Polymeric Nano formulations in the Management of HIV Associated Neurocognitive Disorder

Karamot O Oyediran *

Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos, Lagos, Nigeria

Review Article

Received: 09-Mar-2022, Manuscript No. JPPS-22-56465; **Editor assigned:** 12-Mar-2022, PreQC No. JPPS-22-56465 (PQ); **Reviewed:** 25-Mar-2022, QC No JPPS- 22-56465; **Revised:** 30-Mar-2022, Manuscript No. JPPS-22-56465(R); **Published:** 06-Apr-2022, DOI: 10. 2320-0189.11.2.001

For Correspondence:
Karamot O Oyediran, Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos, Lagos, Nigeria

E-mail:
md.Karamahtobi@yahoo.com

Keywords: Polymeric nanoformulations; HIV/AIDS; neurocognitive disorder; antiretrovirals; CNS penetration

ABSTRACT

Background: HIV-Associated Neurocognitive Disorder (HAND) is one of the most prevalent comorbidities in the era of ART. The reported HAND prevalence ranges from 21% to 86%. The use of encapsulated nanosized antiretrovirals in various polymers has shown potential for enhanced permeation into the CNS and other latent viral reservoirs thus providing hope for prevention and treatment of neurocognitive disorders in HIV positive patients.

Main text: Methodology HIV-Associated Neurocognitive Disorder (HAND) has been used to describe the spectrum of neurocognitive dysfunction associated with HIV infection. Neurocognitive disorders are a result of a deficit in neurological activity. There is no specific treatment for HAND;however, ART has been used to reverse the disease process and improve cognitive function. Polymeric nanoformulations are solid colloidal systems consisting of a polymer matrix loaded with active therapeutic compounds within or adsorbed on it. Such compounds have particle sizes ranging from 1 to 1000 nm. Polymeric nanoformulations offer the potential for controlled release of a range of hydrophilic, hydrophobic drugs, vaccines, peptides, and biological macromolecules *via* several routes of administration.

Conclusion: Active targeting with PNPs has yielded promising results in preclinical studies and some cases early clinical trials. There is a need for further studies on polymeric nanoformulations for management of HAND with the potential for eliminating latent viral reservoirs and formulation of novel antiretroviral that is safe, effective, easily administered in adult and pediatric HIV positive populations.

LITERATURE REVIEW

Background

According to the WHO, there were approximately 37.7 million people worldwide livings with HIV/AIDS at the end of 2020. Of these, 36 million were adults while 1.7 million were children (<14 years old). 68% of people living with HIV are in sub-Saharan Africa. An estimated 1.5 million individual worldwide became newly infected with HIV in 2020, while 689,000 people died from AIDS-related illnesses. 27.5 million People (73% of PLWHA) worldwide had access to antiretroviral therapy in 2020. 74% of adults aged 15 years and older living with HIV had access to treatment, as did 54% of children aged 0–14 years. Each day in 2020, approximately 850 children became infected with HIV and approximately 330 children died from AIDS-related causes, mostly because of inadequate access to HIV prevention, care, and treatment services (UNICEF 2021). Most of these children live in sub-Saharan Africa and were infected by their HIV-positive mothers during pregnancy, childbirth, or breastfeeding. Currently, only 84% of people with HIV know their status, 73% had access to treatment and 66% were virally suppressed in 2020, hence the 90-90-90 target for 2020 was not achieved (UNAIDS 2021).

The newly proposed global 95–95–95 targets were set by UNAIDS in 2014 aims to end the AIDS epidemic by 2030. This implies that about 95% diagnosed of all PLHIV should be diagnosed, 95% of all diagnosed patients should be on antiretroviral drugs and 95% of all patients on antiretroviral medications should be achieving viral suppression.

Advancements over the years have been made to ensure improvement in quality of care offered to HIV-infected individuals. The introduction of Antiretroviral (ARV) drugs has prolonged survival and improved the quality of life among people living with HIV/AIDS (PLWHA) (CDC. 2007). However, complications especially of neurological nature remains a great source of concern as it is attributed to increased morbidity, and decreased the quality of life among these patients and the informal caregivers of PLWHA [1,2].

HIV-Associated Neurocognitive Disorder (HAND) is one of the most prevalent comorbidities in the era of ART. The reported HAND prevalence ranges from 21% to 86% [3]. However, varying results were achieved across studies. Studies carried out in the Sub-Sahara Africa region estimated the prevalence of HAND to be over 30% [1,2]. Perinatally infected children may present more frequently than adults with central nervous system disease due to the vulnerability of the developing brain, resulting in more severe brain degeneration occurring during a period of rapid brain and development [4].

Factors that are associated with the prevalence of HAND range from demographic factors notably age, sex, educational level and demographic region. Clinical factors such CD4 count; HCV coinfection, antiretroviral drug regimen, and psychosocial factors such as anxiety, depression and stigma are determinants of HAND [5]. Factors specific to the pediatric HIV-positive population include complexity in dosage measurement, non-palatability of drugs, and difficulty in swallowing pills. This problem persists especially in children on the lopinavir/ritonavir-based regimen [6]. Currently, the first line regimen for infants and young children are LPV/r-based regimens.

The use of encapsulated nanosized antiretrovirals in various polymers has shown potential for enhanced permeation into the CNS and other latent viral reservoirs thus providing hope for prevention and treatment of neurocognitive disorders in HIV positive patients especially those with unsuppressed viral load [7]. Several studies investigating naturally occurring compounds (for example resveratrol) have revealed the potential for nanoformulation of such compounds to achieve high CNS penetration and targeted drug delivery to the latent viral reservoir. These might help solve the impending problems inherent in presently available antiretroviral formulations. Thus a review was performed to provide researchers with recent advancement on polymeric Nano-formulations for active targeting of latent viral reservoirs especially the CNS.

Neurocognitive disorder in HIV infection

HIV-positive individuals often experience neurological complications such as cognitive deficits commonly called HIV-Associated Neurocognitive Disorders (HAND). Neurocognitive disorders are a result of a deficit in neurological activities, motor activities, psychological functioning, daily activities, and activities involved in job execution. HIV-Associated Neurocognitive Disorder (HAND) describes the spectrum of neurocognitive dysfunction, a complication of HIV infection. HIV can enter the CNS during the early stages of infection, and persistent CNS HIV infection and inflammation probably contribute to the development of HAND. As a result, latent reservoir is established in the brain, which serves as a source of reinfection and replication even when systemic viral suppression has been achieved. HAND can remain in patients treated with HAART, and its effects on survival, quality of life, and everyday functioning make it an important unresolved issue. Replication of the HIV virus takes place in the brain, macrophages and microglia, resulting in inflammatory and neurotoxic host responses. HIV may cause cognitive, behavioral, and motor difficulties. These difficulties may range in severity from very mild to severe and disabling [8].

According to the American Academy of Neurology (AAN), there are three categories of HIV-Associated Neurocognitive Disorder (HAND).

- Asymptomatic Neurocognitive Impairment (ANI) is determined by neurocognitive testing and is not apparent clinically.
- Mild Neurocognitive Disorder (MND) presents as mild functional impairment and may be diagnosed clinically if neurocognitive testing is unavailable.
- HIV-Associated Dementia (HAD) involves moderate to severe functional impairment.

Risk factors for developing an HIV-associated neurocognitive disorder include the following [8]

- Old age
- Female gender
- More advanced HIV disease (including CD4 count of <100 cells/μL, wasting)
- High plasma HIV RNA (viral load)
- Comorbid conditions (especially anemia and infection with cytomegalovirus, human herpesvirus 6, and JC virus)
- History of injection drug use (especially with cocaine)
- Psychiatric comorbidity: depression, anxiety disorders history of delirium and bipolar disease.

Despite increasing knowledge and understanding of HAND, there is still no definitive marker or specific treatment: HAART offers numerous advantage of preventing or delaying the progression of HAND in a small subset of affected patient. The development of HAND remains an important issue for HIV+ patients, as it affects not only survival and quality of life, but also everyday functioning [9]. Worldwide, HAND remains a common cause of cognitive impairment and has persisted even in individuals who have received HAART [9,10]. As HAART becomes more widely distributed in resource-limited settings and improves survival, the long-term global impact of HAND will become even more significant. Early HIV infection of the CNS has been attributed to the development of HAND, and evidence suggests that the CNS can subsequently serve as a reservoir for ongoing HIV replication, thereby limiting the opportunity for a sterilizing cure or eradication [11].

The Brain as a reservoir of HIV persistence

The characteristics of tissues/cells considered to be biologically significant HIV-1 reservoirs include [12]

- Cells must contain a replication-competent integrated provirus.
- Cells must have a mechanism that allows the virus to escape from biochemical decay processes or immune mechanisms and persist for long periods.
- Cells must possess a mechanism that suppresses viral replication and establish a latent infection.
- Cells must be infected in significant numbers to contribute to the establishment of a viral reservoir.
- Finally, cells must have the ability to produce new viral particles once activated. This leads to reseeding of the HIV infection.

Several HIV-1 reservoirs have been identified in multiple anatomical sites that harbor cells that fulfill some or all of these characteristics. These include resting memory CD4+ T cells in the blood, lymph node, gut-associated lymphoid tissue, and genital tract; resting naïve CD4+ T cells in the bone marrow; macrophages in lymph nodes, gut-associated lymphoid tissue, lung, kidneys, genital tract; and astrocytes, microglia and perivascular macrophages in the CNS. The largest latent viral reservoir for HIV-1 is attributed to resting memory CD4+ T cells [13,14].

Studies have demonstrated that the brain cells harbor genome integrated HIV [15]. HIV infects the astrocytes, perivascular macrophages, and microglial cells in the brain.In addition, mechanisms such as epigenetic regulation have been ascribed to induction of viral latency in the astrocytes and microglial cells. Evaluation of human brain-infected cells for their capacity to produce replication competent viruses remains a challenge due to ethical and technical problems. However, there are several indirect shreds of evidence showing that CNS is a reservoir for HIV. Indeed, HIV DNA has been detected in brain tissues isolated from autopsies of HIV patients whose infection has been controlled by Cart [16]. Moreover, there is a strong correlation between the amount of HIV DNA found in astrocytes and HIV-Associated Dementia (HAD) [17].

Various animal models have been used to show the persistence of HIV infection in the CNS as brain biopsy is not possible. Macaque, rats, and humanized BLT mouse have been used as models to mimic the condition of HIV-infected patients on HAART. These animal studies have confirmed the presence of viral RNA or viral proteins in the brain [18,19]. A mechanism of establishment of latent HIV transcription in the CNS has been suggested in the macaque model.They notably showed that interferon-beta repressed SIV LTR activity by inducing C/EBPγ expression, a dominant-negative isoform of C/EBPβ[20]. There are also several pieces of evidence supporting continuous CNS perturbation despite an efficient HAART [19] with an increase of the prevalence of a milder HAND. Moreover, in patients under suppressive HAART activation of the immune system is still observed in the CNS with some biomarkers, such as neopterin being detected in the Cerebrospinal Fluid (CSF).

One explanation is the existence of an inflammatory process that might be driven by low-level HIV replication in infected cells [21]. Interestingly, neuroimaging data are also in favor of persistent CNS inflammation in patients on HAART [22]. Finally, the development of highly sensitive methods, such as Single-Copy Assay (SCA), has allowed the detection of HIV RNA in the CSF from infected patients on HAART or from elite controllers whose HIV RNA level was initially undetectable in the plasma and CSF [23]. The recent discovery of a CSF viral escape in patients on HAART with undetectable plasma HIV RNA but with neurological impairment argue also for the existence of a persistent HIV reservoir in the brain[24].

Management of HIV associated neurocognitive disorder

There is no specific treatment for HAND; however, ART has been used to reverse the disease process and improve cognitive function. Reduction in patient's viral load and increased CD4 count has resulted in improved cognitive function. The CNS penetrating ability of antiretrovirals and high concentration in the cerebrospinal fluid has a strong correlation to viral load reduction and alleviation of neurocognitive deficits. Various antiretrovirals have been ranked based on drug concentration in the cerebrospinal fluid, chemical properties, and CNS effectiveness in clinical studies[25] assigned a penetration rank of 0 (low), 0.5 (intermediate), or 1 (high) with efavirenz, lamivudine, and zidovudine scoring high while abacavir had a low penetration rank score. Other studies have demonstrated cognitive improvement is associated with high penetrability of the antiretroviral [26]. However, the high penetrability of antiretrovirals has been linked to neurotoxicity, hence little or no improvement in HAND despite viral suppression[27]. Treatment of HAND requires a multidisciplinary approach that involves specialists such as a neurologist, psychiatrists, psychologist, nurses, and social workers. Neurocognitive impairment in patients with HIV infection often is multifactorial. Several medical conditions, such as psychiatric ailment, endocrinological anomalies, adverse drug effects that adversely affects the brain must be treated and eliminated before HAND can be diagnosed.

For patients using alcohol or illicit or nonprescribed drugs, implement strategies to reduce their use; these agents can further impair cognition. Antidepressants including SSRIs, TCAs have shown moderate symptomatic relief of HAND [28-30]. Selegiline has also shown efficacy. Efforts led by academic researchers have led to the development of intranasal insulin as a possible therapeutic agent for HAND. Several studies have successfully used intranasal insulin to improve cognitive function in healthy individuals, and in individuals with impaired cognitive performance as a result of aging or Alzheimer's disease [31]. The mechanistic explanation for these protective effects is not well understood, but insulin has a variety of metabolic and trophic effects and might directly protect neurons and dampen inflammatory cytokine expression [32,33]. Insulin has the potential to be delivered intranasally to multiple target organs as well as selectively target the CNS, an attribute that has made intranasal insulin an attractive candidate for neuroprotective therapy in HAND.

Challenges for treatment of HAND

The complex nature of the blood-brain barrier, poor pharmacokinetic profile, and poor bio-distribution of antiretroviral are barriers to effective drug delivery to the CNS [34]. A major obstacle to molecule passage through the BBB is the Brain Microvessel Endothelial Cells (BMVECs), which contribute to the formation of brain capillaries. Poor transportation rate of ARV drugs across the BBB may be attributed to high mitochondria and low pinocytotic activity in the BMVECs. Overexpression of P Glycoprotein (P-GP) on the BBB limits the entry of many drugs, including protease inhibitors, notably indinavir and ritonavir, epileptic drugs, and anti-inflammatory agents [35]. Despite the numerous challenges in the development of novel therapeutics for the treatment of HAND, notable achievements have been made in elucidating the mechanism of establishment, diagnosis and mechanism of neuroAIDS [36]. Novel therapy approaches have been explored to improve antiretroviral drugs delivery to the brain for the management of HAND.

Methods of enhancing HAART delivery to the brain

The selectively permeable blood-brain barrier reduces the bioavailability of HAART in the brain due to highly efficient drug efflux systems in the brain [37]. Several attempts to increase penetration of HAART into the brain include development of nanoformulations with increased BBB permeability, disruption of the BBB, uptake by brain microvascular endothelial cells *via* the adsorptive-mediated transcytosis, and cell-mediated delivery [38].

The rate of penetration of HAART through the blood-brain barrier is dependent on particle size, shape, and protein and lipid coatings. These properties affect drug uptake, release, and ingress across the barrier. Various attempts have been made to target HIV latent reservoirs in the brain. A variety of approaches including utilization of natural compounds such as resveratrol with proven cryoprotectant ability modified antiretroviral medications (nanoformulations and complexation with polymeric nanocarriers, liposome-based Nanomedicines, Dendrimers, Micelles, Proliposomes, Cubosomes, Nanoformulations optimized by use of artificial neural network) as well as coformulation of antiretrovirals with naturally occurring compounds with proven efficacy in the management of neurological disorder[39].

Natural compounds for management of HAND

Currently available antiretroviral medications used for HIV management and HAND have some limitations predominantly side effects and resistance, hence the need to consider the use of naturally occurring compounds predominantly plant-originated compounds, and plant extracts with anti-HIV and neuroprotective activity. Research groups have analyzed many plants and their extracts for the treatment of different diseases. However, knowledge of herbal medicines used to manage HIV and HAND are few, vague, and poorly documented. Natural products such as alanolides (Coumarins), Betulinic acid (a Triterpene), Baicalin (a Flavonoid), Polycitone A (an Alkaloid), Lithospermic acid (a Polyphenolic) can be mentioned as promising for anti-HIV agents whereas Withanolides and some polyphenolic compounds notably resveratrol for HIV-associated neurocognitive disorders [40]. Resveratrol, a naturally occurring polyphenolic found in grapes has been shown to possess the ability to inhibit HIV 1 replication with minimal toxicity due to its ability to increase the activity of SIRTI, a protein that reduces the transcription rate of the proviral genome. Resveratrol also synergistically increased the antiviral activity of nucleoside derivatives due to its ability to inhibit Ribonucleotide Reductase Inhibitors (RNRIs) [41].

Nanotechnology-based approach

Nanotechnology can improve the delivery of antiretroviral drugs across the blood-brain barrier. Antiretrovirals have the potential to be formulated as solid lipid nanoparticles, polymeric nanoparticles, nanogels, nanoemulsions, nanosuspensions, nanospheres, nanomicelles and liposomes, Lipid Drug Conjugates (LDC), and Nanostructured

Lipid Carriers (NLC) to increase the bioavailability and dissolution rate across the blood-brain barrier [42]. These nanoparticles are captured by monocytes, transported and housed within these cells as they are carried across the BBB, released into the CNS. These cells have the potential to be employed cell-mediated drug delivery to the CNS. Other laboratories have conjugated nanoparticles with Tat, which has an affinity for nuclear transport mechanisms [43]. This results in a nanoparticle that has high CNS penetrability while still bypassing efflux transporters to prolong exposure within the CNS [44].

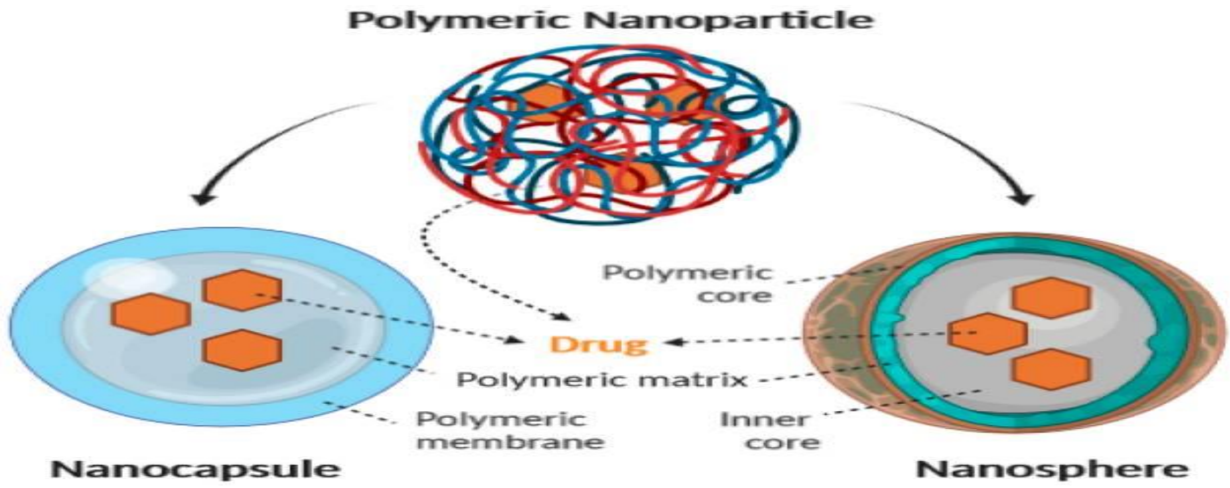
The sustained drug delivery and cell-specific targeting properties of nanoparticles have been employed to deliver conventional drugs, recombinant proteins, vaccines, and nucleotides. This in turn reduces toxicities associated with these drugs. Nanoparticles of Lopinavir, ritonavir, and efavirenz encapsulated in PLGA core has been studied and result obtained revealed optimal targeting of macrophages and monocyte. Other studies revealed a traceable concentration of encapsulated antiretroviral drugs in peripheral blood mononuclear cells *in vitro* after 28 days of drug administration [45] compared to the non-encapsulated drugs that were undetected with two days of administration.The novel integrase inhibitor, encapsulated elvitegravir nanoparticle formulation had shown improved ability to cross BBB *in vitro*[46]. Investigated the feasibility of developing a trojan horse prodrug that could simultaneously inhibit P-gp and have anti-HIV properties [47].

This could be a very promising approach that will need further investigation. In addition developed a magnetic nanoformulation consisting of genome editing Cas9/gRNA bound with magneto-electric nanoparticles to target HIV-1 long terminal repeat, thereby stopping viral transcription and eradicating latent HIV infection. This approach has enormous potential and further studies need to be carried out on its utility in the management of HIV infection of the brain [48]. A major concern with the reformulation of a drug is maintaining its stability during manufacturing and storage. Encapsulation of drug moieties with biodegradable and biocompatible polymers such as Poly (Lactic-Co-Glycolic Acid) (PLGA) have been employed to protect drug molecules from enzymatic degradation and provide physicochemical stability 48 nanoparticles can be coupled with protein, lipid coatings as well as ligands to facilitate their drug release, cellular uptake, and improve permeability across the blood-brain barrier and other physiological barriers. Formulation with ligands with immune-modulating effects, such as chitosan modifies the immune response and enhances intracellular drug delivery [49,50].Chitosan (CS), a natural polymer that carries a positive charge has gained attention in the nanomedicine field due to its ability to deliver nanoparticles to cellular and anatomic site[51].The electrostatic interactions between positively charged CS- NPs and the negatively charged cell surface have been shown to enhance nanoparticle uptake[52]. By using a PLGA core in conjunction with a CS shell, both hydrophobic and hydrophilic drugs can be encapsulated within the nanoparticle.

Polymeric nanoformulations

Polymeric nanoformulations are solid colloidal systems consisting of a polymer matrix loaded with active therapeutic compounds within or adsorbed on it with particle sizes ranging from 1 to 1000 nm. Polymeric nanoformulations offer the potential for controlled release of a range of drugs such as hydrophilic, hydrophobic drugs, vaccines, peptides, and biological macromolecules *via* several routes of administration. The formulations protect active moieties against harsh environmental degradation hence conferring improved bioavailability and therapeutic index. Polymeric nanoformulations may be formulated as nanocapsules and nanospheres.In nanocapsules, the active drug moieties are dissolved in an oily core and surrounded by a polymeric shell that controls the release profile of the drug from the core while nanospheres have the drug moiety dissolved in or adsorbed on the polymer network [53] as shown in Figure 1.

Figure 1. Schematic representation of the structure of nanocapsules and nanospheres [54] .



Polymeric nanoparticles can be prepared from natural or synthetic polymers. Synthetic polymers commonly used include Polylactide, Polylactide–Polyglycolide copolymers, Polycaprolactones, and Polyacrylates. Numerous studies have been carried out on Lactide–glycolide copolymer various natural polymers that have been explored include Alginate, Albumin, or Chitosan [55]. There are two main methods of preparation of polymeric nanoparticles. This includes the "top-down" approach and the "bottom-up" approach.Top down approach employs the use of preformed polymers to product polymeric nanoparticles in contrast to the bottom –up method that utilizes monomers that are subsequently polymerized to form polymeric nanoparticles. Factors such as particle size, types of solvents and polymers used in the synthesis, area of application, and nature of the drug will influence the choice method. The choice of biocompatible and biodegradable starting material is critical. The "top-down" methods frequently used are solvent emulsification–evaporation (emulsion evaporation method [43]. Solvent emulsification–diffusion (emulsion diffusion method [56,57]. Coacervation [58,59] and nanoprecipitation (solvent displacement method [60,61].The bottom-down methods reported includes emulsion polymerization [62,63].interfacial polymerization interfacial polycondensation, and molecular inclusion (Figure 2) summarizes the various methods of producing polymeric nanoparticles.

Commonly used synthetic polymers and monomers are utilized in the top-down and bottom-up nanoformulation method. Polymers include poly (d, l-lactide-co-glycolide), poly (ethyl cyanoacrylate), poly (butyl cyanoacrylate), poly (isobutyl cyanoacrylate), and poly (isohexyl cyanoacrylate) with poly vinyl alcohol and didecyldimethylammonium bromide being used as stabilizers and dichloromethane and ethylacetate, benzyl alcohol, cyclohexane, acetonitrile, acetone serves as vehicles/solvent for dissolving the polymers and monomers [64].

Polymer Nanoparticles (PNP) designed to target the central nervous system

Research has shown that drug moieties stand a chance of 1% or less for crossing the BBB for targeting purposes. Major research has confirmed that polymeric nanoparticles can be designed for therapeutic delivery to the CNS systematically and locally *via* endothelial cell endocytosis (PNPs release drug within these cells to reach the brain), Endothelial cell transcytosis, high concentration gradient due to accumulation of PNPs in brain capillaries, which will raise the transport rate across the BBB and boost delivery toward the brain cells, lipid solubilization of the endothelial cell membrane owing to the PNP surfactant effect, which results in membrane fluidization and enhanced drug permeability across the BBB, the opening of tight junctions between the endothelial cells of brain blood vessels, allowing the drug to then pass through the tight junctions in free form or entrapped within PNPs, brain vasculature toxicity(done at a minimal level), efflux system inhibition (Polysorbate is used to coat agent components of PNPs) and possible combination of all these mechanisms[65]. Polymeric nanoparticles serve the function of controlled delivery of conventional medications, proteins, nucleic acid, and diagnostic agents to the desired site of action in the body. Polymeric nanoparticles have a better safety and stability profile when compared to other Nano carrier systems. As the rate of HAND continues to increase, research involved in optimization of antiretroviral for enhanced CNS penetration has increase enormously. Notable examples are summarized in Table 1.

Table 1..Studies undertaken to date using polymeric nanoparticles for ARV drug delivery to the CNS.

Drugs	Drug Class	Polymeric System/s	Incorporation Method	Remarks	Reference
Darunavir, Darunavir prodrugs(M1DRV and M2DRV)	Protease inhibitor	Polaxamer 407(0.5%w/v in PBS)	High pressure homogenization at 20,000 psi	Sustained drug retention and antiretroviral effect for 15 days and 30days respectively in mice	[66]
Cabotegravir and Cabotegravir prodrug NMCAB,NM2CAB and NM3CAB)	Integrase inhibitor	Polaxamer 407	High pressure homogenization at 12,000 psi	Substantial inhibition of virus production for 30 days after single exposure, improved intracellular drug delivery, enhanced potency and sustained antiretroviral effect.	[67]
Dolutegravir prodrug (MDTG)	Integrase inhibitor	Polaxamer 407	high-pressure homogenization at 1.24 × 108 Pa	Plasma CAB levels above the protein-adjusted 90% inhibitory concentration for up to a year in mice and rhesus macaques, prolonged drug release, plasma circulating time and tissue drug concentration after 45 mg/kg body weight intramuscular prodrug injection	[68]
Lamiuvine microparticles	Nucleoside Reverse Transcriptase Inhibitor(NRTI)	Poly-ε-Caprolactone	High speed homogenization	HIV reverse transcriptase activity in culture fluids for more than 30 days, increase half-life of drug from 62 to 330 hours. Single IM injection can provide plasma drug levels above PA-IC90 for one month	[69]

Darunavir and ritonavir	Protease inhibitor	calcium alginate/chitosan microparticles that were film-coated with a series ofpoly(methacrylate) copolymers	Ring opening polymerization	The study highlights crucial uptake enhancing parameters for solid, surface modified particles. Results indicate particle diameter and surface hydrophobicity are the most influential parameters.	[70]
Myristolated Cabotegravir(NMCAB)	Integrase inhibitor	Polaxamer 407	Nanoprecipitation, solvent diffusion and evaporation	Increased oral bioavailability due to localized release of encapsulated nanoparticles in the small intestine.	[71]
Efavirenz, Saquinavir	Non-nucleoside reverse transcriptase inhibitor and protease inhibitor	poly(lactide-co-glycolide)	High pressure homogenization with drug polymer ratio 10:1 w/w	NMCAB demonstrated enhanced cellular entry, retention and intracellular drug depots for sustained and effective drug delivery	[72]
Indinavir,/ Ritonavir/ Efavirenz	Protease inhibitor and non-nucleoside reverse transcriptase inhibitor	Ethylene oxide and propylene oxide (poloxamer 188 (P-188), 1,2-distearoyl-phosphatidyl-ethanolamine-methyl-poly(ethylene-glycol) (DSPE-mPEG2000), poly(lactic-co-glycolic acid) (PLGA; ratio 50:50 of lactide to glycolide;), (1-oleoyl-2-(6-((7-nitro-2–1,3-benzoxadiazol-4-yl)amino]hexanoyl)-3-trimethylammonium propane) (DOTAP; Genzyme) and Cetyltrimethyl Ammonium Bromide (CTAB)	Emulsion or nanoprecipitation	50 fold increase I 50% inhibitory concentration compared to free drug	[73]

			High pressure homogenization	Cells pretreated with nanoART were protected against viral challenge for up to 15 days. Rapid drug uptake and slow release of drugs in clinically significant amount.	[74]
Lopinavir/ritonavir/efavirenz	Protease inhibitor, non-nucleoside reverse transcriptase inhibitor	Poly-(Lactic-co-Glycolic Acid) (PLGA)	Homogenization solvent extraction	Antiretroviral drug release for over 14 days , dose dependent reduction in progeny virion production and HIV-1 p24 antigen	[75]
Zidovudine	Nucleoside reverse transcriptase inhibitors	PLA and PLA/PEG blend	Double emulsion solvent evaporation	The addition of the PEG to the formulation, in the form of a physical mixture with the PLA modified the characteristics of the nanoparticles and resulted in different profiles of phagocytosis by rat neutrophils.	[76]
Efavirenz	Non-nucleoside reverse transcriptase inhibitor	β-cyclodextrin (β-CD),hydroxypropyl β-CD (HPβCD)	Physical mixing, kneading and freeze-drying	Enhaced dissolution of Efavirenz/CD complexes, increased rate of absorption of EFV/CD compared to Efavirenz.	[77]
Saquinavir	Protease inhibitor	polysorbate 80, Stearylamine (SA) and Dioctadecyldimethyl Ammonium Bromide (DODAB) and nonionic Compritol 888 ATO (CA) and	Microemulsion formation	Sustained release of saquinavir with no apparent initial burst.	[78]

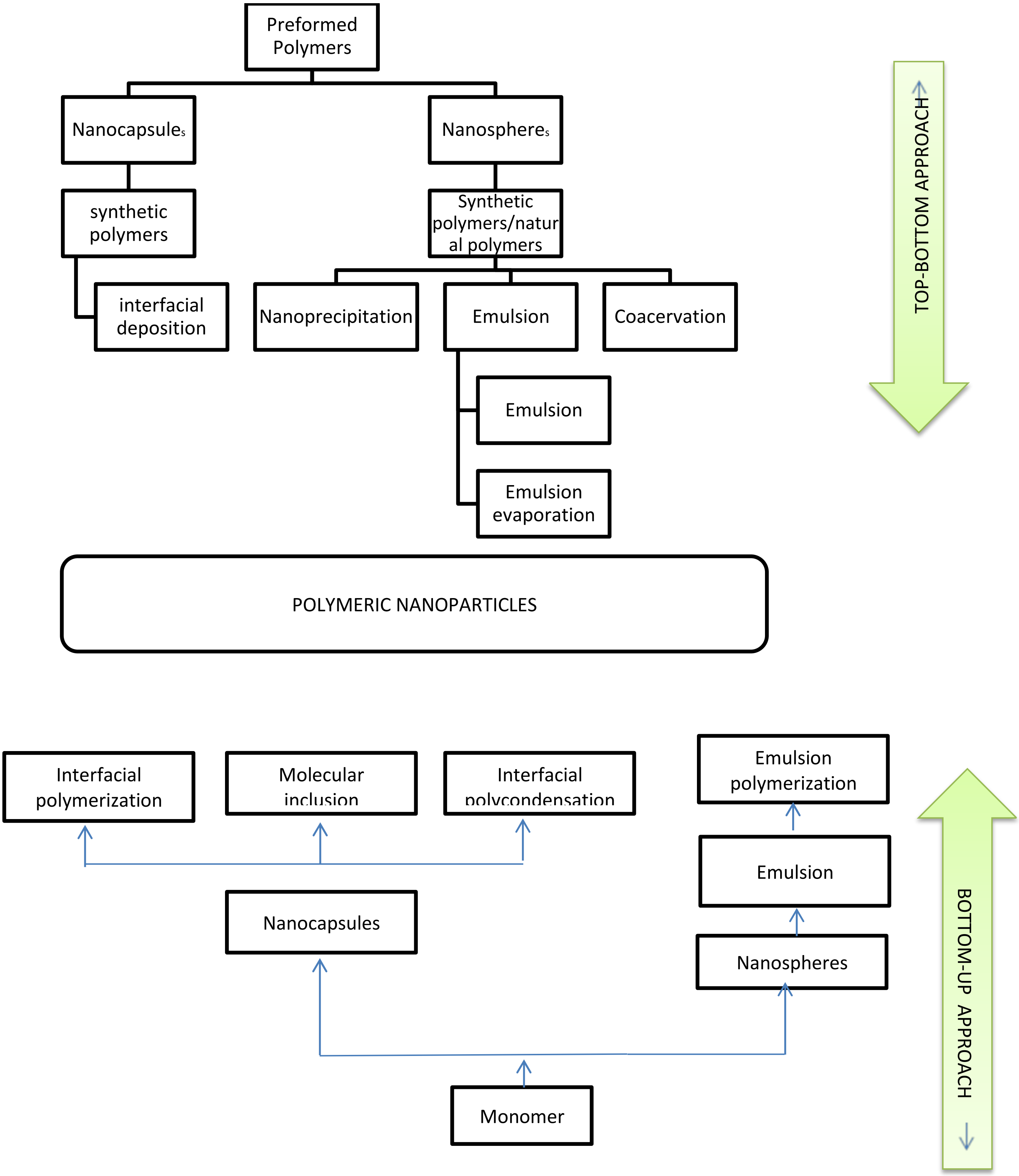
		ATO (CA) and cacao butter			
Stavudine	Protease inhibitor	Polybutylcyanoacrylate, methylmethacrylate/sulfopropylmethacrylate, and solid lipid (tripalmitin, phospatidylcholine, cholesterylhemisuccinate,taurocholate	Emulsion polymerization	Significantly enhanced permeation across the blood brain barrier	[78]
Saquinavir (SQV), delavirdin(DLV), stavudine(D4T)	Protease inhibitor, non-nucleoside reverse transcriptase inhibitor and nucleoside reverse transcriptase inhibitor	Polybutylcyanoacrylate(PBCA), Methylmethacrylate(MMA)/Sulfopropylmethacrylate(SPM), and solid lipid (tripalmitin, phospatidylcholine, cholesterylhemisuccinate, taurocholate)		The permeability of the three drugs enhanced about 12–16 folds on PBCA, 3–7 folds on MMA-SPM, and 4–11 folds in SLNs. For DLV and SQV, the order of permeability promotion was PBCA>SLNs>MMA-SPM; for D4T, PBCA>MMA-SPM>SLNs	[78]
Indinavir	Protease inhibitor	Lipoid E80(phosphatidylcholine, phosphatidylethanolamine, and hydrolyzed lyso)	High pressure homogenization	NP–IDV was readily taken up and released by MDM and showed sustained and potent anti-retroviral activities. The anti-retroviral activities were superior from what was observed for the IDV free form (soluble drug)	[79]
Saquinavir	Protease inhibitor	Poly (ethylene oxide)-modified poly (epsilon-caprolactone)	Solvent displacement method	The intracellular concentrations of saquinavir when administered in the nanoparticle formulations was significantly higher than from an aqueous solution, hence this can serve as a targeted drug delivery system for viral eradication in HIV latent reservoir.	[80]

CGP70726 -Novartis (HIV-1 protease inhibitor)	Protease inhibitor	Eudragit L100-55 poly (methacrylic acid-co-ethylacrylate) copolymer, Poly vinyl alcohol.	Emulsification-diffusion method	There was selective release of CGP 70726 in a highly dispersed/amorphous state and creation of high concentrations close to its absorption site.	[81]
Zidovudine	Nucleoside reverse transcriptase inhibitor	Hexylcyanoacrylate	Emulsion polymerization	Study shows that AZT bound to nanoparticles is selectively taken up by organs rich in macrophages.	[82]
Zidovudine	Nucleoside reverse transcriptase inhibitor	Trilaurin, dipalmitoylphosphatidylethanolamine- N –(poly (ethylene glycol) 2000) (PE-PEG).	Emulsion polymerization	Study revealed enhanced bioavailibility of incorporated AZT-P. The pharmacokinetic behaviour of the incorporated drug can be modified by changing the surface characteristics of SLNs by using the amphiphilic solvation enhancer PE-PEG.	[83]
Zidovudine	Nucleoside reverse transcriptase inhibitor	Hexylcyanoacrylate	Emulsion polymerization	The AUC in the organs that are mainly Infected by the HIV-virus, namely the reticuloendothelial cell containing organs, the blood, and the brain was increased when nanoparticles were used per orally.	[84]
Saquinavir, zalcitibine	Protease inhibitor, nucleoside analoque reverse transcriptase inhibitor	Poly(hexylcyanoacrylate)	Emulsion polymerization in an acidic medium	Nanoparticles as a drug carrier system could improve the delivery of antiviral agents to the mononuclear phagocyte system *in vivo*, overcoming pharmacokinetic problems and enhancing the activities of drugs for the treatment	[85]

				of HIV infection and AIDS.	
CGP 57813 (Peptidomimetic inhibitor of HIV-1 protease)	Protease inhibitor	Poly (lactic) acid	Salting out or the emulsification diffusion method	PLA nanoparticles did not provide significant plasma concentrations of CGP 57813, hence intact PLA nanoparticles pass slowly across the intestinal mucosa	[86]
Zidovudine ,zalcitabine	Nucleoside reverse transcriptase inhibitor	Human Serum Albumin(HSA) and Poly Hexyl Cyano Acrylate(PHCA)	PHCA NP was prepared by emulsion polymerization, while HAS NP was prepared by emulsification and subsequent heat denaturation as well as by precipitation	Antiviral effects of the drugs were similar with HSA and PHCA. The short incubation period of 2 hours suggest phagocytotic uptake of NP by human MAC is a very fast process. Therefore, it may be possible to accelerate the cellular uptake of some antiviral drugs by reducing release of these drugs before the target cells are reached, thereby producing a depot effect. .	[87]
Zidovudine	Nucleoside reverse transcriptase inhibitor	Polyalkylcyanoacrylate, polymethylmethacrylate, Human Serum Albumin	Suspension of freeze dried particles in buffer containing 1% of pluronics	NP made of Polyhexylcyanoacrylate (PHCA) or human serum albumin with a diameter of about 200 nm were found most useful for targeting antiviral substances such as azidotymidine to macrophages.	[88]
Stavudine	Nucleoside reverse transcriptase inhibitor	Polybutylcyanoacrylate(PBCA), Methylmethacrylate/Sulfopropylmethacrylate(MMA-SPM)	PBCA NPs by Emulsion polymerization, MMA-SPM NPs by free radical polymerization	PBCA NPs are superior to MMA-SPM NPs for oral D4T administration, and for intravenous D4T	[78]

				treatment, MMA-SPM NPs can be better tha n PBCA NPs	
Zidovudine ,lamivudine	Nucleoside reverse transcriptase inhibitor	Polybutylcyanoacrylate (PBCA), Methylmethacrylate/Sulfopropylmethacrylate (MMA-SPM).	PBCA NPs and MMA–SPM copolymer NPs were synthesized, respectively, by emulsion polymerization and free radical Polymerization	BBB permeability of AZT and that of 3TC became, respectively, 8–20 and 10–18 folds. Application of MMA–SPM NPs lead to about 100% increase in the BBB permeability of the two drugs. In the presence of 0.5% ethanol, 4–12% enhancement in the BBB permeability of the two drugs was obtained in the current carrier-mediated system.	[78]
Zidovudine	Nucleoside reverse transcriptase inhibitor	Poly (isohexylcyanoacrylate)	Emulsion polymerization	Poly (isohexylcyanoacrylate) nanoparticles are able to target and concentrate AZT in the intestinal epithelium and the associated immunocompetent cells of the GALT.	[89]
Saquinavir	Protease inhibitor	Combined hydroxypropyl-cyclodextrin and Poly(alkylcyanoacrylate)	Emulsion polymerization	The apparent solubility of saquinavir was increased 400-fold at pH 7.0 in presence of hydroxypropyl-cyclodextrin owing to the formation of a drug–cyclodextrin complex	[90]

Figure 2. Different methods for producing polymeric nanoparticles (Krishnaswamy and Orsat, 2017).



DISCUSSION AND CONCLUSION

Research on polymeric nanoparticles towards developing a formulation for the management of HAND has attracted the attention of recent. This review has highlighted studies and progress of polymeric nanoformulation for CNS targeting of the HIV viral reservoir and controlled release of antiretroviral to latent viral sites over the years. Active targeting with PNPs has yielded promising results in preclinical studies and some cases early clinical trials. Some studies however remained inconclusive as therapeutic efficacy in humans could not be established. There is a need for continued research geared towards development of polymeric nanoformulations for management of HAND with the potential for eliminating latent viral reservoirs and formulation of novel antiretroviral that is safe, effective, easily administered in adult and pediatric HIV positive populations.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

There was no special funding for the research. The authors funded the research.

Authors' contributions

KOO conceived the project and was a major contributor in writing the manuscript; CPA and MOI supervised the project. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

REFERENCES

1. Lawler K,et al. Neurocognitive impairment among HIV positive individuals in Bostwana: a pilot study. J Int AIDS Soc.2010;13:15–21.
2. Josker, et al.The Neurobiology of HIV dementia: implications for practice in South Africa. Afr J Psychiatry.2011;14:17–21.
3. Rosenthal LS,et al. A novel computerized functional assessment for human immunodeficiency virus-associated neurocognitive disorder.J Neurovirol.2013;19:432–41.
4. Phillips N,et al.HIV-Associated Cognitive Impairment in Perinatally Infected Children: A Meta-analysis.Pediatrics.2016;138:e20160893.
5. Wei J,et al.The Prevalence of Frascati-Criteria-Based HIV-Associated Neurocognitive Disorder (HAND) in HIV-Infected Adults: A Systematic Review and Meta-Analysis. Front Neurol.2020;11:581346.
6. Adrienne F,et al.The Need for Pediatric Formulations to Treat Children with HIV. AIDS Res. Treat.2016;8.
7. Joshi G,et al. Bioavailability enhancement, Caco-2 cells uptake, and intestinal transport of orally administered lopinavir-loaded PLGA nanoparticles.Drug Deliv.2016;23:3492–3504.
8. Saylor D,et al.HIV-associated neurocognitive disorder — pathogenesis and prospects for treatment. Nature Reviews. Neurology.2016;12: 234-248.
9. Heaton R, et al. HIV-Associated Neurocognitive Disorders (HAND) persist in the era of potent antiretroviral therapy: The CHARTER Study.Neurology.2010;75:2087–2096.
10. Tozzi V, et al. Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: prevalence and risk factors.J Acquir Immune Defic Syndr.2007;45:174–182.
11. Fois AF, et al.The potential of the CNS as a reservoir for HIV-1 infection: implications for HIV eradication.Curr HIV/AIDS Rep.2015;12:299–303.
12. Blankson JN, et al. The challenge of viral reservoirs in HIV-1 infection. Annu Rev Med.2002;53:557–593.
13. Gray LR,et al.Is the central nervous system a reservoir of HIV-1? Current Opinion in HIV and AIDS.2014; 9: 552–558.
14. MarbanC,et al. Targeting the Brain Reservoirs: Toward an HIV Cure. Front. Immunol.2019;7:397.
15. Kramer-Hämmerle S,et al. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus.Virus Res.2005;1:11:194–213.
16. Thompsonet al. Synthesis and Characterization of Long-Acting Darunavir Prodrugs. Molecular pharmaceutics.2020;17:155–166.
17. Churchill MJ, et al. Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia.Ann Neurol.2009;66:253–8.
18. Petry H, et al. Infection of Macaque monkeys with simian immunodeficiency virus: an animal model for neuro-AIDS. Intervirology.1997:40:112–21.
19. Lukeee,et al. Chapter 14 - Polymer nanoparticle drug-nucleic acid combinations, Editor(s): 2019;241-255.
20. Schwartz C,et al. Functional interactions between C/EBP, Sp1, and COUP-TF regulate human immunodeficiency virus type 1 gene transcription in human brain cells. J Virol.2000; 74:65–73.
21. Edén A,et al. Immune activation of the central nervous system is still present after >4 years of effective highly active antiretroviral therapy. J Infect Dis.2007; 196(12):1779–83.

22. Harezlak J, et al. Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment.2018;25:625–33.
23. Dahl V, et al. Single-copy assay quantification of HIV-1 RNA in paired cerebrospinal fluid and plasma samples from elite controllers. AIDS.2013; 27(7):1145–47.
24. Peluso MJ, et al.Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load.AIDS.2012;26:176574.
25. Letendre, S,et al. Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system.Archives of neurology.2008; 65:65–70.
26. Cysique LA, et al. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. Neurology.2009;73:342-8.
27. Robertson KR,et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort.Neurology.2010;74:1260-6.
28. Robertson KR, et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. Neurology.2010;74:1260-6.
29. Rabkin JG,et al. Effects of fluoxetine on mood and immune status in depressed patients with HIV illness.J Clin Psychiatry.1994;55:92-7.
30. Perkins DO, et al. Mood disorders in HIV infection: prevalence and risk factors in a nonepicenter of the AIDS epidemic.Am J Psychiatry.1994;151:233-6.
31. Mitra, P,et al. HIV Neurocognitive Disorders.In StatPearls.2021.
32. Craft S, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol.2012; 69:29–38.
33. Craft S,et al.Insulin and Alzheimer’s disease: untangling the web.J Alzheimers Dis.2013; 33:S263–S275.
34. Cholerton B, et al. Insulin and Alzheimer’s disease: untangling the web. J Alzheimers Dis.2013; 33:S263–S275.
35. Das MK, et al. Nano-ART and NeuroAIDS.Drug Deliv Transl Res.2016;6:452-472.
36. Zhang YL, et al. Blood-brain barrier and neuro-AIDS. Eur Rev Med Pharmacol Sci.2015;19:4927-4939.
37. Mahajan SD, et al. Anti-HIV-1 nanotherapeutics: promises and challenges for the future.Int J Nanomedicine.2019;7:5301-5314.
38. Ene L,et al. How much do antiretroviral drugs penetrate the central nervous system? J Med Life.2011;4:432.
39. Nowacek AS, et al. NanoART synthesis, characterization, uptake, release and toxicology for human monocyte-macrophage drudelivery.Nanomedicine.2009;4:903–917.
40. Saravanan M,et al. Nano-Medicine as a Newly Emerging Approach to Combat Human Immunodeficiency Virus (HIV).Pharm Nanotechnol.2018;6:17-27.
41. Kurapati KR,et al. Natural Products as Anti-HIV Agents and Role in HIV-Associated Neurocognitive Disorders (HAND): A Brief Overview. Frontiers in microbiology.2016;6:1444.
42. Clouser, CL,et al. The anti-HIV-1 activity of resveratrol derivatives and synergistic inhibition of HIV-1 by the combination of resveratrol and decitabine. Bioorganic & medicinal chemistry letters.2021; 22(21), 6642–6646.
43. Kumar, S,et al. Global Perspective of Novel Therapeutic Strategies for the Management of NeuroAIDS: Novel drug delivery methods for NeuroAIDS.Biomolecular Concepts.2018; 9:33-42.

44. Rao,et al.Polymeric Nanoparticles for Enhancing Antiretroviral Drug Therapy.Drug Deliv.2008;8:493-501.
45. Alfahad and Nath, et al. Polymeric nanoparticles for drug delivery to the central nervous system, Adv. Drug Deliv. Rev.2016; 64:701-705.
46. Kuo and Chen.Transport of stavudine, delavirdine,and saquinavir across the blood-brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmetheorylate, and solid lipid nanoparticles.Int J Pharm. 2007; 340:143–152.
47. Gong Y,et a. Novel elvitegravir nanoformulation for drug delivery across the blood-brain barrier to achieve HIV-1 suppression in the CNS macrophages.Sci Rep.2020;10 :3835.
48. Agrawal N,et al. Potential tools for eradicating HIV reservoirs in the brain: development of trojan horse prodrugs for the inhibition of P-glycoprotein with anti-HIV-1 activity. J Med Chem.2020;63:2131–8.
49. Kaushik A,et al.Magnetically guided non-invasive CRISPR-Cas9/gRNA delivery across the blood-brain barrier to eradicate latent HIV-1 infection. Sci Rep.2019; 9(1):3928.
50. Nagpal ,et al. CNS reservoirs for HIV: implications for eradication.J Virus Erad.2015 1:67–71.
51. Dube ,et al. Can humanized mice reflect the complex pathobiology of HIV-associated neurocognitive disorders? J Neuroimmune Pharmacol.2014;7:352–62.
52. Kutscher UNAIDS Global HIV & AIDS statistics — Fact sheet.2021.
53. Dai.UNICEF. Global and regional trends.2021.
54. Zielińska, A,et al. Polymeric Nanoparticles: Production, Characterization, Toxicology, and Ecotoxicology.Molecules (Basel, Switzerland).2016;25:3731.
55. Zazo HinojaL,et al. Current applications of nanoparticles in infectious diseases, J Control Release.2016;224:86-102.
56. Bilati, U, et al. Protein-loaded nanoparticles prepared by the double emulsion method processing and formulation issues for enhanced entrapment efficiency.J. Microencapsul.22:205–214.
57. Sabliov CM,et al.Chapter 12 - Encapsulation and controlled release of antioxidants and vitamins, Editor(s): Nissim Garti, In Woodhead Publishing Series in Food Science, Technology and Nutrition, Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals, Woodhead Publishing.2013;297-330.
58. Cegnar M, et al. Cystatin incorporated in poly(lactide-co-glycolide) nanoparticles: development and fundamental studies on preservation of its activity. Eur J Pharm. Sci.2004; 22: 357-364.
59. Csaba N,et al. PLGA:Poloxamer and PLGA:Poloxamine Blend Nanoparticles: New Carriers for Gene Delivery. Biomacromolecules.2005;6:271-278.
60. Jincheng W,et al. Preparation and properties of nanocapsulated capsaicin by complex coacervation method.Chemical Engineering Communications.2010;197:919-933.
61. Galindo-Rodríguez Sergio A,et al. Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. Eur J Pharm Sci.2005; 25:357-367.
62. Radwan MA .The effect of oral absorption enhancers on the in vivo performance of insulin-loaded poly(ethylcyanoacrylate) nanospheres in diabetic rats. J Microencapsul.2002;19:225-235.
63. Reis Catarina Pinto,et al. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomed. Nanotechnol. Biol Med .2016;2.8-21.
64. Watnasirichaikul,et al.Preparation of Biodegradable Insulin Nanocapsules from Biocompatible Microemulsions.Pharmaceutical Research.2000;17:684-689.

65. Kiruba Krishnaswamy,et al.Chapter 2 - Sustainable Delivery Systems Through Green Nanotechnology, Editor(s): Alexandru Mihai Grumezescu, Nano- and Microscale Drug Delivery Systems, Elsevier.2012;17-32.
66. Banoub.Drug synergy of tenofovir and nanoparticle-based antiretrovirals for HIV prophylaxis.PloS one.2020;8:e61416.
67. Smith N,et al. A long acting nanoformulated lamivudine ProTide. Biomaterials.2019; 223:119476.
68. Kulkarni TA,et al. A year-long extended release nanoformulated cabotegravir prodrug. Nature Materials.2012;19(8), 910–920.
69. NLM Sillman B et al. A long acting nanoformulated lamivudine ProTide. Biomaterials.2019;223.119476.
70. Urbaniak T,et al. Microparticles of Lamivudine-Poly-ε-Caprolactone Conjugate for Drug Delivery *via* Internalization by Macrophages.Molecules.2019.24:723.
71. Augustine, R,et al. Nanoparticle-in-microparticle oral drug delivery system of a clinically relevant darunavir/ritonavir antiretroviral combination. Acta biomaterialia.2018; 74:344–359.
72. Zhou et al.Creation of a long-acting nanoformulated dolutegravir. Nature communications.2018;9:443.
73. Chaowanachan, et al. Drug synergy of tenofovir and nanoparticle-based antiretrovirals for HIV prophylaxis. PloS one,2001;8: e61416.
74. Nowacek A, et al. NanoART, neuroAIDS, and CNS drug delivery.Nanomedicine.2009;557–574.
75. Destache CJ,et al. Combination antiretroviral drugs in PLGA nanoparticle for HIV-1. BMC infectious diseases,2009;9:198.
76. Mainardes RM,et al. Zidovudine-loaded PLA and PLA-PEG blend nanoparticles: influence of polymer type on phagocytic uptake by polymorphonuclear cells. J Pharm Sci.2009; 98: 257–267.
77. Sathigari, S,et al. Physicochemical characterization of efavirenz-cyclodextrin inclusion complexex.AAPS PharmSciTech.2009;10: 81–87.
78. Kuo and Chen Schafer, et al. Phagocytosis of nanoparticles by human immunodeficiency virus (HIV)-infected macrophages: a possibility for antiviral drug targeting.Pharm. Res.1992;9:541–546.
79. Dou et al.Centers for Disease Control. HIV/AIDS Surveillance Report. Atlanta: U.S. Department of Health and Human Services.2007.1-63.
80. Shah and Amiji,et al. Chapter 23 - Nanoarchitectures for Neglected Tropical Protozoal Diseases: Challenges and State of the Art. Editor(s): Alexandru Mihai Grumezescu, Nano- and Microscale Drug Delivery Systems, Elsevier.2017;439-480.
81. De Jaeghere F,et al. Oral bioavailability of a poorly water soluble HIV-1 protease inhibitor incorporated into pH-sensitive particles: effect of the particle size and nutritional state. J Control Release.2009;68:291–298.
82. Lobenberg R,et al.Body distribution of azidothymidine bound to hexyl-cyanoacrylate nanoparticles after i.v. injection to rats. J.Control Release.1998;50:21–30.
83. Heiati H,et al. Solid lipid nanoparticles as drug carriers II. Plasma stability and biodistribution of solid lipid nanoparticles containing the lipophilic prodrug 3-azido-3-deoxythymidine palmitate in mice.Int J Pharm.1998;174:71–80.
84. Kreuter (1997)et al. Creation of a nanoformulated cabotegravir prodrug with improved antiretroviral profiles. Biomaterials.2018;151:53–65.
85. Bender AR,et al. Efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages in vitro.Antimicrob Agents Chemother.40:1461-471.

86. Leroux, JC,et al. Pharmacokinetics of a novel HIV-1 protease inhibitor incorporated into biodegradable or enteric nanoparticles following intravenous and oral administration to mice. J Pharm Sci.1995;84:1387–1391.
87. Bender A,et al. Inhibition of HIV in vitro by antiviral drug targeting using nanoparticles.Res Virol.1994;145:215–220.
88. SchaferV,et al. Phagocytosis of nanoparticles by human immunodeficiency virus (HIV)-infected macrophages: a possibility for antiviral drug targeting. Pharm Res.1992;9:541–546.
89. Dembri A,et al.Targeting of 3-azido 3-deoxythymidine (AZT)-loaded poly(isohexylcyanoacrylate) nanospheres to the gastrointestinal mucosa and associated lymphoid tissues. Pharm Res.2001;18:467–473.
90. Boudad H,et al. Formulation and cytotoxicity of combined cyclodextrin poly(alkylcyanoacrylate) nanoparticles on Caco-2 cells monolayers intended for oral administration of saquinavir.STP Pharma Sci.2001; 11:369–375.