



Published in final edited form as:

Adv Drug Deliv Rev. 2016 August 1; 103: 202–217. doi:10.1016/j.addr.2016.02.008.

Getting into the Brain: Potential of Nanotechnology in the Management of NeuroAIDS

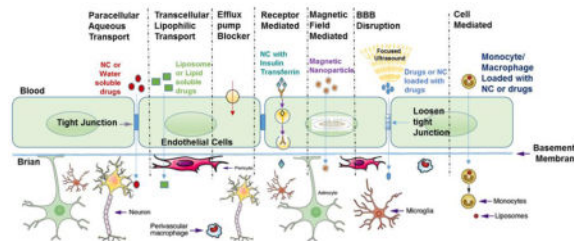
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Abstract

In spite of significant advances in anti-retroviral (ARV) therapy, the elimination of human immunodeficiency virus (HIV) reservoirs from the periphery and the CNS remains a formidable task. The incapability of ARV to go across the blood-brain-barrier (BBB) after systemic administration makes the brain one of the dominant HIV reservoirs. Thus, screening, monitoring, and elimination of HIV reservoirs from the brain remain a clinically daunting and key task. The practice and investigation of nanomedicine possesses potentials for therapeutics against neuroAIDS. This review highlights the advancements in nanoscience and nanotechnology to design and develop specific sized therapeutic cargo for efficient navigation across BBB so as to recognize and eradicate HIV brain reservoirs. Different navigation and drug release strategies, their biocompatibility and efficacy with related challenges and future prospects are also discussed. This review would be an excellent platform to understand nano-enable multidisciplinary research to formulate efficient nanomedicine for the management of neuroAIDS.

Graphical Abstract



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Chemical compounds studied in this article

Ferrous Oxide or Iron (II) oxide (PubChem CID: 14945); Pluronic-P85 (PubChem CID: 24751); Mannitol (PubChem CID: 6251); Bradykinin (PubChem CID: 439201); Saquinavir Mesylate (PubChem CID: 60934); Tenofovir disoproxil fumarate (PubChem CID: 6398764); Foscarnet Sodium (PubChem CID: 44561); Enfuvirtide (PubChem CID: 16130199), Lamivudine & Zidovudine (PubChem CID: 160352); Neopterin (PubChem CID: 4455)

Conflict of interest: Authors state no conflict of interest.

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Keywords

Anti-retroviral (ARV) therapy; Nanotechnology; HIV-1; Blood-Brain-Barrier (BBB); CNS drug delivery; neuroAIDS; Magnetic Nanoparticle; HIV-Monitoring

Introduction

1. Brain pathogenesis of HIV and neuroAIDS

Human immunodeficiency virus (HIV) neuroinvasion associated neurologic condition *i.e.* neuroAIDS prevails among acquired immune deficiency syndrome (AIDS) patients. The HIV presence in the brain jeopardizes the health and function of nerve cells resulting from inflammation mediated damage of the brain region and spinal cord involved in learning and information processing[1]. Nearly ~ 50% HIV patients demonstrate neuropathological signs or symptoms such as loss of sensation, cognitive impairment, seizures, behavioral changes [2], etc., and nearly 80% autopsies shows a range of neuropathology in AIDS patients [3]. AIDS associated neurologic condition is caused by HIV infection to the brain cells, by opportunistic infections via bacteria, fungi or other viruses, toxic effects of antiretroviral drugs or by HIV associated oncogenesis. Major neurological complications associated with AIDS are: HIV-associated dementia [4, 5] (HAD), central nervous system (CNS) lymphomas, chronic meningitis, peripheral neuropathies, neurosyphilis, vacuolar myelopathy, progressive multifocal leukoencephalopathy etc. [6]. Some neurological disorders of unknown origin has also been reported during HIV infection [7, 8]. Nonetheless, the onset of neuroAIDS condition in HIV infected patients remains debatable among scientific group due to multifaceted symptoms and pathologies and lack of specific diagnosis tools or protocols. Many studies suggest that neuroAIDS may develop as soon as HIV infects the brain. Contrary to initial belief, it has been proven that HIV infects the brain during early phase when HIV concentration is as high as the late infection stage. As such, HIV particles, its DNA and proteins can be detected in early during the infection. The HIV entry to the brain is mediated via mononuclear phagocytes ('Trojan horse' mechanism) *i.e.* monocytes and blood-borne macrophages in response to specific cytokines/chemokines (e.g. monocyte chemoattractant protein-1)[6, 9]. Initial HIV infection in brain triggers production of factors that alter the integrity of the blood-brain-barrier (BBB) (e.g. matrix metalloproteinase) which induces movement of infected/non-infected leukocytes across BBB from peripheral circulation [1, 10]. This intensifies the HIV infection in various brain cells. While HIV infection of astrocytes and microglia has been established, the direct or indirect invasion mechanism executed by HIV in nerve cells remains debated among scientific group. It is believed that HIV in subpopulations of infected brain cells acquires latency and, in turn, escapes the deleterious effect of antiretroviral therapy (ART) and immune response. Latency can persist for years where no or little virus is produced due to low transcription of host-integrated HIV genome. An appropriate endogenous or exogenous stimulus can reactivate the latent cell causing production of fresh infectious virions [11]. Thus, latent cells are the primary cause of HIV persistence and are reservoirs of rebound viremia.

The HIV virion and its neurotoxic proteins induce significant pathology across various brain cells (Figure 1) [11]. HIV-infected monocytes differentiate into macrophages, which activates astrocytes and resting microglia, which elicits severe neuroinflammation and releases neuron-damaging products such as Tumor necrosis factor (TNF- α), Interleukin (IL-1 β), reactive oxygen species (ROS), nitric oxide (NO), quinolinic acid, etc. The envelope protein gp-120/gp-41 activates neuronal chemokine receptors (CXCR4 or CCR5) and subsequent elevation of intracellular Ca²⁺ leads to apoptosis. In astrocytes, gp120 downregulates glutamate uptake to cause excitotoxicity and nitric oxide synthase production leading to cell death. In macrophages and microglia, gp120 elevates the level of proinflammatory factors such as IL-1 β , TNF- α , β -chemokines, arachidonic acid, etc. The gp120 also inhibits proliferation and migration of neural progenitor cells (NPCs) and affects the component of BBB by inducing apoptosis in brain microvascular endothelial cells (BMVECs). The p-53 pathway mediates the activation of apoptosis by gp120/gp41 occurring in all brain cells i.e., neurons, astrocytes, and macrophages/microglia. Similar to gp120, HIV-Tat protein induces multiple adverse effects on neurons: (i) induce N-methyl-D-aspartate (NMDA) receptors, (ii) decrease dopamine, (iii) inhibits tyrosine hydroxylase, and (iv) activate nitrous oxide (NO) and Calcium (Ca)-release. All of these lead to neuronal death by apoptosis or other cytotoxicity means. HIV Tat mediated upregulation of MCP-1 and reductions of glutamate uptake are seen in astrocytes; in macrophages and microglia, the levels of proinflammatory factors such as TNF- α and IP-10 are elevated. Tat exposure also induces apoptosis in Brain microvascular endothelial cells (BMVECs) and inhibits neural progenitor cells (NPCs) neurogenesis. The HIV Vpr (Viral Protein-R) and Nef proteins induce apoptosis in neurons, astrocytes, and BMVECs. Furthermore, the Vpr modulates ion channels and H₂O₂ upregulation in neurons. Vpr exposure to NPCs causes mitochondrial dysfunction and impaired neuron maturation. The Nef in neurons modulates [K⁺] channels and induces proinflammatory factors such as TNF- α , Macrophage inflammatory protein-1-alpha (MIP-1), IL-6, and elevates superoxide release in macrophages and microglia. In astrocytes, Nef upregulate complement factor C3, IP-10, monocyte chemoattractant protein-1 (MCP-1), and Matrix metalloproteinase-9 (MMP-9) activity. Thus, HIV induced injury of brain cells is mediated via various cell-specific mechanisms [12]. These pathological mechanisms aggravate the neuroAIDS condition. Nonetheless, synaptodendritic injury by HIV is believed to be a primary cause of HIV-associated neurocognitive disorders (HAND) symptoms in HIV patients [13]. Recently we reported that HIV infection to the brain causes significant damage to synaptic plasticity. Atluri et al showed remarkable loss of spines, dendrite diameter, decreased spine density, and dendrite and spine area in HIV-1 clade B and clade C infected neuroblastoma cells [14, 15]. The inter-clade variation in density and morphology of spine and dendrite was seen where HIV clade C was found less injurious than Clade B. This inter-clade variation can be attributed to difference in potency of HIV neurotoxic peptides between both clades. Such as, Thangavel et al found that HIV Tat of clade B and C exert non-similar effect on morphology and spine density, with clade C Tat being less potent [16]. The change in synaptic plasticity was substantiated by the HIV clade and protein specific differential expression of Immediate-early genes (IEGs), long-term potentiation (LTP) genes, and long-term depression (LTD) genes. These are three major groups of genes that play a central role in synaptic plasticity regulations. Moreover, cell specific variations in the expression of synaptic plasticity genes are seen during HIV infection. This may reflect

infectivity and latency intensity where one cell type establishes persistence HIV infection while the other is active. The changes in these genes adversely affect synaptic connections. In fact, subjects with HIV associated neurological disorders exhibit decreased synaptic and dendritic density with atrophy of grey and white matter [17]. As such, ARV and anti-latency agents will not be sufficient to improve the neuroAIDS condition; neuro-protection or neuron-resuscitating therapeutics would be required.

2. State of the art for Current HIV/AIDS treatment

The present state-of-the-art treatment for HIV/AIDS is highly active antiretroviral therapy (HAART), which consist of combination or single formulation of different classes of ARV drugs. In spite of the significant success using the current HAART treatment, numerous challenges are still to be resolved. One of the main drawback in the successful eradication in HIV treatment is poor patient compliance with drug therapy [18]. Due to lifelong continuous medication, patients did not sticks to the treatment schedule and leads to ineffective drug concentration which promotes the ricochet of viral reproduction [19–21]. Despite the best efforts on the part of the healthcare professionals, non-adherence of patients with HAART remains very high. Clinical trial data on HIV drugs shows that missed doses of ARV drugs were occur due to forgetfulness and being away from home (43%), sleeping (36%), busyness to take the dose (22%), feeling sick (11%), and depression (9%) [22, 23]. Also, neurocognitive diseases in PLWH/A (people living with HIV/AIDS) can be greatly aggravated by concurrent intake of drugs abuse e.g. cocaine, methamphetamine etc., which decreases likelihood of adherence to HAART regimens [24]. No single intervention approach will likely improve the adherence; efforts to increase patient adherence will likely rest on set of key factors. Among all strategies, decreasing the frequency of medication administration may have the greatest potential to improve compliance and achieve better treatment outcomes [25, 26].

Moreover, the other issues with HAART are toxic side effects and virological resistance with high economic burden [27]. The key mechanism of ARV drug resistance is due to the great genetic diversity of HIV-1 and their constant mutation during infection. Resistant issue can be solved by developing personalized therapy, wherein the resistance testing can be performed to decide the right kind of therapy with respect each individual patients [28]. Once the body is infected with HIV, due to weak immune system, chances of other diseases progression increases e.g. heart disease, diabetes, neurodegeneration, and immunological disorders [19]. Main reason for above mentioned diseases could be due to the HIV infection in combination with other co-infection (e.g. hepatitis C) and cytotoxicity due to HAART [27]. The main perpetrator for the HIV cure is the presence of resilient viral reservoirs known as “latent reservoirs”. [19, 20]. Mainly these latent cells resides in explicit anatomical location (e.g. lymphoid tissue, testes, liver, kidney, gut and CNS) [29–32]. Along with the presence of latent reservoir in presence of HAART, additional cryptic viral sources also exist along with small amount of active virus replication that follows even during the therapy [33–35]. Therefore, there is a great need to discover new approaches/methods/drugs for developing non-toxic, low-dosage treatment that provide more sustained dosing coverage and efficient eradication of the virus from the reservoirs, circumventing the need for life long treatments [21, 27]. Current HAART regimen is sufficient for complete eradication of

the virus from the body, therefore, understanding the HIV persistence mechanism and development of novel purging strategies of viral reservoirs should be the key areas of research center for complete HIV eradication.

2.1 Constraint for CNS delivery—The available recommended drugs or therapeutics can be delivered to the body in general *via* several routes of administrations. (E.g. oral, intravenous, dermal, subcutaneous, inhalational or intraperitoneal). Drugs absorption process starts immediately once it interact with receptor or biological components and subsequently reaches to the target site in the body. By the time drug is administer and reached to the target site, it may or may not be actively modified or metabolized and due to this it may or may not be recognized by receptors to show its effects at target organs/cells [36]. The unique properties of each therapeutic agent may have desirable or undesirable properties at each of these steps and may affect the pharmacological activity. Throughout this expedition, (i.e. from the time of administration to the site of action/delivery), ARV drugs or nanoformulation (NF) may come across various biological “barriers” as shown in Fig. 2.

For the CNS drugs delivery, the key hindrance is the presence of the BBB tight junction [11, 31, 32, 37–43]. The BBB is an active interface between the blood and the CNS, which rigorously controls the molecular and cellular traffic between the blood and the brain, thus maintains the proper homeostasis of neuronal function [44–47]. Scientists have explored many novel strategies to improve the permeability of ARVs across BBB and to study these parameters of drug transport various types of *in-vitro* BBB model have been developed [11, 36, 48, 49]. To mimic the real *in-vivo* BBB scenario, various *in-vitro* models have been developed using various type of cell line of different origins and culture environments. To achieve more realistic situation, constantly new improvements have been done in the well characterized and established protocols by the scientists. For detailed information on BBB model developments, please read review by Wilhelm and Krizbai [50]. Based on their observation, we have summarized (Table-1) the list of most commonly used *in-vitro* BBB model to study the drug transport or nanoformulations (NF) for CNS delivery.

2.2 Therapeutics approaches for HIV treatment—To achieve the effective treatment for an HIV-infected patient, all sources of virus replication must be abolished. To achieve this objective, we may need the amalgamation of diverse methods, to counter attack the replication of virus from active infection along with the supply from viral reservoirs [21, 51, 52]. To overcome the limitations of the CNS delivery, a number of different strategies have been explored to increase the penetrability of ARVs through the BBB. Each of these strategies presents strengths and limitations for the CNS delivery [53]. At preclinical levels, ARVs delivery to the brain using nanotechnology has shown promising results so far [27, 31, 32, 36, 38, 39, 49, 53–59]. Based on the delivery mechanism, the chemical modifications of ARVs may or may not be needed for effective delivery. The following sections discuss the strategies for enhanced brain delivery using different approaches.

2.3 Approaches for ARV penetration across the BBB—Over the years, conventional and non-conventional approaches have been developed to overcome the BBB constraints to allow drug administration into the brain. The well-known techniques or

approaches that have been explored for BBB delivery of drugs or other therapeutic agent are described below.

2.3.1. ATP-binding cassette (ABC) transporters blocking approach: Most of ARVs are large, high molecular weight lipophilic compounds. They seem probable candidates to be an ABC transporter substrate. Over the years, numerous new chemical entities (NCEs) capable of blocking specific ABC-transporters have been developed [60] and their efficacies have been tested at *in-vivo* level using different animal studies [61]. E.g., treatment with P-gp inhibitor LY-335979 in mice have resulted in higher concentration for many Protease Inhibitors (PIs) (i.e., saquinavir, indinavir, amprenavir, and nelfinavir) in CNS [62]. Similar *in-vivo* results were also observed for Saquinavir using other class of agents i.e. GF120918 (P-gp/ABCG2 blocker) and MK571 (MRP inhibitor) [63]. Another study conducted by Megard et al also proved that P-gp is the key player and plays an vital part in regulating the penetrability of PIs across the BBB [64]. Since, the abundant presence of ABC transporters, these blockers are not very CNS-specific and due to increase ARV delivery across BBB it may results in higher chances of drug toxicity/drug-drug interactions.

2.3.2. The BBB opening approach for navigation of drug to the brain: The BBB functions to maintain a delicate homeostatic environment by regulating ion and neurotransmitter concentrations, while simultaneously preventing the access of toxins, immune cells and pathogens from the peripheral circulatory system [65]. The BBB regulates the transport of nutrients into the brain and assists with removing waste products. While these functions are necessary for maintaining the health of the brain, the BBB prevents the access of therapeutic agents when brain diseases develop, thus makes brain diseases treatment difficult. It has been estimated that the BBB prevents the access of over 98% of potential therapeutics from passing into the brain [66]. To overcome these issues, an alternate strategy known as hyper-osmotic opening of the BBB has been explored for transient opening. Mannitol and urea hypertonic solution have been extensively used for opening of BBB tight junction transitorily due to shrinkage of capillary endothelial cells which induces water efflux and successively paracellular transport of the drugs or nanoformulation materials across the BBB [67, 68]. This approach has been explored *in-vivo* with some success due to the risky nature of this procedure, it is not well accepted in general. Most common side effects includes seizures and impulsive long-term neurological complications, thus restricts it application in day to day procedure. Another alternate strategy used for the transient BBB opening is the usage of cytotoxic agents, e.g. alkylating agents (etoposide and cisplatin), they main works by disrupting the BBB tight junctions and generate small opening with in the endothelial cells [69]. Likewise, intra-arterial delivery of vasoactive agents (e.g. bradykinin, peptidase inhibitors and angiotensin-II) may also transitorily upsurges the BBB permeability [70–72]. The enhanced brain concentration of testing agents, therapeutic and drug nanoformulations has been detected after systemic delivery. But due to toxic nature of these compounds, this strategy is not recommended for the enhanced delivery of ARVs at the CNS. Another minimal invasive approach of focused ultrasound and microbubble (fine gas bubbles with diameter of > 50 μm) are also being explored for the BBB opening for therapeutic delivery [73–75]. Microbubbles on exposure to focused ultrasound, help the cavitation nuclei to focus and transduce the acoustic energy

into mechanical power and this force at the BBB interface induces the transitory opening of endothelial tight junctions [76–78]. Chen et al have used focused ultrasound transiently to allow the permeability of the BBB to increase as to allow more drug diffusion. They have used non-invasive external magnetic field for the improved localization of therapeutic agent immobilized on the magnetic nanoparticle. Results showed that this combinative approach significantly improved the *in-vivo* delivery of anti-cancer drug [BCNU: 1,3-bis(2-chloroethyl)-1-nitrosourea] for the treatment of brain tumor [79]. Due to safety concerns, this strategy is also not suggested for long term application and to make this strategy works more standardization is required. In addition, the biggest challenge with the above technique is that the BBB opening is uncontrolled and may allow unwanted or other neuro damaging entities entry into the brain [75, 80, 81]. Especially in case of HIV infected patient, the passage of virus from periphery to CNS is unwanted.

2.3.3. Prodrug based approaches: Presently, only two traditional prodrugs have been developed in HIV therapy, fosamprenavir and tenofovir disoproxil fumarate [82]. Recently, Tenofovir alafenamide fumarate (TAF, before known as GS-7340), a new pro-drug of the widely used tenofovir (Nucleoside reverse transcriptase inhibitor-NRTI), have similar antiviral efficacy reaches and better penetration power in HIV harboring cells than the parent tenofovir disoproxil fumarate (TDF) molecule [83]. The prodrugs with the good lipophilic profile, once reaches to the endothelial cells can be hydrolyzed and releases the parent ARVs molecule and crosses the cell membrane of the endothelial cells by passive diffusion [84]. Although the BBB penetration is restricted, TDF dose is capable of passing the blood-CSF barrier of the choroid plexus gaining access to perivascular and meningeal macrophages [85]. Generally a prodrug is recognized as a separate chemical entity than the parent molecule, considerably more drug purification steps are needed along with the screening tests and clinical studies to prove the efficacy of the drugs are foreseen, which is generally not lucrative or possible to derive all pharmaceutical agents to prodrug states. Hence this technique may not be as effective as parent ARV molecule delivery approach.

2.3.4. Nanomaterials based approach: There are numerous valid reasons for which nano based drug delivery systems (NDDS) are attractive option for the development of formulation towards the treatment of neuroAIDS. The most important reason is the increased availability of charged surface molecules on carriers with respect to the total number of drug molecules to design efficient nanoformulation systems. Due to the higher surface area of nanoparticles, it results in higher drug binding/adsorption and can also be used to carry for diverse kinds of compounds such as biological molecules (siRNA, gene or plasmids) and even large proteins. The nanomaterials/nanoformulations used for the CNS drug delivery should have following salient features

- Soluble, safe or non-toxic, and biocompatible
- Have good bioavailability and long circulation life
- Size of nanoparticle should not block blood vessel
- Protects or avoid the drug from enzymatic and hydrolytic degradation
- Can be used to deliver short half-life drugs in sustained or controlled manner

- Increase or should not delay the dissolution rate of drug and onset of therapeutic action
- Helps in avoiding loss of drug through rapid RES clearance and its metabolism

Nanoscale drug delivery systems are available in variety of forms e.g. nanoparticles, liposomes, dendrimers, fullerene, nanotubes, quantum dots, nanocapsule, nanosphere, nanocrystals etc. are believed to have promises to transform drug delivery systems [Fig. 3]. Additionally nanomaterials also used for device or instruments development in variety of industries e.g. lab-on-chips, nano-robotics etc. Nanoparticle can be made using organic or inorganic materials have certain advantage and disadvantages, but can be used smartly to achieve desired therapeutic action. Thus, nanomaterials can be explored for the development of novel drug delivery systems and redeveloping the current drugs delivery techniques to enhancement the efficacy, patient-compliance, better safety of drugs and economic burden of health care system [86]. The next section discusses each type of nanomaterial explored for the ARV drug delivery to CNS and its state of the art with respect to neuroAIDS treatment.

2.4 NanoART for neuroAIDS treatment—The ARVs can be efficiently delivered to the brain using drug loaded on nanocarriers (NCs) [11, 38, 53, 58]. Depending on the chemical/physical nature of NCs, chemical modifications of ARVs may or may not be essential for effective loading and its targeted delivery. There is an extensive variety of NCs are available (as shown in Fig. 3) that are in practice and being tested for the brain drug delivery applications to cure CNS diseases. Their versatility of tuning properties allows them to deliver ARVs across the BBB [49, 55, 58, 87, 88]. The following sections will discuss the application NCs for ARVs delivery to the brain in details.

2.4.1 Magnetic nanoformulations for neuroAIDS treatment: The MNPs certainly possess advantage over other NCs because of its inherent super-paramagnetic property which allows the control over its magnetization i.e. movement/speed and, in turn, attached drug can be delivered to specific body locations by applying non-invasive magnetic force from exterior. Simultaneously, neuroimaging techniques such as magnetic resonance imaging (MRI) can be applied and based on quantification analysis of MNPs-associated drugs can lead to determine site-specific optimal or suboptimal dosing [38, 89–93]. Nonetheless, applications of the MNPs for the brain drug delivery has been explored a little. The release of loaded drugs from MNPs, in entirety, depends on cell or tissue based physiological phenomenon such as change in temperature, pH, intracellular Ca^{2+} level, etc., which cannot be manually controlled. Biomolecules such as proteins, enzymes, drugs, etc. can be tagged on MNPs and navigated magnetically to targeted sites including brain pathologies [94]. Recent improvement in synthesis techniques allow achieving super paramagnetic MNPs size of as small as 10 nm which can cross the BBB without affecting its integrity [95]. Moreover, MNPs encapsulations into liposomes i.e. “magnetoliposomes” can be excellent module to protect loaded drug from peripheral enzymatic decomposition and can reduce the entrapment by reticuloendothelial systems as well [38, 96]. Our group (Saiyed et al) already shown that magnetoliposomes used packed in monocytes/macrophage and used for the drugs delivery across BBB [58]. The movement/speed of these magnetized carriers can be operated in the same way as for naked MNPs. In recent years, our laboratory

has investigated on the transendothelial delivery of MNPs bound anti-retroviral drugs. Nanoformulation of 3'-azido-3'-deoxythymidine-5'-triphosphate (AZTTP) showed a comparable efficiency to the free drug in suppressing HIV replication. An AZTTP-magnetoliposomes were developed which showed sustained AZTTP release for 14 days with intact anti-HIV potency [58]. Both, AZTTP-magnetic and AZTTP-magnetoliposomes resulted in nearly 3 fold increased *in vitro* BBB transmigration when compared to free AZTTP and did not affect the BBB integrity. Wen et al also have explored the magnetoliposomal formulation [Magnetic-PLGA/lipid nanoparticles (MPLs)] for trans-activating transcription factor (Tat peptide) delivery across BBB. Therapeutic efficiency of the drug-loaded Tat-MPLs versus drug-loaded MPLs was compared in bEnd.3 cells (endothelial cell line). Results showed dose and time dependent accumulation of higher concentration of Tat-MPLs than MPLs in bEnd.3 cells and proves that Tat-conjugated MPLs may serve as an effective drug delivery approach that crosses the BBB [97].

Furthermore, to address the issue of non-adherence in HIV infected subjects, it is necessary to develop long-term release of drug from formulation to achieve better patient compliance and therapy adherence. Our group (Jayant et al) have recently explored this aspect and investigated a novel ARV loaded magnetic nanoformulation for sustained release by assembling layer-by-layer (LbL) of polymer molecules (dextran sulphate) and ARV drug (Tenofovir) on MNPs [55, 98]. The sustained release of tenofovir showed 2.8 times higher drug loading with 30-fold increase in the drug release period. Also, nanoformulation showed a higher BBB transmigration ability ($37.95\% \pm 1.5\%$) and improved *in vitro* antiviral efficacy (~33% reduction of p24 level) over a period of 5 days after HIV infection in primary human astrocytes compared to free tenofovir. Also our group (Raymond et al) have explored MNP based delivery of Nef peptides containing the Nef myristoylation site across an *in vitro* BBB, which results in reduced nef-transfected microglia release of Nef exosomes and prevented the loss of BBB integrity and permeability. This study shows the preliminary evidence of the role of exNef in HIV neuroimmune pathogenesis and the possibility of a nanomedicine-based therapeutics targeting exNef to treat HIV-associated neuropathogenesis [99]. Recently, Fiandra et al investigated amphiphilic polymer (MYTS) to improve the permeation of a high-weighted ARV drug [Enfuvirtide-Enf] across the BBB both in *in-vitro* and *in-vivo* models. Results showed good efficacy and trans-BBB permeation of nanoformulation across BBB in mice model [100]. The advantage of MYTS coating on nanoparticles is that it help in loading of multiple drugs of different classes. Notably, cell viability of all aforementioned magnetic nanoformulations has been > 95%, making them very promising therapeutic agents. To overcome this constraint of uncontrolled release of drug from the MNPs, our group have discovered a novel magneto-electric nanoparticle (MENPs). MENP exhibits dual effect (magnetic and electronic) and therefore possesses both ferroelectric and ferromagnetic parameters in a single Nanocarrier. The biggest advantage of MENPs is that there is no heat dissipation on application alternate current (a.c.) field and thus leads to unprecedented high release efficacy of the drug without any side effects or toxic effect to target or neighboring cells. Our group have explored this novel dual property (Magnetic and electrical property) nanomaterial for the delivery of ARV drug i.e., AZTTP across *in-vitro* BBB model for the potential application of neuroAIDS treatment [49]. MENPs under the influence of external ac trigger allows on-demand drug release *via* disturbing the original symmetry of ionic

bonding (charge distribution) between drugs molecules and MENPs as shown in Fig. 4. The salient features of MENPs to prove it as a potential CNS drug delivery agent were explained by Kaushik et al. [39]. MENP-AZTTP nanoformulation showed 100% drug release at low alternating current (44 Oe at 100 HZ) without losing the drug integrity due to ME (magneto-electro) effect and showed excellent HIV-p24 inhibition at *in vitro* level with very good transendothelial BBB transmigration efficiency [49].

The results are encouraging but at the moment this novel technique needs more validation at *in-vivo* levels to prove its future clinical application. Currently we are testing these MENP nanoformulation at *in vivo* levels (both at rodent and non-primate levels) to prove it clinical efficacy for the treatment of neuroAIDS. While these anti-retroviral magnetic nanoformulations certainly show tremendous promise in reducing or eliminating HIV load from the brain, still lot of process optimization and technique validation at *in-vivo* level is required before it can be adapted in clinical settings.

2.4.2 Polymeric NCs for ARV delivery: Polymers are the important and well-explored NCs utilized for drug delivery system. Most of (>90%) controlled and sustained drug delivery systems basically contain polymers as important formulation constituent. Due to versatile tunable features such as safety profiles, biodegradability, high encapsulation/ entrapment efficiency, anticipated physical properties e.g. controlled rate of disassociation, permeability, degradation rate along with targeting capabilities and easy modulation of release kinetics polymeric NCs (synthetic or natural) have been explored for ARV drug delivery across the BBB [11, 21, 53, 101, 102]. Polymers like Poly (butyl cyanoacrylate) (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) have been explored for Zidovudine, Lamivudine and stavudine delivery at *in-vitro* and *in-vivo* levels. Kau et al reported delivery of stavudine, zidovudine and lamivudine using MMA-SPM polymer nanoformulation leads to increase in ARV permeability (8–20 folds) across *in-vitro* BBB model [103]. Same group also studied SLNs (tripalmitin and cocoa butter mixture) as carriers for stavudine, delavirdine and saquinavir delivery across the in-vitro BBB model and showed enhanced permeability of the ARV compared to free forms [104]. Poly- D-L-lactide-co-glycolide (PLGA) and polylactide (PLA) nanoparticle formulations also have been explored for various ARVs (e.g. zidovudine (AZT), lamivudine) delivery across mice brain [105]. Mainardes et al showed higher bioavailability (2.7 times) AZT in PLA-PEG nanoparticle compared to AZT-loaded PLA nanoparticle and 1.3 times higher relative to free drug solution, thereby showing PLA-PEG blend NPs as potential carrier of drug to delivery drugs across BBB and also suggested intranasal route as a better route of delivery as a potential therapeutic approach for HIV-1 infection treatment [106]. Table. 2 summarized all type of polymeric nanotherapeutics that have been explored for the CNS delivery of ARV drug for the treatment of HIV-1.

2.4.3 Dendrimer based BBB ARV delivery: Dendrimers have been used as carriers of antiretroviral peptides and genes for HIV inhibition and more astonishingly, many recent studies indicated that they themselves can be used as antiretroviral agents [107–109]. Mannose-capped [poly (propyleneimine)-PPI] and polyamidoamine [PAMAM] dendrimers loaded with lamivudine were evaluated *in-vitro* for antiviral activity in HIV-1 infected MT-2

cells. Lamivudine loaded dendrimer NCs showed 21-fold increase in cellular uptake and 2.6-fold reduction in the viral p24 levels when compared to the group treated with free drug solution [110], again proving the importance of nanoformulation for BBB delivery. Dutta et al also have prepared efavirenz loaded tufstin conjugated 5th generation poly (propyleneimine) dendrimers (TuPPI). Tuftsin [natural macrophage activator tetrapeptide (Thr-Lys-Pro-Arg)] bind specifically to mononuclear phagocytic cells and enhance their phagocytic activity. They reported that the dendrimer were able to extend the *in vitro* drug release up to 144 hr in comparison to 24h of the PPI polymer. Furthermore, a 34.5 times higher cellular uptake and reduced viral load by 99% at a concentration of 0.625 ng/ml was also reported; this activity was more significant in HIV infected macrophages than uninfected cells. In a similar study the same group prepared t-Boc-glycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimer [111]. The mannose conjugated dendrimer exhibited a higher (12 times) cellular uptake of efavirenz by monocyte/macrophage cells compared to free drug solution. Regardless of these promising results, unpredictable drug release kinetics and long-term safety issues hampers their wide use in BBB delivery like other polymers (e.g. PLGA). More modification in the parent molecule are needed to further authenticate the use of dendrimeric nano-systems for ARVs delivery to the CNS.

2.4.4 Micelle-based NCs and ARV delivery: Due to unique properties of smaller size and higher drug solubilization make micelles a promising candidate for drug and protein delivery across BBB. Amongst all the dendrimers, Pluronic micelles have shown very good promise in the BBB drug transport across *in-vitro* and *in-vivo* model [11, 60, 112]. The influence of Pluronic P85 on the permeability of a broad range of structurally unrelated ARV compounds was examined by Kabanov's group *in vitro* using bovine brain microvascular endothelial cell (BMVECs) model [113]. Results showed 19-fold increases in the drug permeability in *in-vitro* BBB model compared to free drug solution. Most remarkable permeability results were obtained for PI class of drugs e.g. ritonavir due to strong bond formation with P85. Batrakova and co-workers investigated the co-administration of ARV drugs such as zidovudine, nelfinavir, and lamivudine with P85 and reported an improvement of the drugs permeability *in-vitro* in BMVECs and macrophages [113]. At *in vivo* levels, Pluronics have shown increased ARV delivery to the brain of wild-type mice, but results were opposite in *mdr1a/b* knockout mice, showing that the drug permeability effect by Pluronics is facilitated in part by P-gp inhibition at the BBB [112]. The enhancement of the drug's efficacy upon co-administration with P85 was also confirmed *in-vivo* in SCID mouse model of HIV-1 encephalitis [114]. Along with so many promising advantaged, micelles biological stability and their slower rate of drug dissociation, long drug retention time limits their clinical use for CNS drug delivery.

2.4.5 Lipid-based NCs for the brain Delivery: Lipid-based NCs have shown strong potential for delivery of ARVs drugs to the CNS or other target specific delivery. There are a wide range of biological lipids and phospholipids accessible for lipid NCs development {please refer these reviews for more details [115–118]}. Liposomes are vesicles made of phospholipids bilayers, biocompatible in nature and have very good biodegradable profile. Due to unique lipophilic nature they possess the natural affinity to target the BBB and thus

presents the usefulness for CNS delivery of ARVs [118]. Lipid based NCs exists in many forms including liposomes, microemulsion, nanoemulsion and solid lipid nanoparticles (SLN). The unique and advantageous property of liposomes lies in their recognition as a foreign body by the cells of the mononuclear phagocytic system (MPS) e.g. monocytes/macrophages [119, 120]. Since HIV largely exist in the macrophages and MPS, the liposome based nanoformulations of ARV drugs helps in decreasing cytotoxicity associated with free drug solution both at *in-vitro* and *in-vivo* levels. Largely, there are four types of the liposomes (cationic, anionic, sterically stabilized and immune-liposomes) largely explored for the anti-HIV/AIDS drug delivery so far [101]. Liposomes have been mostly used for the delivery of hydrophobic anti-HIV drugs like ddCTP, zidovudine, didanosine and zalcitabine, which is not possible with magnetic or other polymeric nanoparticle. In one of the study, liposomal foscarnet (antiviral used as a rescue therapy for late-stage HIV patients with multidrug resistance) nanoformulation helps in increasing the drug level in rat brains by 13-fold when compared to the free foscarnet solution [121]. Study on encapsulated 2',3'-dideoxycytidine-5'-triphosphate (ddCTP) in liposomes and compared its antiviral effect with the dideoxycytidine and dideoxycytidine-triphosphate in cultured human monocyte-macrophages (M/M) infected with HIV-1 by Szebeni et al [122] showed better drug stability and cell uptake compared to free drug, thus suggest the ability of liposomal formulation for targeting drugs to macrophages *in-vivo* and can be a better choice to increase the therapeutic index of ARV drugs. AZT-loaded liposomes was also studied by Phillips et al [123] for the antiviral effect and bone marrow toxicity of in C57BL/6 mice, results showed improved antiviral activity for liposomal nanoformulation when compared with free drug solution in the HIV infected mice. Also, Mannosylated anggalactosylated liposomes were also investigated for the delivery of stavudine in order to improve the stability of liposomes, which is an intrinsic limitation for these carriers [124, 125]. Makabi-Panzu et al. have investigated the cellular accumulation, tissue distribution, and antiviral efficacy of liposomal and free zalcitabine in RAW264.7 and U937 cell lines and reported a considerably higher drug uptake of the liposomal formulation compared to free drug in both the cell lines [126, 127]. Ramana et al developed have neviravine (NVP) loaded liposomal formulation and studied its different parameters with respect drug loading, release kinetic and BBB transmigration [128]. The *in-vitro* results showed an efficient targeted delivery of the NVP to the selected compartments with reduced systemic toxic effects. All the studies support the encapsulating ARV drugs in liposomes is more effective approach for delivering drugs across BBB for the treatment of HIV-associated CNS complications. Vyas et al evaluated oral formulation of flaxseed oil-based nanoemulsion of saquinavir for CNS delivery. Results showed 3 folds increase in the drug concentration in the systemic circulation and 5-fold increase in the area-under-the-curve (AUC) values and maximum drug concentration in the brain for saquinavir nanoemulsion when compared with free drug solution in male balb/c mice, respectively. Results indicates due to small size of the nanoemulsion formulation, it has better BBB permeability and may also help in bypassing gastrointestinal tract barriers when administered orally [129]. The SLN are another relatively new class of lipid-based NCs. As per one of the study, SLN showed better profiles drug release profile and showed less non-specific cell toxicity when compared to PLGA nanoparticles formulation [130], which is FDA approved and more standard biomaterial. Chattopadhyay et al have examined role of SLN for atazanavir delivery using a human brain microvessel endothelial cell line *in-*

vitro BBB model. Results showed significant improvement in the accumulation of SLN-[3H]-atazanavir when compared with free drug [131]. Thus suggest that SLN clearly have strong potential as nanocarrier for brain delivery of ARVs, especially for highly lipid soluble compound e.g. PIs. In table 2, we have compiled the most recent strategies for the HIV-1 nanotherapeutics for BBB delivery and treatment of neuroAIDS. Other than the HIV-1 nanotherapeutics discussed above, different types of therapeutics (genes, proteins, peptide, DNA vaccine etc.,) and modifications of carriers [cyclodextrins (CDs), carbon nanotubes (CNT) etc.,] are being investigated for the treatment of HIV-1 in CNS. However, we are not discussing them here as they falls outside the scope of present review manuscript, but the related references and the strategies for the CNS delivery have been cited in Table 2 for the benefit of readers. For more details about the advantage, disadvantages and BBB transmigration potentials and suggested improvement all type of NCs systems, their physico-chemical properties, cytotoxicity and immunologic responses to other neighboring cell/ organs have been described in details in our previously published review by Sagar et al [11].

3. Monitoring and management of NeuroAIDS

Effective prevention of HIV/AIDS needs timely diagnosis, introduction of therapy, and routine plasma viral load monitoring of the infected patient; viral rebound rate assessment by precise and sensitive assays is desirable to increase HIV prevention [159]. Hence, more reasonable and user-friendly technologies capable of providing continuous monitoring and early diagnosis is very much needed. In the past few years, we have seen massive scientific and technical developments to achieve simple, cost-effective, and rapid diagnostic tests for HIV detection. HIV/AIDS has quickly risen and thus there is a high demand globally to find improved and simplified diagnostic tools while maintaining good patient care. In this regards, the monitoring of HIV-1 at CNS is crucial to manage early diagnosis and treatment strategy for neuroAIDS treatment [39, 160, 161]. Qualitative and quantitative strategies have been used for the diagnostics of HIV-1, staging of HIV-1, progression and selection of ARV therapy [162, 163]. Point-of-care (POC) technologies and other novel detection assays are increasing access to HIV monitoring services in developing countries. These technologies are durable, portable, easy to use, and provide adequate accuracy in ARV therapy. This section highlights the recent advancements and potentials to combat HIV using POC, image guided HIV therapy, smart optical and electrical assay based on nanotechnology.

3.1 Biomarkers for HIV-1 in CNS for disease detection and its progression—

Currently biomarkers of CNS diseases are being used to study the progression of HIV as the brain and spinal cord cannot be assessed due to its inaccessibility. Unfortunately, biomarkers with high accuracy and quantifiable assessments have not been found. Due to these limitations, a combination of different markers or multi-marker assay or technique is needed to predict the HIV-1 disease progression [164, 165]. CD4+ cell count (CD4) and RNA viral loads (RNA) are the two most frequently used as analytical markers in the clinical assessment of HIV infection progression [166, 167].

However, these markers have variable predictive values that depend on which stage the disease is in and cannot explain all discrepancies of disease progression. As a result, further studies investigating markers of immune activation has occurred. Additionally, other types of

HIV progression markers exist e.g. p24 antigen, CD8+ cell count, platelet concentration, anti-HIV antibodies, erythrocyte sedimentation rate (ESR) and (Hb) hemoglobin concentration that can be used to along with main markers to predict the diagnosis. In addition to all these markers, the serum concentrations of beta-2-microglobulin [168], IgA, interleukin-2 receptors and p24 antigen (as shown in Fig. 5) have also been utilized. Cellular activation and inflammation, in conjunction with the discovery of the above markers, have been used to assess HIV infection progression; consequently, focus has been turned to these markers and their function in the CNS. In order to monitor progress, which is thought to serve as the substrate for neuropathology in HAND, soluble CSF markers of macrophage activation (neopterin), chemokines stimulators of macrophages and lymphocytes across the BBB (CCL2/MCP1 and CXCL10/IP10), and molecules involved at various phases in the pathways for cell turnover and activation within the CNS compartment are used. Brew et al observed that progression to HAD can be predicted with the monitoring of CSF neopterin, CD4 cell count and cerebrospinal fluid concentrations of β -2-microglobulin [169]. However, immune activation for HIV infection lacks specificity and so although moderately high levels of the previous markers have been correlated to disease activity, they have not been clinically used for diagnosis or monitoring of NeuroAIDS. Next section deals with techniques and assays that have been used for detection of HIV in laboratory setting for disease detection and its progression.

3.2 Techniques and technology used HIV-1 diagnosis and monitoring—HIV/ AIDS diagnosis can be categorized into three different stages/test: (i) tests to enable preliminary diagnosis, (ii) tests to stage the patient, and (iii) tests to monitor the patient (before and after commencement of ART) [159]. Currently, delivering therapeutics across the BBB for treatment of CNS disorders signifies the most challenging and emergent field in neuro-pharmaceuticals. The image-guided therapy in combination with nanotechnology is emerging as a novel tool for HIV-1 detection, monitoring and treatment (theranostics) for CNS diseases, but this type of therapy has not been implemented with the management of neuroAIDS. We believe that the combination of personalized nanomedicine with non-invasive imaging techniques (anatomical imaging modalities) e.g. magnetic resonance imaging (MRI), positron emission tomography (PET), ultrasound (US), single-photon emission computed tomography (SPECT), fluorescence molecular tomography (FMT-based on fluorescence), optical and thermal imaging, X-ray computed tomography (CT), and NIR (Near infrared) [89, 160, 170]. Comprehension and treatment of CNS diseases has been further advanced due to drug and diagnostic molecules that can be delivered to the brain across the BBB as personalized nanomedicine with the help of highly specific and multifunctional NPs using drug delivery images. Recently, to cure neuroAIDS, drug delivery across the BBB is being developed with NFs consisting of MNP, imaging agents and optimized ratio of therapeutic agent [56]. This efficient therapeutic approach can be coupled with a suitable imaging technique to monitor HIV progression, drug efficacy and neurobehavioral changes. For example, magnetic NCs and anti-HIV drug based NFs have demonstrated HIV eradication across BBB [55, 171]. Such investigated NFs can be navigated cross to BBB in mice by applying external magnetic field and ultrasound. An MRI technique can be coupled with such systems to monitor stimuli responsive targeted drug release and its efficacy [56, 172]. This brain imaging research technology has potential for

real time tracking of therapeutic cargo and therapeutic mechanism. However, wide application is sometimes limited because these methods possess neuronal alteration (CT) and low penetration (NIR based imaging); furthermore, they require high expertise, and a laboratory set-up of sophisticated equipment such as cryogenic, cyclotron, sensitive probes. Fig. 6 explains the advantages and disadvantages of image guided and nano-enabling POC devices for neuroAIDS monitoring and management.

Diagnostics assays such as flow cytometry, western blot (WB), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA) are being used to detect and monitor HIV progression in spite of high costs. Shafiee et al have summarized potential emergent technologies for Point-of-care (POC) management of HIV infection and proposed standards of ASSURED (affordable, sensitive, specific, user-friendly, rapid, robust, equipment-free and deliverable) to patients and clinics [162]. This ASSURES criteria should be a minimum landmark to technologist for developing advanced analytical devices for HIV management. Exploring POC detection system for HIV monitoring do not need expensive laboratory set-up, multi-step detection protocol, complicated operational parameters, and high expertise [162, 163]. Thus, developing such devices for POC will facilitate the gathering of on-site bioinformatics to decide timely treatment. The POC detection of HIV is performed via estimation of CD⁺ T lymphocytes count, viral load detection, and drug resistance measurements. Various tools have been utilized for detection of physiological range and to generate enough informatics to manage and cure HIV [162]. Details of state-of-the-art of the technologies involved in HIV screening and its detection is summarized in Table-3.

In consideration with the aims of this review, we are summarizing the performance of recent advancements in selected technologies that are capable of detecting HIV at an early stage. To begin with, flow cytometry, a conventional reliable technique to detect and quantify CD⁺ T lymphocytes has been well explored [173]. The captured CD⁺ T lymphocytes from whole blood (3 μ L) [174] is detected using specific antibody that binds with cell surface markers and is quantified by using a bright field or fluorescent image processing method integrated with a microfluidic system [175–177]. A sensitive (97%) and selective (80%) ELISA integrated with an automated reader was also developed for CD⁴⁺ T lymphocytes count up to 350 cell/ μ L using whole blood as real sample within 40 min [178]. ELISA was used to estimate viral load < 200 RNA copies/mL [179]. This ELISA system needs a high volume of real sample i.e., 1 mL of plasma, and a long assay time of 48 hours to perform a test [180]. These issues limit this tool to be used at POC application. The nucleic acid based PCR approach extracts HIV-RNA from blood and amplifies RNA detection at 100–200 copies/mL in the case of HIV-1 and HIV-2 category [181]. This PCR system coupled with microfluidic system for precise and accurate RNA extraction from real sample (100 μ L) can detect DNA at 5,000 copies/mL, which seems suitable for diagnosing HIV at a mature stage [182]. Thus investigating novel tools for rapid selective screening and monitoring of HIV at CNS would be of use for timely diagnostics to optimize cure. According to ASSURED landmark, nano-enabled biosensors emerged as a potential analytical device to detect HIV-viral load. The nano-plasmonic resonance based sensing method was able to quantify (~100 copies/mL-HIV-1 subtype D) with a smaller volume (100 μ L) of whole blood and plasma [183]. Meanwhile, Nanostructure photonic crystals based biosensors have been found suitable for

label free optical detection (10^4 to 10^6 copies/mL) and capturing of HIV-1 in blood plasma [184]. Both bio-sensing methods do not need pre-processing of sample and can be integrated in a POC system. Miniaturized nano-enabled electro-chemical biosensor surfaced recently as a potential approach to detect CD⁺ T lymphocytes and viral load in real samples [185]. Lab-on-chip (LOC) based on highly sensitive electrochemical biosensor on MEMS integration are capable of capturing cells to quantify and detect CD⁺ lymphocytes [186]. These microchips exhibited a detection limit of 9 cells/ μ L and a detection range from 100 to 700 cells/ μ L [187]. LOC functionalized with specific antibodies demonstrate viral lysate detection in an automated manner [188].

Moreover, paper-based sensors based on microfluidic principle or Microelectromechanical systems (MEMS) are made of a disposable sensing chip that has been integrated to detect early stage HIV-1 infection at POC application. Regrettably, accuracy and sensitivity limit paper-based sensors; nevertheless, future advances in the improvement of analytical techniques can develop more state-of-the-art technology based on these sensors. Figure 7 summarizes the commercially available diagnostics kit/tools that are used commonly for the detection and monitoring of HIV-1 in rural and urban clinical settings. However, there is always a considerable scope to explore engineered novel sensing strategies and the integration of miniaturized portable sensing devices of reduced form factor to detect all possible biomarkers related with HIV progression before and after therapeutics. The outcomes of such devices that satisfy ASSURED criteria would be valuable bioinformatics, which will be of use for complete HIV management.

4. Challenges and Future prospects

As a proof-of-concept, varieties of nanoformulations consist of therapeutics agents and nanocarriers (NCs) have been navigated across BBB for eradication of HIV. Unfortunately, mostly such investigated nanoformulations (NFs) and approached are limited to only *in-vitro* model. The potential of NCs for developing personalized nanomedicine to achieve targeted or on-demand release of therapeutics without depending on the physiological conditions has been demonstrated. The natural integrity of BBB found as a major obstacle to achieve the CNS delivery of nanomedicine due to BBB permeability size constraints, technically NFs of size > 150 nm has less chances to cross BBB. Thus scientists are exploring surface bioengineering and formulation strategies to develop nanomedicine with size limitation (< 120–150 nm) for higher efficacy. To achieve this, efforts are being made to fine tune the particles size, surface engineering, toxicity profiles, and BBB transmigration ability of NCs for effective brain delivery without loss of drug payload. These NCs can be promoted to develop non-invasive deep brain stimulation for the treatment of neurological disorders such as Parkinson's, Alzheimer's, dementia, etc. Beside this, few transient BBB opening approaches as discussed above have been demonstrated for the CNS delivery of therapeutics (ARVs) cargo. To overcome related issues with transient BBB opening, efforts are being made to explore novel CNS navigation methods which can delivery NFs to the brain non-invasively and without generating side effect. For, example, our group extensively working on magnetically guided CNS delivery of magnetic NFs can be used as a potential alternate delivery strategy to achieve combat against HIV infection at CNS. Recent investigation of on-demand drug release of therapeutic agent in the CNS has also shown significance step

towards the eradication and cure of neuroAIDS treatment. MENPs based NFs for magnetically responsive, MNP-LBL based NFs approach to sustained release, and optical responsive based NFs have demonstrated site-specific on-demand drug release for the eradication of HIV. However, execution of these approaches using animal model is not yet well established. Another challenge in the CNS nanotechnology is the optimization of toxicity associated with the NCs and or its NFs for *in-vivo* application. A low dose of NCs such as metal, metal oxides, polymers, and composites are generally non-toxic but continuous delivery such agent may lead to long term accumulation and its related toxic effects. Thus, more comprehensive studies are required to assess the short and long term toxicity using *in-vivo* models (small animals to non-human primates). Advantage of such type of studies will be that they will help in understanding the organ specific toxicity and also alteration in neuro-behavioral to access changes in motor coordination and associated factors. The outcomes of these finding will helps in confirming the clinical application the developed NF and safety for human subjects. Due to the limitation of ARV in penetrating into the latent reservoirs, an alternate strategy would be to deliver latency reactivating agents (LRAs) along with ARV therapy. This dual agent delivery will be helpful in complete eradication of HIV-1 from the CNS reservoir as shown by us previously [55]. Also prolong delivery of multiple ARV agents to the brain may cause neurotoxicity and may lead to neuroinflammation. To avoid these challenge with ARV therapy, recently efforts are being made to investigate a single therapeutic agent which is capable of recognition and eradication of HIV-1 from the CNS reservoirs. One of such recent discovery was the advancement of clustered regulatory interspaced short palindromic repeat [CRISPR], a powerful type of genome editing technology appears promising. A Cas9/gRNA system has been designed to target highly specific sequences within the HIV-1 LTR U3 region that were proficiently modified by Cas9/gRNA [189–191]. However, delivery of this powerful Cas9/gRNA complex across the BBB is limited and an effective delivery and release will be high significance in eliminate the HIV-1 from the latent and active reservoir in the brain with any neuroinflammatory side effects. With the help of nanotechnology, the delivery of CAS9/gRNA to the brain could be possible and may be a novel future research direction. We believe that targeting latent virus and permanent elimination of integrated DNA proviral copies of HIV-1 in brain is possible using our newly invented [MENP+Cas9/gRNA] on-demand release technology. Additionally, considerable efforts are being to develop novel sensing technologies, novel signaling transduction, and imaging pathways to monitor HIV infection progression and mechanism of HIV eradication. Nano-enable sensing immunoassays and biosensor have been used for screening and detection HIV markers at pM (picomolar) level. At clinical level, such investigated nano-enabling sensing systems technologies on integrated with drug delivery systems can be promoted for the management of neuroAIDS. We proposed that a multidisciplinary nano-engineered approaches must be investigated to develop site-specific targeted delivery of NFs to brain to combat against HIV. Thus, highly specific on-demand drug release and HIV progression monitoring devices would be a 3-D diagnostics approach towards developing a personalized nanomedicine approach for the treatment and management of neuroAIDS.

5. Conclusions

This review has summarized some of the significant progresses and prospects of nanomedicine to investigate an effective therapeutics to screen, recognize and eradicate HIV-1 infection from the CNS. State-of-the-art of HIV eradication using nanotechnology suggested that developing a nanomedicine for site-specific controlled targeted drug delivery to CNS and smart assaying to assess or monitor HIV progression is in high demand for neuroAIDS management. Thus, significant efforts should be made to accelerate fundamental and applied nanoscience research to investigate smart biocompatible NCs, site-specific navigation approaches, stimuli responsive release of desired drug without side-effects to combat against neuroAIDS. On the other hand, high-quality diagnostics are also crucial to fight against HIV and to diminish its transmission and its management. Currently available diagnostic needs more advancement in without increasing the cost of treatment and its availability at underdeveloped countries. Hence, timely advancement of proposed delivery systems/testing strategies will be important to move into a new era of HIV free world. In summary, getting medicine in to the brain seems possible on exploring and optimizing nano-enable compartmentalization based personalized nanomedicine along with suitable monitoring technologies for the management of neuroAIDS. Once well characterized and validated, an optimized NFs strategy will be developed, it may be explored to cure CNS diseases/neurological disorders other than neuroAIDS e.g. Huntington's disease, Amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease, wherein site-specific drug delivery is the key to manage diagnosis and treatment.

Acknowledgments

Authors acknowledge NIH grants namely R01-DA037838, RO1-DA 034547, R21-MH 101025, RO1-DA027049, and 1R01-DA-040537.

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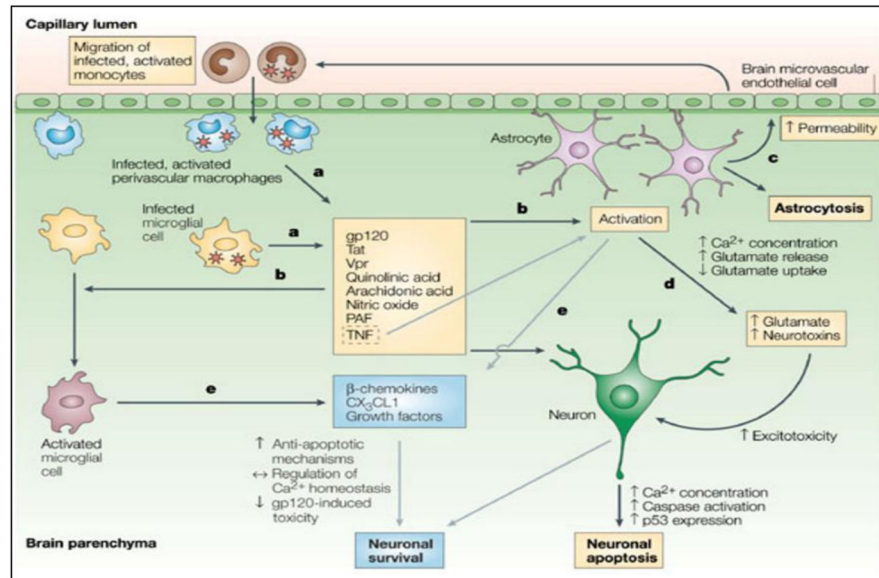


Figure 1. Mechanisms of neurodegeneration and neuroprotection in AIDS [6]

a) Infected perivascular macrophages and microglia are responsible for producing HIV but might also release viral proteins that can be deleterious to the central nervous system. The HIV-envelope protein gp120 (glycoprotein 120), Tat (transcriptional transactivator) and Vpr (viral protein R) have all been shown to be toxic in vitro to neurons and/or astrocytes, although their relevance in vivo is unknown. Infected and activated cells also produce other factors — such as cytokines (including tumour-necrosis factor, TNF), quinolinic and arachidonic acid, platelet-activating factor (PAF) and nitric oxide—which are known to have neurotoxic effects; b) Importantly, they promote the further activation (and to some extent, proliferation) of macrophages and/or microglia, as well as the proliferation and activation of astrocytes; c) Activated astrocytes modify the permeability of the blood–brain barrier and promote the migration of more monocytes into the brain; d) In addition, through increases in release of intracellular Ca^{2+} and glutamate and through decreases in glutamate uptake, the brain concentration of glutamate and other neurotoxins increases and results in excitotoxic death of neurons; e) Activation of macrophages and/or microglia, and TNF-mediated activation of astrocytes, also results in the release of beta-chemokines, CX3C-chemokine ligand 1 (CX3CL1) and growth factors, all of which are known to regulate Ca^{2+} homeostasis in neurons, to promote anti-apoptotic signaling pathways and to decrease gp120-mediated and excitotoxic neuronal cell death, thereby promoting neuronal survival. Grey arrows indicate neuroprotective pathways. (Reproduce with permission from authors-González-Scarano and Martín-García - Nature Reviews Immunology, 2005).

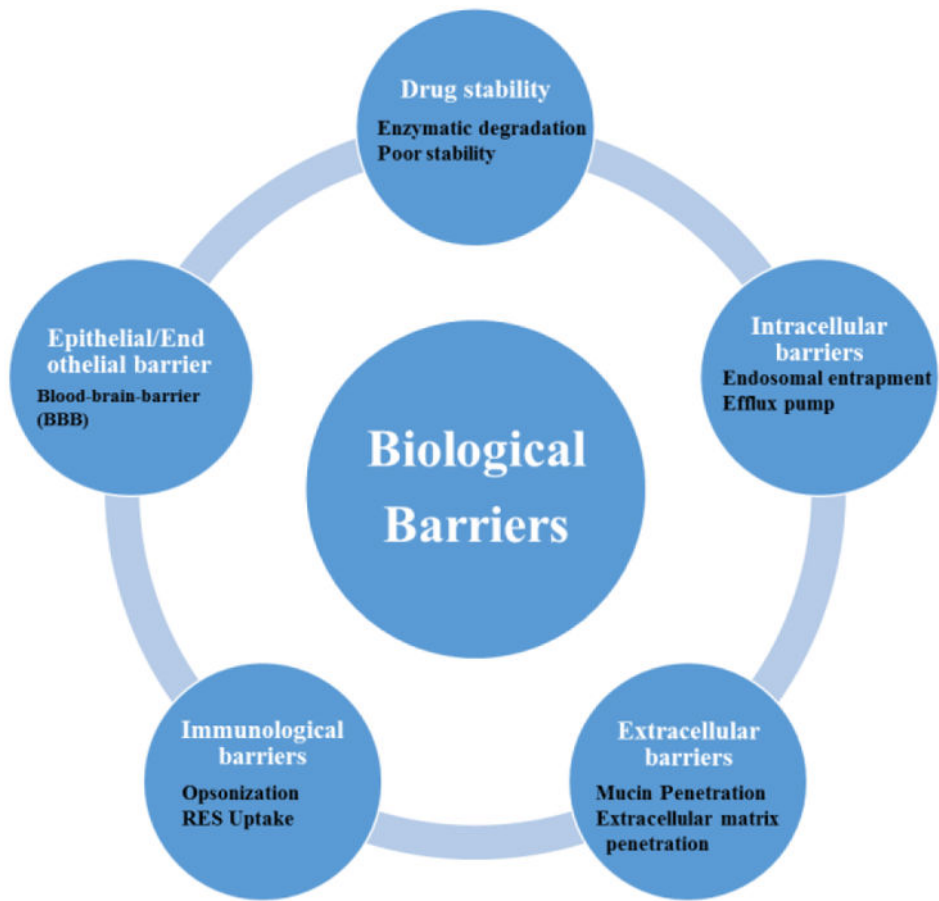


Figure 2. Different types of biological barriers for ARV drugs for CNS drug delivery

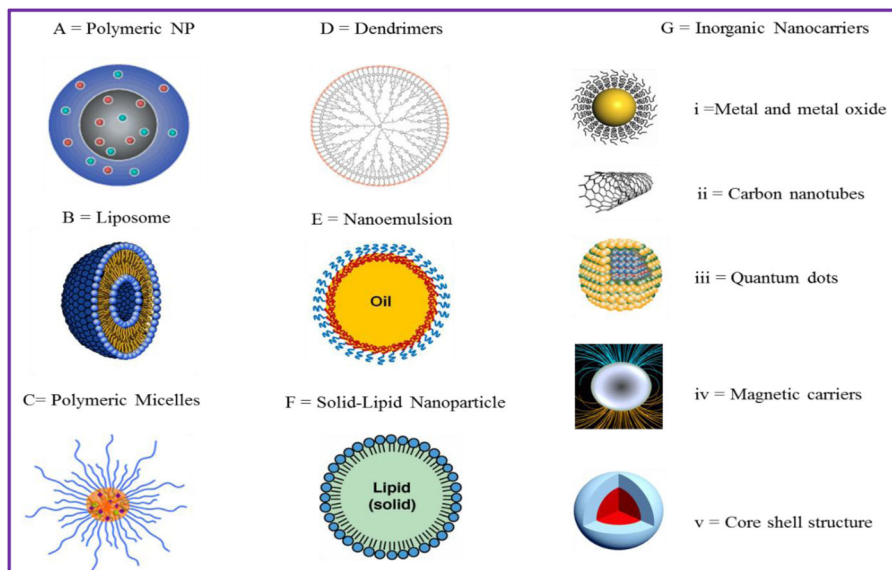


Figure 3. Illustration of various nanocarriers (NCs) exploited for CNS drug delivery
 A) Polymeric nanoparticle (PLGA, PLA PBCA); B) Liposomes; C) Polymeric micelle; D) Dendrimers; E) Lipid micro & nanoemulsion; F) Solid lipid nanoparticle and G) Inorganic NCs including: (i) Metal & metal oxide nanoparticles; (ii) Carbon nanotubes; (iii) Quantum dots; (iv) Magnetic nanoparticles (MNP) and (v) Core-shell nanoparticles (MENP)

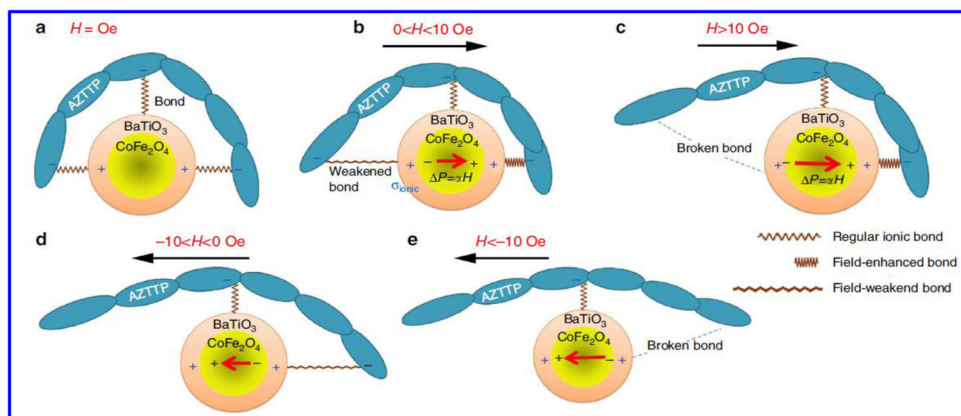


Figure 4.

A simplified (one directional) illustration of the concept of on-demand drug release by MENPs stimulated by a uniform a.c. magnetic field in X direction. (a) At zero field, only the ionic charge is present in the MEN shell. (b) An additional dipole moment (proportional to the magnetic field) breaks the original symmetry of the charge distribution in the shell. (c) As the field is increased above the threshold value ($\sigma_{\text{ionic}} \sim \sigma_{\text{ME}}$), the bond on one side is broken. (d,e) The field is reversed to break the bond on the opposite side of the nanoparticle. The red arrows show the electric dipole due to the ME effect. In practice, owing to the random configurations of nanoformulations with respect to the field, the effect is present along every central bond orientation. (Reproduce with author permission, Nair et al, Nature Communication, [49])

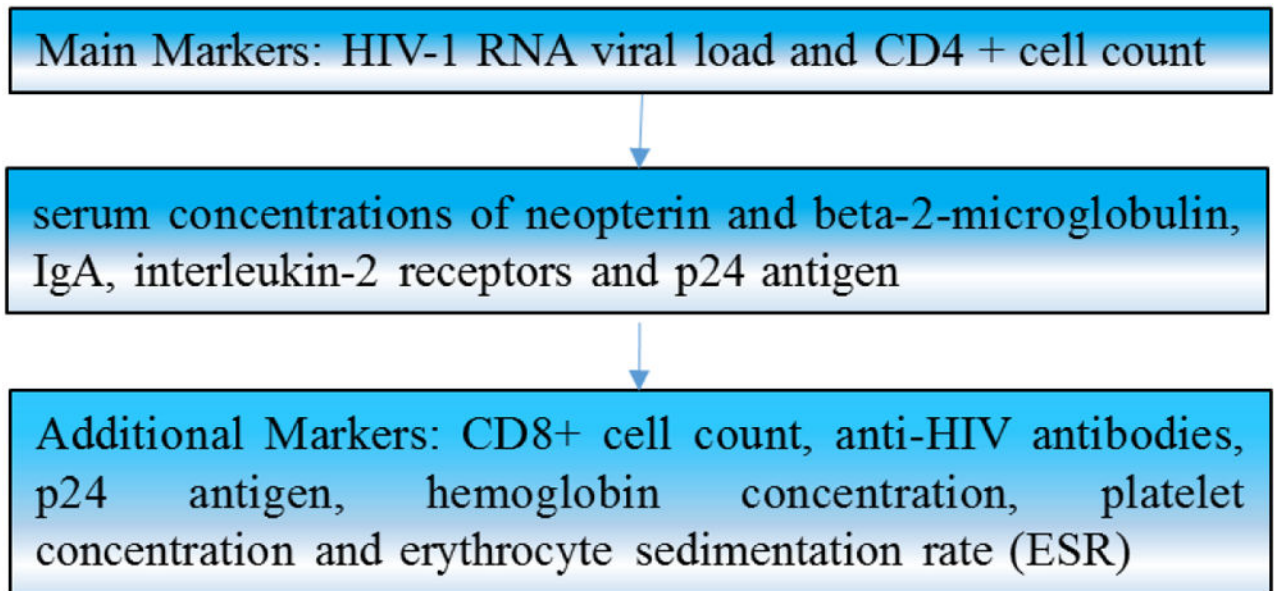


Fig. 5.
Types for biomarkers used to detect and monitor HIV-1 progression

NeuroAIDS monitoring & management	
<p><u>Image Guided neuroAIDS for monitoring</u></p> <ul style="list-style-type: none"> ✓ Qualitative and semi quantitative approach ✓ Drug delivery and release monitoring ✓ Diseases progression ✓ Drug efficacy ✓ Brain mapping ✓ Neuro- impairment assessment ✓ Neuro- behavioral assessment <p><u>Drawbacks</u></p> <ul style="list-style-type: none"> ✓ Sophisticated equipment ✓ Expensive and time consuming ✓ Sometime exhibits cell derange ✓ Need to develop novel biocompatible multifunctional imaging agents 	<p><u>Nano-enabled smart assay for neuroAIDS management</u></p> <ul style="list-style-type: none"> ✓ Selective and sensitive quantitative approach ✓ Portable and can be used at POC ✓ ARV therapy ✓ Viral load; CD+ counts ✓ Drug resistance ✓ Mother-to-child transmission ✓ Provide bioinformatics for diagnostics <p><u>Drawbacks</u></p> <ul style="list-style-type: none"> ✓ Need to optimized device integration with reduced form factors, easy to use, need less operational power, good data storage and easy calibration

Figure 6. Comparative analysis of images guided and nano-enabled techniques for neuroAIDS monitoring and management

Diagnostic Test/Tool	Detection technique	Sensitivity/Specificity
Low cost, rapid HIV monitoring tests <ul style="list-style-type: none"> Ora Quick Rapid HIV-1/2 Antibody Test Aware HIV-1/2 U (alternative to urine tests) Anti-HIV antibody and p24 antigen (early infant diagnosis and for HIV-1 and HIV-2 subtype differentiation) HIV-1/2Ag/Ab Combo (antigen and antibody) is a rapid 	<ul style="list-style-type: none"> Oral Fluids/blood sample Blood/Serum ELISAs Immunocromatographic 	<ul style="list-style-type: none"> 97.2% / 100% 88.7% / 99.9% 12.5 to 200 pg/ml 50% / 86%
HIV viral load monitoring <ul style="list-style-type: none"> Reverse transcriptase (RT) Assay: (ExaVir Load) Nucleic acid-based tests (NAT): COBAS AmpliCor HIV-1 Monitor v1.5; (ii) COBAS Ampli Prep/COBAS TaqMan v2.0; (iii) Real-Time HIV-1; (iv) VERSANT HIV-1 RNA 1.0 assay (kPCR) non-NAT (HIV viral enzymes and proteins) p24 Antigen Technologies (NEN HIV-1 p24 ELISA) p24 antigen+anti-HIV, IgM and IgG antibody (HIV-1/2Ag/Ab Combo) 	<ul style="list-style-type: none"> RT activity in blood Detect and quantify viral RNA via RT-PCR N/A Detect and quantify HIV viral enzymes and proteins Detect blood p24 antigen 	<ul style="list-style-type: none"> 200-600,000 copies/mL 50 to 750,000 copies/mL with 99.85% specificity N/A 10,000 -30,000 RNA copies/ mL 30,000-50,000 RNA copies/mL
Microfluidics diagnostic <ul style="list-style-type: none"> p24 test for early infant diagnosis (EID) CD4+ cells from whole blood (Daktari Diagnostics) 	<ul style="list-style-type: none"> Plasma Whole blood 	<ul style="list-style-type: none"> 95% / 99% 97% / 86%
POC <ul style="list-style-type: none"> Ultrasensitive p24 Antigen Assay (NNGHF) PanNAT Diagnostic Platform Portable CD4+ T-cell count (PointCare NOW, CyFlow miniPOC) 	<ul style="list-style-type: none"> Heel-stick blood Blood Blood 	<ul style="list-style-type: none"> 95% / 99% 95% / 99% 90% / 96.5%

Fig. 7. Summary of marketed available diagnostic test or tools for HIV-1 detection & monitoring

Table 1

types of *in-vitro* BBB models for drugs/nanoformulation transport. [50]

Model Type	Advantages	Disadvantage
Epithelial cells overexpressing Transporters models	<ul style="list-style-type: none"> Economical Easy to standardize 	<ul style="list-style-type: none"> Differences between epithelial and endothelial cells Non-physiologically high levels of transporter
Transwell monoculture models <ul style="list-style-type: none"> Cerebral endothelial cells on microporous membranes 	<ul style="list-style-type: none"> Uses brain endothelial cells Economical 	<ul style="list-style-type: none"> Effect of other cellular components of the neurovascular unit (NVU-astrocytes, pericytes) is ignored No shear stress
Co-cultures models <ul style="list-style-type: none"> Co- culture of cerebral Microvascular endothelial cells with astrocytes Co-culture models using pericytes Triple Cell Co-culture models (Astrocytes, Endothelial and Pericytes) Co-culture of brain endothelial cells with neuronal precursors. 	<ul style="list-style-type: none"> Consider the effect of other essentials of the neurovascular unit (NVU) 	<ul style="list-style-type: none"> Moderately expensive and laborious No shear stress
Dynamic in-vitro (DIV) models	<ul style="list-style-type: none"> Simulates real <i>in-vivo</i> condition of co-cultures 	<ul style="list-style-type: none"> Expensive No chance to monitor cells optically Special skills prerequisite
Microfluidic models	<ul style="list-style-type: none"> Mimics <i>in-vivo</i> situation possibility of co-culture 	<ul style="list-style-type: none"> Not well established models Very Expensive Laborious

Table 2

Summary of preclinical NCs/Nanomaterials based CNS delivery of ARV drugs or other therapeutic compounds for prevention/treatment of HIV-1

Type of Therapy	Therapeutic agents	Nanomaterials/Nanocarriers	References
Antiretroviral therapy (ART)	ddCTP, Zidovudine, Stavudine, Didanosine, Zalcitabine, Foscarnet & Indinavir	Liposome	[104, 121, 122, 124, 132, 133]
	Stavudine	Liposome-laden macrophages	[124, 125]
	Zidovudine	Mannose- and galactose-targeted liposome Mannose-targeted liposome	[27, 134]
	Lamivudine	Mannose-targeted dendrimer	[111, 135]
	Efavirenz	Tufts dendrimers	[111, 136]
	Ritonavir, lopinavir, zidovudine & Stavudine	PLGA nanoparticles	[105, 106, 137]
	Nevirapine Ritonavir & Efavirenz	Tat-conjugated nanoparticle	[53, 97, 138, 139]
	Ritonavir, Darunavir & Atazanavir	Poly(ϵ -caprolactone) nanoparticles	[140, 141]
	Dapivirine & Saquinavir	RMP-7/MMA-SPM nanoparticles	[132]
	Saquinavir, Stavudine, delavirdine & Atazanavir	Solid lipid nanoparticles (SLN)	[104, 131]
	Tenofovir, Enfuvirtide & AZTTP	Magnetic nanoparticle	[55, 58, 100]
	AZTTP	Magneto-electric nanoparticle	[49]
	Efavirenz, Lamivudine	Cyclodextrins	[113, 142]
	Rilpivirine	Poloxamer 338/TPGS 1000	116
	Zidovudine, Lamivudine, Efavirenz, Nelfinavir & Ritonavir	Micelles P85	[112–114]
	Zidovudine, Lamivudine, Efavirenz, Indinavir, Ritonavir & Atazanavir	Monocyte-derived macrophages-nanoparticle	[111, 139, 143–145]
	Stavudine, Zidovudine, lamivudine & Delavirdine	PBCA, MMA-SPM	[103, 104, 132, 146]
Ampenavir & Saquinavir	Transferrin (Tf)-conjugated QD	[138, 147]	
Lamivudine	CNT	122	
HIV gene therapy (RNA- and DNA-based therapies)	Antisense RNAs, aptamers, siRNA therapeutics to HIV infected cells	PLGA nanoparticles CNT; Dendrimers	[27, 138, 148–153]
Protein or peptide vaccine (Immunotherapy)	Gp-41, 120, 160, p24 protein, Env, Gag, Tat	PLGA nanoparticles	[153–155]
DNA Vaccine	Env, rev, gag, tat, ODN	Liposomes, nanoemulsion, PLA nanoparticles	[154–158]

PBCA-Poly(butylcyanoacrylate); **MMA_SPM**-Methylmethacrylatesulfopropylmethacrylate; **PLA**-Polylactide; **PLGA**-Poly (D,L-lactide-co-glycolide); **Tf**-Transferrin; **ddCTP**- 2',3'-Dideoxycytidine-5'-Triphosphate; **AZTTP** -3'-Azido-2',3'-dideoxy thymidine-5'-Triphosphate; **RMP-7**: Bradykinin agonist; **Tat**- Transactivator of transcription; **TPGS**- D- α -Tocopherol polyethylene glycol succinate; **P85**- Pluronic; **CNT**- Carbon Nanotubes; **QD**- Quantum dots; **RNA**- Ribonucleic acid; **DNA**- Deoxyribonucleic acid; **siRNA**- Small interfering RNA; **gp**-HIV envelope proteins e.g. gp 41, 120, 160; **p24**- HIV-1 viral protein; **ODN**- CpG oligodeoxynucleotides; **rev**- transactivating protein HIV-1; **Env**- HIV-1 envelope; **gag**- Protein of HIV-1

Table 3

Summary of various HIV detection techniques useful for HIV management

Techniques	CD+ T Lymphocytes Counting	Viral Load Estimation	Remarks
Flow Cytometry	Conventional well-established for both		<ul style="list-style-type: none"> Assay time: 8–40 min Sample volume: 10 μL of blood Laboratory set-up Not suitable for POC
Image Processing	Bright field or fluorescent image of CD+ T lymphocytes		<ul style="list-style-type: none"> Assay time: 20–30 min Sample volume: 30 mL of whole blood Power: AC/battery Only qualitative Not suitable for POC
ELISA	Centrifuged based approach	Nano-enabled ELISA	<ul style="list-style-type: none"> Assay time: 40 min for CD+ and 48 hrs for viral load Sample volume: 30 μL of blood and 1 mL of plasma Power: DC/battery
RT-PCR		DNA based Virus capture based	<ul style="list-style-type: none"> Assay time: 35 to 90 min Sample volume: 150 μL of blood, 75 μL of fingerstic blood, and 1mL of plasma Power: AC/DC/battery
Electrical Sensing	Best suitable for HIV detection and monitoring		<ul style="list-style-type: none"> Sensing time: 15–30 min Sample volume: 10 μL Power: AC/battery/solar Sensitive and selective Suitable for detection and monitoring Suitable for POC application

ELISA-Enzyme-linked immunosorbent assay; **RT-PCR**- Reverse transcription polymerase chain reaction; **CD**- Cluster of differentiation; **POC**- Point-of-care; **AC**-Alternate current; **min**-minutes; **μ L**-microliters; **mL**- milliliters.