Contd.)

	Association with endogenous ligand	Cholesterol-conjugated siRNAs and LNPs bind to serum apolipoproteins conferring uptake in hepatocytes
Endosomal release	Membrane-destabilizing Lipids	Lipid nanoparticles contain lipid bilayer-disrupting lipids that are activated by low endosomal pH
	Membrane-destabilizing peptides and polymers	Masked endosomolytic peptides or polymers become unmasked (positively charged) in acidic endosomes and enhance endosomal escape of siRNA
	Increase endosomal Accumulation	Even if endosomal release is inefficient, efficient uptake can compensate for poor release, as only a few hundred cytosolic siRNAs are needed for maximal knockdown

The *si*RNA-mediated knockdown of gene-specific messenger RNA (mRNA) levels is a great therapeutic strategy [61] in which double-stranded RNAs are cleaved by the cellular nuclease Dicer into short 21–22-mer fragments referred to as *si*RNA, which enter a ribonuclease protein complex called the RNA-induced silencing complex. This complex mediates a specific degradation of the corresponding mRNA. Nanoparticles can form stable complexes with *si*RNAs, called nanoplexes, which can overcome all the impediments associated with *si*RNA in the free form. A nanoplex is a generic term for a nanoparticle complexed to another biological component, which could be a drug, a *si*RNA, an imaging agent and antibody, a peptide, etc. Delivery is a key determinant as to whether or not RNA interference based therapeutics will have clinical relevance and delivery encompasses extracellular transport of the nanoplex to target cells, its intracellular RNA trafficking, and processing [36]. The first HIV-1 gene therapy treatment to progress to a phase 2 clinical trial was a combination approach employing a tat-vpr-specific anti-HIV ribozyme, called OZ1, which was delivered in transduced autologous CD34+ HSC [64]. This study demonstrated that the RNA therapeutic was safe and efficacious, with CD4+ lymphocyte counts being higher in the OZ1 treated group compared to the placebo group throughout the 100 week trial, despite there being no

statistically significant differences in viral load reported at the primary end point of 47 to 48 weeks [64].

Gold nanoparticles are particularly attractive for therapeutic applications due to their biocompatibility and ease of complex formation with biomolecules. Gold nanorods (GNR) have far-reaching potential for the study of intracellular processes at the single-molecule level, using high-resolution cellular imaging, long-term observation of cell trafficking *in vivo*, and gene silencing [65, 66]. The hydrodynamic size of these GNR-nanoplexes under physiological condition is, 100 nm, making them ideal as intracellular delivery agents. Gold nanoparticles have been used for more than a decade as a gene carrier for plasmid DNA and oligonucleotides, but recently it was the first to report the use of GNR for the delivery of *si*RNA against dopamine- and cyclic-adenosine monophosphate-regulated phosphoprotein of molecular weight 32,000 (DARPP-32) *in vitro* [67]. These nanoplexes were also found to transmigrate across an *in vitro* model of the BBB without compromising the integrity of the barrier, while retaining their gene-silencing efficiency. These results have enormous implication in the treatment of drug addiction in HIV-1-infected drug-abusing patients. These observations have further underscored the tremendous benefits that nanotechnology can offer towards the safe and efficient delivery of *si*RNA-based therapeutics in the brain and other organs.

To enhance the cellular uptake of *si*RNA molecules, cell-penetrating peptides (CPP)-mediated *si*RNA delivery employing a disulfide bond formation between peptide transduction domain (PTD)/CPP and *si*RNA is typically utilized. Some of the most well characterized CPPs are TAT peptide, penetratin, transportan, polyarginine and MPG. Recently, studies evaluated the *in vivo* efficacy of structurally flexible, cationic PAMAM dendrimers as a *si*RNA-delivery system in a humanized mouse model for HIV-1 infection [68, 69]. They have also developed novel dual inhibitory anti-glycoprotein-120 aptamer–*si*RNA chimera with potent anti-HIV activities and have further constructed a chimerical RNA nanoparticle that contains a HIV gp120- binding aptamer escorted by the packaging RNA (pRNA) of bacteriophage phi29 DNA-packaging motor. These pRNA– aptamer chimeras specifically bind to and are internalized into cells expressing HIV gp120 and inhibit HIV function by blocking viral infectivity. This nanoplex represents a potential HIV-1 inhibitor, and provides a cell-type-specific *si*RNA-delivery vehicle, showing promise for systemic anti-HIV therapy. It was demonstrated that an almost 90%

viral suppression using a nanoparticle (QR)-conjugated well validated *si*RNA (*si*510) that targets the poly-A/TAR (transactivator of the HIV-1 LTR) site and suppresses viral replication in the THP-1 monocytic cells [70]. Dendrimers have also been successfully used to deliver and transfect *si*RNA to various HIV-infected human cell types resulting in gene silencing without causing cytotoxicity [71, 72]. It was observed that a decrease in endothelial permeability, as reflected by reduction of transendothelial resistance across an *in vitro* BBB on treatment with QR-conjugated matrix metalloproteinase-9 (MMP-9)-*si*RNA due to down regulating the expression of MMP-9 gene in brain micro vascular endothelial cells that constitute the BBB [67]

A novel drug delivery system comprised of ferric-cobalt electro-magnetic nano-material (CoFe2O4@ BaTiO3; MENP) bound to *si*RNA targeting Beclin1 (MENP-siBeclin1) to cross the blood-brain barrier (BBB) and attenuate the neurotoxic effects of HIV-1 infection in the central nervous system following on-demand release of *si*RNA using an *in vitro* primary human BBB model [73]. This study shows that the nano-formulation can silence the BECN1 gene as an effective mechanism to attenuate HIV-1 replication and viral-induced inflammation in the context of the BBB.

Thus *si*RNA therapeutics for HIV infection have been demonstrated in many studies, but limitations of this strategy include successful strategies to deliver *si*RNA to the desired target cells such as T cells, macrophages, dendritic cells, and tissues. Despite these limitations, *si*RNA therapeutics possesses great potential in HIV therapy.

Conclusion

*si*RNA therapeutics for HIV infection have been demonstrated in many studies, but limitations of this strategy include successful strategies to deliver *si*RNA to the desired target cells such as T cells, macrophages, dendritic cells, and tissues. With improvements in current strategies, like delivery, immunogenicity, toxicity, and viral mutagenesis, the potential for RNAi to be used against HIV in the clinic would be feasible. To reduce immunogenicity modified base incorporation and improved *si*RNA specificity showed successful results. As a therapeutic, *si*RNAs have great promise due to their target specificity, potency, and ability to be chemically synthesized for manufacturing. They are widely adaptable, which is advantageous in the case of HIV mutagenesis. By developing a platform for clinical use of RNAi

against HIV, targets can be changed rapidly as the virus mutates. The mode of delivery is one of the limitations in bringing *si*RNA to the clinic to treat neuroAIDS. Delivery must be efficiently reached the intended cells and tissues, by crossing the BBB, to have an effective *si*RNA therapeutic. To improve each of these shortcomings, continuous progress is required to fulfill the promise of *si*RNA therapeutics for neuroAIDS.

References:

1. http://www.unaids.org/en/resources/fact-sheet accessed on 16/02/2018 8.30pm.
2. Gilbert PB, McKeague I.W, Eisen G, Mullins C, Guéye-NDiaye A, Mboup S, Kanki PJ. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Stat Med.* 2003; 22(4):573-93.
3. Reeves JD, Doms RW. Human Immunodeficiency Virus Type 2. *J Gen Virol*. 2002; 83(6) :1253-65
4. Moore RD; Chaisson RE. Natural history of HIV infection in the era of combination antiretroviral therapy. *AIDS*. 1999: **13** (14): 1933–42.
5. Saxena S K, Tiwari S and Nair M P N. NeuroAIDS: Mechanisms, Causes, Prevalence, Diagnostics and Social Issues. *In Tech.* 2013; 109-124. (open access article http://dx.doi.org/10.5772/55100)
6. Singh R, Kaur M, Arora D (2011). Neurological complications in late-stage hos- pitalized patients with HIV disease. *Ann Indian Acad Neurol*, 14(3), 172-177.
7. Kaul M, Garden GA, Lipton SA (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature, 410(6831), 988-994.
8. Antinori A, Arendt G, Becker JT et al (2007). Updated research nosology for HIV-associated neurocognitive disorders. Neurology, 69:1789–1799.
9. Ghafouri M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: symptoms and causes. Retrovirology. 2006; 3:28. (https://doi.org/10.1186/1742-4690-3-28)
10. Jayant RD and Nair M. Nanotechnology for treatment of NeuroAdis. J Nanomed Res 2016, 3(1): 00047. DOI: 10.15406/jnmr.2016.03
11. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature, 1998;391:806–11.

12. Li M, Li H, Rossi JJ. RNAi in combination with a ribozyme and TAR decoy for treatment of HIV infection in hematopoietic cell gene therapy. Ann N Y Acad Sci. 2006;1082:172–9.
13. Burnett JC, Zaia JA, Rossi JJ. Creating genetic resistance to HIV. Curr Opin Immunol. 2012;24:625–32.
14. Westerhout EM, Ooms M, Vink M, Das AT, Berkhout B. HIV-1 can escape from RNA interference by evolving an alternative structure in its RNA genome. Nucleic Acids Res, 2005;33:796–804
15. Castanotto D, Rossi JJ. The promises and pitfalls of RNA interference-based therapeutics. Nature, 2009; 457:426–433.
16. Antiretroviral Therapy for HIV Infection. https://emedicine.medscape.com/article/1533218 accessed on 19/02/2018.
17. Panel on Antiretroviral Guidelines for Adults and Adolescents. United States Department of Health and Human Services Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Available online:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf (accessed on 19 February 2018).
18. Mallipeddi R, Rohan LC. Progress in antiretroviral drug delivery using nanotechnology. Int J Nanomedicine. 2010; 5:533–547.
19. Rosenbloom DI, Hill AL, Rabi SA, Siliciano RF, Nowak MA. Antiretroviral dynamics determines HIV evolution and predicts therapy outcome. *Nat Med*. 2012:18(9), 1378–1385.
20. Owen A, Rannard S (2016). Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: insights for applications in HIV therapy. Adv Drug Deliv Rev, 103, 144–156.
21. Poveda E, Tabernilla A. New insights into HIV-1 persistence in sanctuary sites during antiretroviral therapy. *AIDS Rev*. 2016:18(1), 55-56
22. Cory TJ, Schacker TW, Stevenson M, Fletcher CV (2013). Overcoming pharmacologic sanctuaries. Curr. Opin. HIV AIDS, 8(3), 190–195.
23. Shiramizu B, Williams AE, Shikuma C, Valcour V (2009). Amount of HIV DNA in peripheral blood mononuclear cells is proportional to the severity of HIV-1-associated neurocognitive disorders. J Neuropsychiatry Clin Neurosci, 21(1), 68-74.
24. Verma AS, Singh UP, Dwivedi PD, Singh A. (2010). Contribution of CNS cells in NeuroAIDS. J Pharm Bioallied Sci, 2(4), 300-306.

25. Wang T, Gong N, Liu J, Kadiu I, Kraft-terry SD, Schlautman JD, Ciborowski P, Volsky DJ, Gendelman HE (2008). HIV-1-infected astrocytes and the microglial proteome. J Neuroimmune Pharmacol, 3(3), 173-186.
26. Gendelman HE, Narayan O, Molineaux S, Clements JE, Ghotbi Z (1985). Slow, persistent replication of lentiviruses: role of tissue macrophages and macro-phage precursors in bone marrow. Proc Natl Acad Sci U S A, 82(20), 7086-7090.
27. Kanmogne GD, Primeaux C, Grammas P (2005). HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. J Neuropathol Exp Neurol, 64(6), 498-505.
28. Woods SP, Moore DJ, Weber E, Grant I (2009). Cognitive neuropsychology of HIV-associated neurocognitive disorders. Neuropsychol Rev, 19(2), 152-168.
29. Morgan EE, Woods SP, Delano-wood L, Bondi MW (2011). Intraindividual variability in HIV infection: evidence for greater neurocognitive dispersion in older HIV seropositive adults. Neuropsychology, 25(5), 645-654.
30. Iannazzo D, Pistone A, Romeo R, Giofrè SV. Nanotechnology approaches for antiretroviral drugs delivery. J AIDS HIV Infect, 2015;1:1–13.
31. Jain KK. Nanobiotechnology-based drug delivery to the central nervous system. Neurodegener Dis, 2007;4(4):287–91.
32. Barbu E, Molnàr E, Tsibouklis J, Górecki DC. The potential for nanoparticle-based drug delivery to the brain: overcoming the blood–brain barrier. Expert Opin Drug Deliv 2009;6(6):553–65.
33. Shaik N, Pan G, Elmquist WF. Interactions of pluronic block copolymers on P-gp efflux activity: experience with HIV-1 protease inhibitors. J Pharm Sci, 2008;97(12):5421–5433.
34. Dou H, Grotepas CB, McMillan JM, et al. Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuroAIDS. J Immunol, 2009;183(1):661–669.
35. Saiyed ZM, Gandhi NH, Nair MP. Magnetic nanoformulation of azidothymidine 5′-triphosphate for targeted delivery across the blood-brain barrier. *Int J Nanomedicine*, 2010; 5:157–166.
36. Mahajan S D, Aalinkeel R, Law W C, Reynolds J L, Nair B B, Sykes D E, Yong K T, Roy I, Prasad P N, Schwartz S A. Anti-HIV-1 nanotherapeutics: promises and challenges for the future. *Int J Nanomedicine*. 2012;7:5301-14

37. Kuo YC, Chen HH. Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier. International Journal of Pharmaceutics. 2006; 327:160–69.
38. Destache CJ, Belgum T, Goede M, Shibata A, Belshan MA. Antiretroviral release from poly(DL-lactide-co-glycolide) nanoparticles in mice. J Antimicrob Chemother. 2010;65(10):2183–7.
39. Rao KS, Reddy MK, Horning JL, et al. TAT conjugated nanoparticles for the CNS delivery of anti-HIV drugs. Biomaterials, 2008;29:4429–38.
40. Basu S, Mukherjee B, Chowdhury SR, Paul P, Choudhury R, Kumar A, et al. Colloidal gold-loaded, biodegradable, polymer-based stavudine nanoparticle uptake by macrophages: an in vitro study. Int J Nanomedicine, 2012;7:6049–61.
41. Kuo YC. Loading efficiency of stavudine on polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate copolymer nanoparticles. Int J Pharm, 2005;290:161–72.
42. Peng J, Wu Z, Qi X, Chen Y, Li X. Dendrimers as potential therapeutic tools in HIV inhibition. Molecules. 2013;18:7912–29.
43. Macri RV, Karlovska J, Doncel GF, Du X, Maisuria BB, Williams AA, et al. Comparing anti-HIV, antibacterial, antifungal, micellar, and cytotoxic properties of tricarboxylato dendritic amphiphiles. Bioorg Med Chem, 2009;17:3162–8.
44. Dutta T, Jain NK. Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly (propyleneimine) dendrimer. Biophys Acta, 2007;1770:681–6.
45. Dutta T, Garg M, Jain NK. Targeting of efavirenz loaded tuftsin conjugated poly (propyleneimine) dendrimers to HIV infected macrophages in vitro. Eur J Pharm Sci, 2008;34:181–9.
46. Ardestani MS, Fordoei AS, Abdoli A, Ahangari Cohan R, Bahrali G, Sadat SM, et al. Nanosilver based anionic linear globular dendrimer with a special significant antiretroviral activity. J Mater Sci Mater Med, 2015;26(5):179.
47. Vacas-Córdoba E, Galán M, de la Mata JF, Gómez R, Pion M, Muñoz-Fernández MA. Enhanced activity of carbosilane dendrimers against HIV when combined with reverse transcriptase inhibitor drugs: searching for more potent microbicides. Int J Nanomedicine, 2014;9:3591–600.

48. Kim S, Scheerer S, Geyer MA, Howell SB. Direct cerebrospinal fluid delivery of an antiretroviral agent using multivesicular liposomes. J Infect Dis, 1990;162:750–2.
49. Jin SX, Bi DZ, Wang J, Wang YZ, Hu HG, Deng YH. Pharmacokinetics and tissue distribution of zidovudine in rats following intravenous administration of zidovudine myristate loaded liposomes. Pharmazie, 2005; 60:840–3.
50. Ramana LN, Sharma S, Sethuraman S, Ranga U, Krishnan UM. Stealth anti-CD4 conjugated immunoliposomes with dual antiretroviral drugs—modern Trojan horses to combat HIV. Eur J Pharm Biopharm, 2015; 89:300–11.
51. Chattopadhyay N, Zastre J, Wong HL, Wu XY, Bendayan R. Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line. Pharm Res, 2008; 25:2262–71.
52. Heiati H, Tawashi R, Shivers RR, Phillips NC. Solid lipid nanoparticles as drug carriers. I. Incorporation and retention of the lipophilic prodrug 3′-azido-3′-deoxythymidine palmitate. Int J Pharm, 1997; 146(1):123–31.
53. Kuo Y-C, Su F-L. Transport of stavudine, delavirdine, and saquinavir across the blood–brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles. Int J Pharm, 2007;340(1–2):143–52.
54. Sagar V, Kanthikeel SP, Pottathil R, Saxena SK, Nair M. Towards nanomedicines for neuroAIDS. Rev Med Virol, 2014;24:103–24.
55. Sathigari S, Chadha G, Lee YH, et al. Physicochemical characterization of efaviren cyclodextrin inclusion complexes. AAPS PharmSciTech. 2009; 10(1):81–7.
56. Yang H, Parniak MA, Isaacs CE, Hillier SL, Rohan LC. Characterization of cyclodextrin inclusion complexes of the anti-HIV non-nucleoside reverse transcriptase inhibitor UC781. AAPS J, 2008;10(4):606–13.
57. Iannazzo D, Piperno A, Pistone A, Grassi G, Galvagno S. Recent advances in carbon nanotubes as delivery systems for anticancer drugs. Curr Med Chem, 2013; 20:1333–54.
58. Cunha C, Panseri S, Iannazzo D, Piperno A, Pistone A, et al. Hybrid composites made of multiwalled carbon nanotubes functionalized with Fe_3O_4 nanoparticles for tissue engineering applications. *Nanotechnology*, 2012;23:465102.

59. Iannazzo D, Piperno A, Ferlazzo A, Pistone A, Milone C, et al. Functionalization of multi-walled carbon nanotubes with coumarin derivatives and their biological evaluation. Org Biomol Chem, 2012;10:1025–31.
60. Liu Z, Winters M, Holodniy M, Dai H. SiRNA delivery into human T cells and primary cells with carbon-nanotube transporters. Angew Chem Int Ed, 2007; 46:2023–7
61. Vyas TK, Shah L, Amiji MM. Nanoparticulate drug carriers for delivery of HIV/AIDS therapy to viral reservoir sites. Expert Opin Drug Deliv, 2006; 3(5):613–628.
62. Xu L, Liu Y, Chen Z, et al. Surface-engineered gold nanorods: Promising DNA vaccine adjuvant for HIV-1 treatment. *Nano Lett*. 2012. 12(4):2003-12
63. Kalkanidis M, Pietersz GA, Xiang SD, et al. Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity. Methods, 2006; 40(1):20–29.
64. Mitsuyasu RT, Merigan TC, Carr A, Zack JA, Winters MA, Workman C, Bloch M, Lalezari J, Becker S, Thornton L, et al. Phase 2 gene therapy trial of an anti-HIV ribozyme in autologous CD34+ cells. Nat Med 2009, 15, 285–292.
65. Prasad PN. Introduction in Biophotonics. New York, NY: Wiley; 2003.
66. Prasad PN. Nanophotonics. New York, NY: Wiley; 2004.
67. Bonoiu AC, Mahajan SD, Ding H, et al. Nanotechnology approach for drug addiction therapy: gene silencing using delivery of gold nanorod siRNA nanoplex in dopaminergic neurons. Proc Natl Acad Sci U S A, 2009;106(14):5546–5550.
68. Zhou J, Neff CP, Liu X, et al. Systemic administration of combinatorial dsiRNAs via nanoparticles efficiently suppresses HIV-1 infection in humanized mice. Mol Ther. 2011;19(12):2228–2238.
69. Zhou J, Shu Y, Guo P, Smith DD, Rossi JJ. Dual functional RNA nanoparticles containing phi29 motor pRNA and anti-gp120 aptamer for cell-type specifc delivery and HIV-1 inhibition. Methods, 2011;54(2): 284–294.
70. Mallipeddi R, Rohan LC. Nanoparticle-based vaginal drug delivery systems for HIV prevention. Expert Opin Drug Deliv, 2010;7(1): 37–48.
71. de Fougerolles AR. Delivery vehicles for small interfering RNA in vivo. Hum Gene Ther, 2008;19(2):125–132.

72. Toub N, Malvy C, Fattal E, Couvreur P. Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. Biomed Pharmacother, 2006;60(9):607–620.
73. Myosotys R, Ajeet K, Jessica L, Seth M, El-Hage D N, Nair M. Electro-magnetic nano-particle bound Beclin1 siRNA crosses the blood-brain barrier to attenuate the inflammatory effects of HIV-1 infection in vitro. *J Neuroimmune Pharmacol*. 2017 ; 12(1): 120–132.

How to cite this article:

Deka T, Das M K. Anti-HIV small interfering RNA (siRNAs) therapeutics in the management of Neuro-AIDS. *Curr Trends Pharm Res,* 2018, 5(1):44-65.