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A review on HIV Blood-Brain Barrier Penetrance and Antiretroviral Drug Delivery Deficiencies: A Paradox Revisited

[1*]Bhoj Raj Bashyal,[2] Yuvraj Singh Sarangdevot, [3]Bhupendra Vyas
Research Scholar, Faculty of Pharmacy
Bhupal Nobles' University, Udaipur (Rajasthan), india-313001

Abstract
HIV has the ability to infect immune cells that can migrate into the brain by crossing the blood-brain barrier. As a result, HIV can disguise itself to evade this biological barrier, which typically restricts the entry of most foreign substances. The majority of antiretroviral drugs are either unable to effectively penetrate the blood-brain barrier or are efficiently eliminated from the brain tissue. Consequently, this leads to the ineffective elimination of HIV from the brain and theformation of reservoirs. Within the brain, there exist diverse cell reservoirs that can harbor dormant HIV. This accumulation within the central nervous system (CNS) can result in viral recurrence and rebound infection. The brain-targeting efficiency of clinically administered antiretroviral drugs is generally low. However, the utilization of nano-formulations of these drugsholds the potential to enhance the bioavailability of effective medications in specific regions while preserving neurological integrity.

Introduction

According to the latest data from the Joint United Nations Programme on HIV/AIDS, the global number of individuals affected by human immunodeficiency virus (HIV) is estimated to be 35.3 million. Despite a decreasing trend in new infections since 2001, the number of individuals living with the virus continues to rise. This can be attributed to the success and increased availability of antiretroviral drugs. The implementation of cART in 1996 brought about a significant transformation in the treatment of AIDS, leading to improved quality of life and increased lifespan for those who are seropositive [2,3]. Several limitations of currently available drugs, including their toxicity and poor pharmacokinetics, the requirement for prolonged or chronic use, and the emergence of viral resistance, continue to pose challenges to the optimal efficacy of cART [4] The inadequate bioavailability of various anti-HIV medications at viral reservoir sites, including the central nervous system (CNS), particularly in brain macrophages and microglia cells, is a matter of significant concern. This issue persists despite the availability of dosage forms.

Low concentration-ratio values of cerebrospinal fluid to blood plasma (CSF: BP) have been documented for various drugs commonly utilized in the management of HIV/AIDS. For instance, protease inhibitors (PIs) exhibit extensive binding to plasma proteins and serve as substrates for permeability glycoprotein (P-gp) and other crucial efflux transporters found at the blood-brain barrier (BBB). This combined action severely restricts their entry into the brain. In addition to hindering the elimination of the virus from the central nervous system (CNS), the limited ability of antiretroviral drugs to penetrate the BBB is associated with elevated CSF viral loads, which can significantly contribute to the development of neurological disorders [5-7].

The potential benefits of utilizing drug nanocarriers in delivering antiretroviral drugs across the blood-brain barrier (BBB) and into the central nervous system (CNS) have been widely advocated. Nanotechnology-based systems offer intriguing features, including enhanced intestinal absorption following oral administration, improved toxicity profiles, increased drug stability, prolonged drug residence in the body (particularly in the CNS), circumvention of efflux pumps at the BBB, and selective drug delivery to specific cells, such as HIV-target cells. While other types of nanocarriers, such as dendrimers, nano-emulsions, liposomes, micelles, and nanogels, have also been proposed for managing HIV infection of the CNS, this review will specifically focus on solid nanoparticles (NPs) of polymeric, macromolecular, lipid, or metallic nature. Additionally, strategies based on antiretroviral drug nanosuspensions will be overviewed [8–13].

The CNS as a Sanctuary for HIV

Antiretroviral therapy (ART) is a crucial tool in the fight against the global epidemic of HIV, which affects nearly 38 million people worldwide. ART works by suppressing viral replication and reducing HIV RNA to untraceable levels in the blood, but its effectiveness in eradicating the virus from the central nervous system (CNS) is limited by the blood-brain barrier (BBB). The BBB presents a major challenge to drug delivery, as successful passage across it is necessary to target viral reservoirs in the CNS that can accumulate HIV proviruses. However, crossing the BBB without altering its neurological barrier integrity and CNS function remains an unsolved problem. In addition to this challenge, ART drug cocktails are accompanied by the risk of comorbidities, including neurological, metabolic, cardiovascular, and cerebrovascular conditions. Addressing these challenges is crucial to achieving the goal of eradicating HIV and improvingthe lives of those affected by this devastating disease [14].

The paradox that is being examined in this text is the ability of foreign viral components to cross the blood-brain barrier (BBB) and enter the central nervous system (CNS), while crucial therapeutic drugs are unable to do so. The review discusses the neuropathology of HIV and the BBB, highlighting how disruption of the BBB facilitates and enhances proviral replication. It also addresses the current therapies available for HIV-infected individuals and their limitations. Furthermore, attention is drawn to promising therapeutic solutions that aim to overcome barriers associated with HIV, such as the use of nanoparticles for efficient delivery of antiretroviral therapy (ART) to the brain. These innovative approaches for drug delivery play a crucial role in advancing efforts to develop drugs that specifically target the CNS [15].

HIV possesses the inherent capability to attach itself to receptors present on circulating leukocytes in the bloodstream, as depicted in Figure 1. Subsequently, these leukocytes can be recruited into the brain by various hypothesized mechanisms, crossing the blood-brain barrier (BBB). The migration of these infected host cells into the brain occurs with remarkable ease, surpassing the efficacy of therapeutic drugs in accomplishing the same task. Although antiretroviral therapy (ART) effectively circulates in the peripheral blood and adequately suppresses HIV replication levels, its inability to penetrate the BBB creates a secure sanctuaryfor HIV to accumulate and remain dormant. This poses a significant challenge in the treatment of HIV and the prevention of disease recurrence [16].

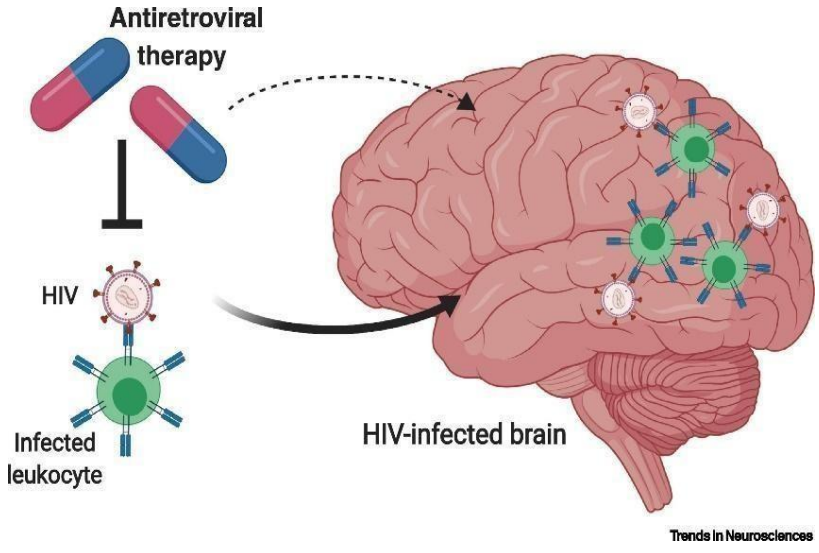

Figure 1

The inherent capability of HIV to attach to receptors expressed on circulating leukocytes in the bloodstream. Subsequently, these leukocytes can be recruited into the brain through various hypothesized mechanisms, bypassing the blood-brain barrier (BBB). The migration of these infected host cells into the brain is remarkably effortless compared to the efficacy of therapeutic drugs in accomplishing the same objective. While antiretroviral therapy (ART) effectively circulates in the peripheral blood and successfully suppresses HIV replication levels, its inability to penetrate the BBB creates a secure sanctuary for HIV to accumulate and

remain dormant. This

Blood–brain barrier (BBB): The anatomy-physiological unit that acts as a crucial interface between the blood and the brain consists of brain vascular endothelial cells. These cells are securely connected by tight junction proteins and interact with neighboring astrocytes and pericytes. Together, they form a functional neurovascular unit that safeguards the brain against pathogens, ensures cerebral homeostasis, and controls the exchange of molecules between the blood and the central nervous system [17].

Clonal expansion: Cells infected with HIV that are present in the brain can continue to replicate the virus. These groups of cells that are multiplying are essentially copies of the originalHIV-infected cells and can remain inactive in the central nervous system for a prolonged period. This phenomenon is known as clonal expansion and is a significant factor in the persistence of viral reservoirs in the CNS, even when antiretroviral therapy is administered [18].

HIV-associated neurocognitive disorder: More commonly known as HAND, this encompassesa wide array of comorbidities that emerge from immune activation caused by HIV infection.

Latent viral reservoirs: Cells infected with HIV have the ability to integrate the virus's genetic material into their own DNA. These cells can then enter a dormant state by halting theirtranscription processes, rendering them undetectable by the immune system and allowing them to evade immune responses. Consequently, the development of effective drugs and the eradication of HIV pose significant challenges [19].

Proviruses: When HIV infects a host, it undergoes reverse transcription, resulting in the formation of a provirus - an inert form of the virus that is integrated into the host cell's DNA. During normal cell replication, the provirus is replicated along with the host cell's genome, leading to the production of viral particles in the cell progeny [20].

QVOA: The quantified viral outgrowth assay (QVOA) is an essential tool for quantifying viral reservoirs, specifically on CD4+ T cells. This assay plays a crucial role in measuring the level of viral reservoirs, particularly after ART administration. By reversing latent cells and initiating HIV transcription, the QVOA enables the detection of high levels of HIV replication, which may indicate the presence of viral reservoirs unaffected by ART. However, the QVOA has limitations due to the heterogeneity of replication-competent cell reservoirs that have recently been identified. Consequently, this assay may significantly underestimate the accurate measurement oflatent HIV in the brain [21].

Rebound viremia: The process of HIV re-entering the bloodstream through the cerebrospinal fluid (CSF) after escaping from CNS viral reservoirs can lead to rebound viremia. This can also be caused by patient noncompliance or premature discontinuation of ART regimens.

Viral load: A widely employed clinical term for assessing the viral load within a designated blood volume is commonly used. In individuals affected by HIV, this measure is typicallyquantified by determining the quantity of HIV RNA copies per milliliter of collected blood [22].

Structural and Regulatory Components of HIV

The structure of the HIV lentivirus consists of two single strands of RNA enclosed within a capsid that contains the viral protein, p24. This protein, p24, serves as a significant marker for detecting HIV infection (Figure I). The RNA genome of HIV is highly organized and consists of nine genes that have the ability to code for 15 different viral proteins. These genes include three structural genes: gag (group specific antigen), pol (polymerase), and env (envelope).Additionally, there are two essential regulatory genes: tat and rev, as well as four additional accessory genes: nef, vpr, vpu, and vif [96]. These genes, along with their associated viral protein products, play a crucial role in viral replication and can be targeted as potential drug targets [23]. To target these specific genomic proteins, anti-HIV peptides and small-molecule inhibitors have been utilized, resulting in successful suppression of HIV RNA levels to below 50 copies/ml when measuring viral load [24]. The HIV RNA-enclosed capsid is surrounded by a lipoprotein-rich membrane.

The membrane of the virus contains two essential proteins, the surface glycoprotein (gp120) and transmembrane glycoprotein (gp41), that facilitate the virus's ability to attack, bind, and infect host cells. The gp120 protein binds to the CD4 receptors found on various types of host cells, including T cell precursors, monocytes, macrophages, and dendritic cells. The initial stages of HIV infection rely on protein-protein interactions, starting with the binding of gp120 to the CD4 receptor. This binding exposes the gp120 protein

domain, allowing for specific binding ofchemokine receptors, such as CCR5 and CXCR4. When gp120 binds to both CD4 and achemokine coreceptor, the gp41 transmembrane protein creates a channel across the host cell's plasma membrane, resulting in the translocation of viral capsid into the host cell and subsequent HIV replication and infection. This mechanism underlies chronic HIV infection [25].

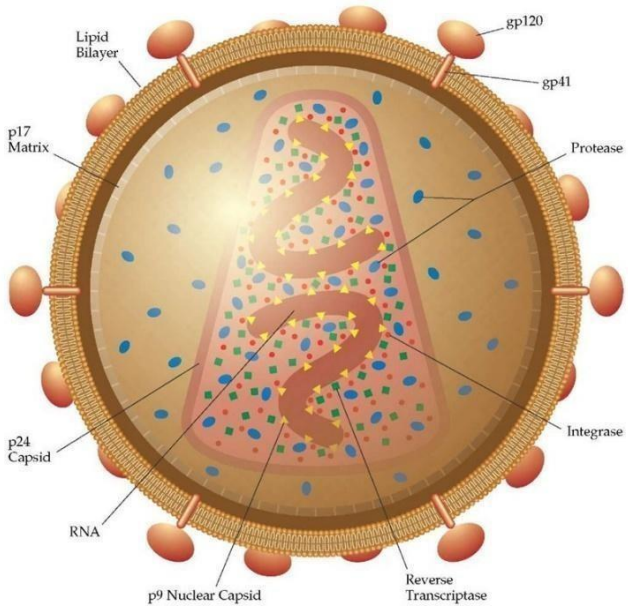

Figure 2. The Structure of HIV.

The schematic provided illustrates the structure of HIV. Enclosed within a protein capsid, the viral RNA genome is surrounded by a lipoprotein-rich membrane. Embedded within this lipid membrane are the significant structural proteins, glycoprotein-120 (gp120) and glycoprotein-41 (gp41), which play a vital role in facilitating HIV's binding to receptors for viral transfection and fusion into the host cell. At the core of the viral capsid, HIV harbors an RNA genome consisting of two single strands. The enzyme reverse transcriptase is responsible for catalyzing the transcription of viral RNA into complementary DNA (cDNA). Another crucial enzyme, HIV integrase, can integrate the HIV DNA into the genome of the host cell once it is transcribed. This viral DNA, once inserted into a host cell, is commonly referred to as proviral DNA.

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There are four molecular transcellular routes into the central nervous system (CNS): lipid-mediated diffusion, carrier-mediated transport, receptor-mediated transport, or active efflux transport. Lipophilic therapeutic molecules with a molecular weight of less than 400 Da can diffuse across the blood-brain barrier (BBB) through lipid-mediated diffusion [27]. However, these small molecules can be quickly eliminated from the brain tissue by ATP-binding efflux pumps, such as p-glycoprotein and multidrug resistance proteins (MRP), which are present in the endothelial monolayer [28-30]. These ATP-binding pumps play a crucial role in the removal and circulation of biomolecules and drugs within the CNS. Consequently, certain antiretroviraltherapy (ART) drugs like abacavir, efavirenz, and protease inhibitors, which are eliminated from the brain, exhibit a high brain efflux and therefore fail to reach therapeutic concentrations in patients [10]. By targeting and providing antagonists for this pathway, it may be possible to enhance the bioavailability of therapeutics beyond

the BBB, prolong circulation time, and improve efficacy. Overall, the penetration of the BBB remains the limiting factor in achieving effective therapeutic interventions for most CNS diseases [31].

HIV Can Stealthily Breach the BBB

The most efficient method of HIV infection is through the binding of the virus to a host cell using CD4 and chemokine receptors, specifically CXCR4 and CCR5. This is followed by the injection of the viral genome for replication [32]. These receptors are predominantly expressed on T lymphocytes and monocytes, which circulate in the peripheral blood and can potentially cross the blood-brain barrier (BBB) [33-35]. It is believed that these infected cells act as carriers,transporting the HIV genome into the central nervous system (CNS) in a Trojan horse-like mechanism. This mechanism is thought to enhance chronic infection by taking advantage of the protective nature of the BBB [36]. Consequently, once the virus has replicated within macrophages and microglia in the CNS, it becomes challenging to eliminate. Another hypothesis proposes that HIV can enter the CNS by being taken up by specific chemokine receptors (such as APJ, CCR3, CXCR4, and CCR5) on the microvascular endothelial cells of the BBB (Figure 2) [37]. Although CD4 receptors have been identified on brain microvascular endothelial cells, active replication of the virus in these cells has not been observed in HIV patients. In an in vitro model of the BBB, inhibiting chemokine receptors does not prevent HIV infection of microvascular endothelial cells [38]. The mechanisms by which HIV invades the CNS remain unclear; however, understanding the consequences of this process is crucial for the development of drugs that can effectively target the brain [39].

Other pathways of viral entry focus on disrupting the integrity of the blood-brain barrier (BBB). This disruption can occur by modifying the tight junction proteins that tightly bind the microvascular endothelial cells. Numerous studies have demonstrated that following an initial HIV infection, there is an increase in BBB permeability, which has significant implications for neurological health [40-42]. This increase in permeability may be attributed, in part, to a decrease in pericyte coverage of the brain endothelium. This reduction in coverage can lead tothe infiltration of monocytes into the brain, including the trafficking of HIV-infected cells, as well as elevated levels of proinflammatory cytokines [43]. Furthermore, dysfunction of the BBB has been observed through the loss of pericytes, contributing to the dysregulation of the BBB induced by HIV and the associated comorbidities [44]. The significance of these events lies inthe fact that endothelial cells are highly susceptible to inflammatory insults [45]. To quantify the occurrence of neuro-invasion, clinical studies have compared the levels of albumin in cerebrospinal fluid (CSF) with the levels of albumin in serum, using the quotient (Qalb) as the standard for determining BBB function.

Dysregulation in patients [46] can be observed through the examination of albumin, a large macromolecule that is unable to penetrate the blood-brain barrier (BBB) and should not be present at high concentrations within the cerebrospinal fluid (CSF). A low (Qalb) indicates a normal functioning BBB, while a high (Qalb) suggests a loss of tight junction protein integrity and subsequent dysregulation of the BBB. Clinical studies have further confirmed that individuals with acute HIV infection experience early neuronal injury, viral infection, inflammatory onslaught of immune cells, and increased CSF to serum albumin quotients [47].

These findings provide evidence of the vulnerability of the BBB following early HIV infection, despite the incomplete understanding of the routes of entry [48].

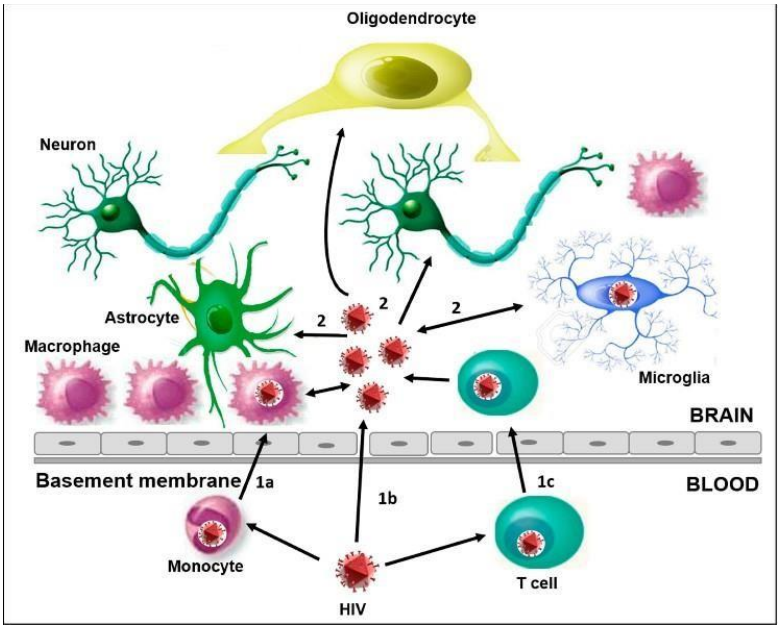

Figure 3. Invasion of HIV into CNS via CD4+

T Cells and Monocytes. *The diagram illustrates the proposed mechanism of HIV-infected immune cell infiltration. CD4+ T cells (green) and monocytes (red) possess receptors that can interact with HIV, facilitating its replication and subsequent injection into a healthy host cell. The blood-brain barrier (BBB) expresses chemokines with chemokine-specific receptors, which aid in the recruitment of immunecells such as T cells and monocytes, as depicted in the diagram. Consequently, HIV can surreptitiously traverse the typically impermeable BBB, resulting in invasion of the centralnervous system (CNS).*

Animal models have been developed to investigate the potential of HIV to enter the brain parenchyma. Studies using simian immunodeficiency virus (SIV) in rhesus macaques have revealed the presence of SIV-infected CD4+ T cells, macrophages, and dendritic cell markers in brain and bone marrow tissues. Furthermore, active division of SIV RNA has been observed, supporting the theory of clonal expansion of latent viral reservoirs in brain compartments. In addition, mice infected with EcoHIV, a chimeric form of HIV, have demonstrated similar results [49].

The enhanced expression of C3, IL-1β, IL-6, CCL2, and STAT-1, which are influential factors in the inflammatory responses to HIV in the brain, illuminated the viral genome. This illumination was observed in the context of the increased expression of these factors [26]. Furthermore, EcoHIV triggered antiviral responses and host cell infections, while concurrently suppressing tissue function and recovery. This suppression increased the brain's susceptibility to cerebrovascular events [50-52].

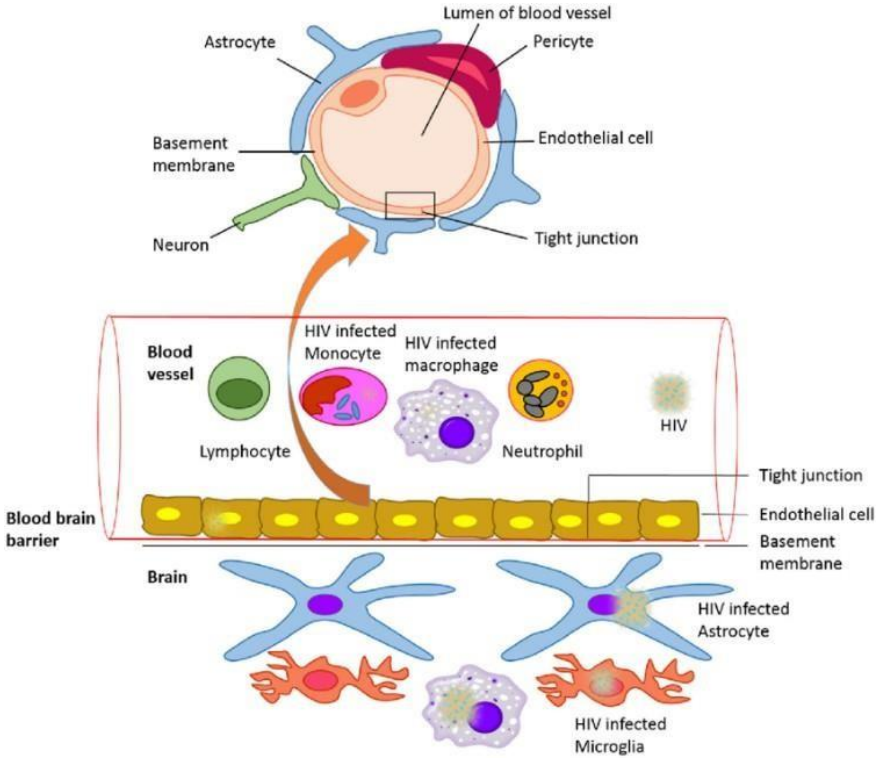

Figure 3. Comparison of Blood–Brain Barrier (BBB) Before and After

Dysregulation and Subsequent HIV Infection. *In individuals with good health, the blood-brain barrier (BBB) serves as an impermeable structure that separates the central nervous system (CNS) parenchyma from the peripheral blood circulation and other pathogens. The BBB primarily consists ofendothelial cells, which are connected by a complex of tight junction proteins. The BBB is composed of three crucial tight junction proteins, namely claudin (with claudin-5 being the primary constituent of the BBB), zonula occludens (ZO-1, ZO-2, and ZO-3), and occludin. On the other hand, HIV infection and the use of long-term antiretroviral therapy (ART) can disrupt the regulation of tight junction proteins, thereby increasing the susceptibility to further CNS HIVinfection. It is important to note that the abbreviation for tight junction is TJ.*

The CNS Safeguards HIV Accumulation in Reservoirs Beyond the BBB

The topic of HIV-associated reservoirs within the central nervous system (CNS) has been a subject of recent debate. Numerous animal models have already demonstrated the existence of these reservoirs. Among the various cell types within the CNS, microglial cells have been identified as the primary reservoir for HIV [53]. In a mouse model consisting solely of T cells, mice were infected with HIV and subsequently treated with antiretroviral therapy (ART). After initiating ART, CD4+ T cells were collected from the mice with suppressed viral loads. It was discovered that HIV expression could be induced ex vivo, indicating that latency had already been established in these isolated CD4+ T cells in vivo [54]. The presence of viral reservoirs within CD4+ T cells, capable of causing rebound viremia if ART drugs are discontinued, has been confirmed through real-time PCR assays and genomic sequencing [55-58]. Furthermore, the development of an intact proviral DNA assay has allowed for the selective detection of proviral DNA levels within CD4+ T cells, distinguishing them from any defective proviruses that may be present [59]. Nevertheless, the significance of T cells in the HIV-infected brain in humans remains a topic of controversy [60].

Studies have explored various cell types as potential hosts for latent HIV reservoirs within the CNS. Pericytes, in particular, remain somewhat mysterious in terms of their interaction with the virus. Recent research has revealed that pericytes can harbor HIV and transition between latent and reactivated viral cycle stages. In fact, when exposed to histone deacetylase inhibitors and tumor necrosis factor, latently infected pericytes

demonstrated increased p24 and HIV RNA levels, indicating viral reactivation. This is likely due to the fact that pericytes express both CD4 and chemokine coreceptors, making them susceptible to direct infection by HIV [61].

Populations of cells capable of harboring HIV can be found in perivascular spaces beyond the neurovascular unit. In a macaque model, SIV genomes were found in perivascular macrophages and microglia, which could be reactivated even after ART suppression. Studies in mice have alsosuggested the importance of macrophages in HIV replication and formation of viral reservoirs. The mouse model involves transplanting CD34+ hematopoietic stem cells into NOD/SCID mice, which lack functional T and B cells. There is growing evidence that macrophages remainsusceptible to HIV even after ART initiation [62].

Various animal models have demonstrated that the central nervous system (CNS) has the ability to compartmentalize pockets of HIV in its latent form. Resting CD4+ T cells can transition into a state of dormancy, making them ideal hosts for viral infections [63-65]. These viral reservoirs, consisting of CD4+ T cells, have been found to have a half-life of 44 months even afterantiretroviral therapy (ART) administration, making viral eradication through natural decayhighly improbable [66]. In fact, a mere 1 million cells within a latent viral reservoir canpotentially remain viable for up to 73 years [67]. Moreover, the presence of multiple heterogeneous reservoirs, each consisting of millions of cells, further complicates the task of eradicating HIV within the CNS. The stability of these cells, combined with physical barriers, provides a protective environment for HIV strains to remain dormant. In human patients, even after initiating ART therapeutic regimens, CD4+ cell counts typically remain below baseline levels, indicating that HIV continues to suppress the healthy replication of CD4+ T cells and highlighting the limited ability of ART to effectively suppress viral replication beyond the blood-brain barrier [68]. Although the complete stability of cell reservoirs is still not fully understood, these models underscore the longevity of HIV and the challenges associated with its eradication through ART or natural decay [69].

Viral entry into the CNS can occur through the choroid plexus in addition to the BBB [70-73]. While both barriers offer protection, the choroid plexus plays a crucial role in producing CSF and circulating molecules throughout the CNS. The epithelium of the choroid plexus is also known to be lined with resident macrophages, which are frequently infected with HIV in the CNS [74]. Studies have shown that feline immunodeficiency virus can cross into the brain through the choroid plexus via macrophages, T lymphocytes, and monocytes [75]. Viral accumulation was observed to be significantly higher on the apical surface of this epithelial barrier, making the choroid plexus a dynamic reservoir for HIV accumulation and a possible path for neuro-invasion events and future ART drug delivery. It is important to note that the ease at which viruses breach this epithelial barrier is coordinated by the high amount of MRP and P-glycoprotein expressed on the surface [78]. Interestingly, the P-glycoprotein pump is oriented in a way that opposes the action of P-glycoprotein efflux transporter located in the BBB. It is important to consider the subsequent modifiers when addressing the query. The provided text elucidates the role of a certain mechanism in impeding the escape of substrates and other molecules from the cerebrospinal fluid (CSF). This intricate association further emphasizes the maintenance of central nervous system (CNS) and blood-brain barrier (BBB) homeostasis in facilitating the transportation of therapeutic agents to the CNS [79].

HIV Therapeutics and Their Limitations in Targeting the Brain

A combination of three therapeutic antiretroviral drugs for HIV treatment was successfully introduced clinically in 1996; however, to date, there are no FDA approved ART agents that can diffuse across the BBB without altering its structural integrity [80]. The mechanism of BBB crossing of present FDA approved ART drugs involves transcellular uptake or rapid efflux across the BBB using transport proteins such as P-glycoprotein, MRP, and breast cancer resistance protein (BCRP). Ineffective ART delivery and suboptimal concentrations reaching the CNS are causes of HIV's ability to manifest in the CNS and maintain a low level of replication [81]. This viral survival event can affect organs and tissues beyond the CNS, as is evident from its associated comorbidities. The ability for these drugs to penetrate the BBB depends on many coordinating factors, including molecular size and weight, protein-to-protein interactions, lipophilicity, molecular pump and uptake mechanisms, as well as physiochemical properties. Due to the recency of newer generations of ART drugs, lifetime longitudinal toxicity studies are somewhat limited. Neurovascular toxicity associated with taking ART as a chronic regimen has been documented as permitting mitochondrial dysfunction, disrupting or altering

the BBB,neural progenitor cell senescence, and reducing electron transport chain function through Complex I [82-84]. While the clinical prescription of ART is the gold standard for suppressing actively replicating viral genomes in HIV-infected individuals, eliminating the virus is notfoolproof and neurovascular toxicity should be further analyzed [85].

Antiretroviral therapeutic drugs face the challenge of being unable to penetrate the blood-brain barrier and effectively eliminate viral reservoirs.

Currently, HIV has no cure, and therefore, ART drugs are the standard for HIV care. These drugs can effectively suppress HIV viral loads in peripheral blood circulation and reduce the risk of HIV transmission. An undetectable HIV RNA viral load is equivalent to less than 50 copies/ml, while HIV suppression is associated with HIV RNA plasma levels below 200 copies/ml. However, rebound viremia occurs when plasma viral loads exceed 500 copies/ml after initial suppression using ART. Although ART drugs can reach the CNS, their concentration is significantly lower than peripheral blood plasma levels. For example, abacavir accumulates in the plasma at 5.2–10.9 μmol/ml but only reaches 0.5–1.8 μmol/ml in the CSF. Similarly, efavirenz accumulates in the plasma at 9.2–16.6 μmol/ml but only reaches 0.006–0.09 μmol/ml in the CSF. While the BBB limits the transport of many ART drugs into the brain, some studies suggest that cation/anion transporters in the choroid plexus can preferentially uptake drugs such as tenofovir disoproxil fumarate (PMPA) and lamivudine (3TC) [86].

Modern day ART therapeutics are commonly administered in combination with each other based on their CNS penetration effectiveness (CPE) scores. A higher CPE score indicates a greater ability to penetrate the blood-brain barrier (BBB), while a lower score suggests limited penetration. However, the potential risks associated with higher CPE scores and consequently higher concentrations of ART drugs remain uncertain. Nevertheless, recent clinical trials havenot observed any impact of CPE score on neurocognitive impairment [87]. For antiretroviral-naïve patients with HIV, therapeutic regimens typically consist of three combined medications from at least two distinct drug classes. These regimens usually include two nucleoside reverse transcriptase inhibitors and one additional antiretroviral therapeutic [88]. These antiretroviral drugs are categorized into eight groups, each targeting a specific stage in the lifecycle of HIV.

The identification of viral reservoirs in the central nervous system suggests that the current antiretroviral therapy may not be effectively penetrating the blood-brain barrier. Consequently, the required therapeutic levels within the CNS may not be achieved to combat latent viruses. Furthermore, these viruses have been observed to undergo clonal expansion, which is not addressed by existing treatments. This is particularly relevant in the case of HIV [89].

The RNA genome possesses a crucial long terminal repeat (LTR) promoter region that drives cell-associated HIV expression. Although certain ART regimens can suppress viral replication, they do not affect the functionality of the HIV LTR promoter region. Consequently, HIV proviruses can continue to express RNA, produce viral loads, and activate T cells, leading to clonal expansion, a significant therapeutic target. It is estimated that more than half of the cells inviral reservoirs are maintained through clonal expansion . Therefore, to ensure the effectiveness of ART, it is necessary to target these reservoirs to prevent clonal expansion into surrounding tissues while preserving the normal functioning of CNS cells [90].

Numerous studies have demonstrated that HIV viral reservoirs in the central nervous system (CNS) can continue to replicate due to inadequate accumulation of antiretroviral therapy (ART) drugs. Consequently, strains of HIV derived from mutations may emerge as a result of multidrug resistance or discontinuation of antiretroviral treatment [91, 92]. Consequently, these HIV strains may exhibit distinct viral genomes, making them challenging to target compared to the original HIV strains that can be effectively addressed by existing medications [93]. Ultimately, this can lead to a resurgence of viremia, resulting in de novo infection [94]. It is important to note that if patients prematurely discontinue the prescribed ART treatments, there will be a lack of targeting of viral reservoirs, making subsequent viremia and disease rebound almost inevitable. The extent of latent reservoir growth is still a subject of debate. Some studies suggest that there is no statistical significance in HIV-associated DNA, RNA, or infected cell levels before versus after treatment interruption; however, the presence of expanded clonal populations cannot be denied. Due to the evidence of viremia rebound, even after achieving undetectable viral plasma levels, reducing the intensity of ART regimens is risky and generally not recommended [95].

With an incomplete efficacy of antiretroviral therapy (ART), there is a potential occurrence of bystander damage, wherein HIV-related secondary mechanisms induce apoptosis in uninfected cells that are in close proximity to infected cells. This phenomenon has been extensively documented in CD4 T cells; however, it may also impact neurons and glia. Consequently, this process can trigger downstream pyroptosis and inflammatory reactions, leading to further comorbidities. Hence, the primary objectives of sustaining ART regimens encompass reducing immune activation and averting immune cell depletion, alongside their capacity to prevent viral rebound [96].

Nanomedicine serves as an effective means to traverse the blood-brain barrier (BBB) while simultaneously safeguarding the neurological integrity.

The administration of ART drugs orally results in reduced drug efficacy and bioavailability in thebrain due to hepatic first-pass metabolism and slow absorption. This necessitates a higher dosage and frequency of dosage to achieve the desired effects. Moreover, the BBB poses a significant challenge in targeting drugs to the brain. However, nanotechnology has emerged as a promising solution to overcome these obstacles by enhancing BBB transmigration, thereby improving drug delivery and minimizing loss of ART drug load to the brain. One potential approach involves using nanotechnology to optimize the shape and size of conventional ART drugs, resulting in a nanoparticle that can address drug solubility and permeability issues [97].

To achieve effective delivery of ART drugs across the blood-brain barrier (BBB), it is crucial to consider the size of the nanoparticles used. Nanoscale size considerations should be taken into account in order to successfully cross the BBB and achieve site-specific drug targeting. In the case of ART nanoparticles, it is recommended to formulate them to be smaller than the typical HIV virus, which has a diameter of approximately 100 nm [98]. For brain-specific drugs, nanoparticles should ideally be less than 120 nm in size and administered intranasally for direct delivery. By adhering to these size guidelines, ART nanoparticles can effectively traverse the BBB through transient pathways, without compromising the neurological integrity. This approach ensures that the innate therapeutic effects of the original drug are preserved. In addition to size considerations, there are other important factors that can limit the passage of drugs across the BBB [99].

Nanoparticles can be modified to enhance their functionality and target specific receptors in the brain. For example, a study utilized PLGA-coated elvitegravir nanoparticles to improve their ability to cross the blood-brain barrier (BBB), reduce inflammation at the brain interface, and effectively inhibit HIV replication. In another experiment, nanodiamonds with modified surfaces were loaded with efavirenz to facilitate BBB penetration. These nanodiamonds served as a safe and inert carbon material for drug delivery, prolonging the drug's availability in the central nervous system (CNS) without causing any harmful effects on neuronal plasticity [100]. The functionalized surface moieties on nanoparticles can also increase the binding surface area for drugs, thereby enhancing drug bioavailability. By improving bioavailability in specific regions, nanomedicine offers the potential to administer lower dosages of potentially neurotoxic ART drugs.

The potential to enhance the treatment of HIV in patients lies in the utilization of nanoparticles and nanodrugs as carriers for therapeutic administration. In rodent models, nanomedicine-based approaches have shown significant improvements in the delivery of antiretroviral therapy (ART), particularly through the uptake of ART drugs by macrophages [101-104]. Another approach, tested in mice, involves the use of long-acting slow-effective release (LASER) ART and CRISPR-Cas9 injections to deliver hydrophobic lipophilic ART nanoparticles into the body. Thismethod allows for gradual drug dissolution, macrophage uptake, and minimal off-target toxicity. A similar observation of macrophage uptake has been made with a long-acting dolutegravir prodrug encapsulated in a poloxamer nano-formulation [105].

To facilitate transmigration across the blood-brain barrier (BBB), nanoparticles utilizing a ferrous magnet-based liposome nanocarrier have demonstrated a 7.3-fold increase in transmigration. This effect is achieved through the synergistic support of transferrin receptors on the epithelium in vitro, without compromising BBB integrity. Additionally, the discovery of magnetic azidothymidine 5′-triphosphate (AZTTP) liposome has shown a threefold increase in transmigration across the BBB compared to free AZTTP. This is achieved by utilizing an external magnetic field to guide the nanoparticle to a specific site of interest. By combining this approach with the conjugation of ART, it may be possible to target sequestered viral genomes and reach brain reservoirs in a controlled, sustained, and nontoxic manner [106].

The use of ART nanoparticles is envisioned to preserve the inherent therapeutic and nontoxic

properties of the original drugs, while also increasing their bioavailability compared to traditional pharmacokinetic properties. Looking ahead, nanomedicine holds promise as a vehicle for drug delivery, not only for targeting HIV viral reservoirs within the brain but also for expanding to other neurological therapeutics aimed at treating brain-related diseases [107].

Concluding Remarks and Future Perspectives

HIV infection results in a swift invasion of immune cells, hindering the body's ability to combat diseases, ultimately leading to AIDS if not treated adequately. Currently, ART is the preferred method for suppressing actively replicating viral genomes in HIV-infected individuals. Although ART has extended the lifespan of patients with this disease, complete eradication of the virus remains elusive. ART has several unresolved limitations, including ineffective BBB penetration, failure to achieve necessary therapeutic concentrations within the CNS, and complete elimination of CNS HIV reservoirs, which can result in chronic HIV rebound viremia and reinfection. The presence of latent viral reservoirs within the brain that can potentiate HIV replication hasprompted the development of drugs aimed at specific targeting. Overcoming the BBB is a significant obstacle in drug delivery, leaving many questions as to how to approach targeting reserves in the brain [108].

Studies conducted in vitro, animal models, and clinical settings have provided valuable insights into the factors that hinder the complete suppression of HIV replication within the body, particularly in the central nervous system (CNS). A key focus in this field is the development of innovative techniques to enhance the delivery of antiretroviral therapy (ART) drugs across the blood-brain barrier (BBB), thereby improving targeted drug efficacy. One potential approach to consider is the augmentation of BBB penetration by FDA-approved ART agents, while ensuring the preservation of normal brain function through the utilization of nanomedicine strategies. In a broader context, nanomedicine has emerged as a crucial tool in the pursuit of HIV vaccines, microbicides, diagnostics, and therapeutics [109].

References

[1] Osborne O, Peyravian N, Nair M, Daunert S, Toborek M. The paradox of HIV blood–brain barrier penetrance and antiretroviral drug delivery deficiencies. Trends in neurosciences. 2020 Sep 1;43(9):695-708.

[2] Phillips AN, Leen C, Wilson A, Anderson J, Dunn D, Schwenk A, Orkin C, Hill T, Fisher M, Walsh J, Pillay D. Risk of extensive virological failure to the three original antiretroviral drug classes over long-term follow-up from the start of therapy in patients with HIV infection: an observational cohort study. The Lancet. 2007 Dec 8;370(9603):1923-8.

[3] Bhaskaran K, Hamouda O, Sannes M, Boufassa F, Johnson AM, Lambert PC, Porter K, Cascade Collaboration. Changes in the risk of death after HIV seroconversion compared with mortality in the general population. Jama. 2008 Jul 2;300(1):51-9.

[4] Schacker TW, Nguyen PL, Beilman GJ, Wolinsky S, Larson M, Reilly C, Haase AT. Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. The Journal of clinical investigation. 2002 Oct 15;110(8):1133-9.

[5] Losina E, Schackman BR, Sadownik SN, Gebo KA, Walensky RP, Chiosi JJ, Weinstein MC, Hicks PL, Aaronson WH, Moore RD, Paltiel AD. Racial and sex disparities in life expectancy losses among HIV-infected persons in the United States: impact of riskbehavior, late initiation, and early discontinuation of antiretroviral therapy. Clinical Infectious Diseases. 2009 Nov 15;49(10):1570-8.

[6] Hammer SM, Eron JJ, Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P, Fischl MA, Gatell JM, Hirsch MS, Jacobsen DM. Antiretroviral treatment of adult HIV

[7] infection: 2008 recommendations of the International AIDS Society–USA panel. Jama. 2008 Aug 6;300(5):555-70.

[8] Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C. Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune defficiency syndrome (AIDS). Revista de investigación clínica. 2004;56(2):126-9.

[9] Kitahata MM, Gange SJ, Abraham AG, Merriman B, Saag MS, Justice AC, Hogg RS, Deeks SG, Eron

JJ, Brooks JT, Rourke SB. Effect of early versus deferred antiretroviral therapy for HIV on survival. New England Journal of Medicine. 2009 Apr 30;360(18):1815-26.

[10] Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, Boden D, Racz P, Markowitz M. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. The Journal of experimental medicine. 2004 Sep 20;200(6):761-70.

[11] Hecht FM, Hartogensis W, Bragg L, Bacchetti P, Atchison R, Grant R, Barbour J, Deeks SG. HIV RNA level in early infection is predicted by viral load in the transmission source. AIDS (London, England). 2010 Apr 4;24(7):941.

[12] Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, Baril JG, Thomas R, Rouleau D, Bruneau J, Leblanc R. High rates of forward transmission events afteracute/early HIV-1 infection. The Journal of infectious diseases. 2007 Apr 1;195(7):951-9.

[13] Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, Kempf DJ,Mellors JW, Coffin JM, King MS. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proceedings of the National Academy of Sciences. 2008 Mar 11;105(10):3879-84.

[14] Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nature medicine. 2006Mar 1;12(3):289-95.

[15] Deeks SG, Hoh R, Neilands TB, Liegler T, Aweeka F, Petropoulos CJ, Grant RM, Martin JN. Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection. Journal of Infectious Diseases. 2005 Nov 1;192(9):1537-44.

[16] Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature medicine. 2006 Dec 1;12(12):1365-71.

[17] Paredes R, Lalama CM, Ribaudo HJ, Schackman BR, Shikuma C, Giguel F, Meyer III WA, Johnson VA, Fiscus SA, D'Aquila RT, Gulick RM. Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure. The Journal of infectious diseases. 2010 Mar 1;201(5):662-71.

[18] Gutiérrez C, Hernández-Novoa B, Pérez-Elías MJ, Moreno AM, Holguín Á, Dronda F, Casado JL, Moreno S. Prevalence of primary resistance mutations to integrase inhibitors in treatment-naive and-experienced patients infected with B and non-B HIV-1 variants. HIV Clinical Trials. 2013 Feb 1;14(1):10-6.

[19] Antinori A, Di Biagio A, Marcotullio S, Andreoni M, Chirianni A, d'Arminio Monforte A, Galli M, Mazzotta F, Mussini C, Puoti M, Lazzarin A. Italian guidelines for the use of antiretroviral agents and the diagnostic-clinical management of HIV-1 infected persons: update 2016. New Microbiologica. 2017;40(2):86-98.

[20] Antinori A, Marcotullio S, Andreoni M, Chirianni A, d'Arminio Monforte A, Di Biagio A, Galli M, Mazzotta F, Mussini C, Puoti M, Lazzarin A. Italian guidelines for the use of antiretroviral agents and the diagnostic-clinical management of HIV-1 infected persons. Update 2015. New Microbiol. 2016 Apr 1;39(2):93-109.

[21] Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, Leechanachai P, Ariyadej S, Leenasirimakul P, Hammer S, Lallemant M. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. New England Journal of Medicine. 2004 Jul 15;351(3):229-40.

[22] Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, Carr A, STEAL Study Group. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-Lamivudine: a randomized, 96-week trial. Clinical Infectious Diseases. 2009 Nov 15;49(10):1591-601.

[23] Eshleman SH, Mracna M, Guay LA, Deseyve M, Cunningham S, Mirochnick M, MusokeP, Fleming T, Fowler MG, Mofenson LM, Mmiro F. Selection and fading of resistance

[24] mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). Aids. 2001 Oct 19;15(15):1951-7.

[25] Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, Koup RA, Mellors JW, Connick E, Conway B, Kilby M. Antiretroviral-drug resistance among patients recently infected with HIV. New England Journal of Medicine. 2002 Aug 8;347(6):385-94.

[26] Wensing AM, Van De Vijver DA, Angarano G, Åsjö B, Balotta C, Boeri E, Camacho R, Chaix ML, Costagliola D, De Luca A, Derdelinckx I. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. The Journal of infectious diseases. 2005 Sep 15;192(6):958-66.

[27] Ferry T, Raffi F, Collin-Filleul F, Dupon M, Dellamonica P, Waldner A, Strady C, ChêneG, Leport C, Le Moing V. Uncontrolled viral replication as a risk factor for non-AIDS severe clinical events in HIV-infected patients on long-term antiretroviral therapy: APROCO/COPILOTE (ANRS CO8) cohort study. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2009 Aug 1;51(4):407-15.

[28] Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomažič J, Jägel-Guedes E, Rugina S, Kozyrev O, Cid JF, Hay P. HLA-B* 5701 screening for hypersensitivity to abacavir. New England Journal of Medicine. 2008 Feb 7;358(6):568-79.

[29] Grund B, Peng G, Gibert CL, Hoy JF, Isaksson RL, Shlay JC, Martinez E, Reiss P, Visnegarwala F, Carr AD. Continuous antiretroviral therapy decreases bone mineral density. AIDS (London, England). 2009 Jul 7;23(12):1519.

[30] Egger M, May M, Chêne G, Phillips AN, Ledergerber B, Dabis F, Costagliola D,Monforte AD, De Wolf F, Reiss P, Lundgren JD. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. The Lancet. 2002 Jul 13;360(9327):119-29.

[31] When To Start Consortium. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. The Lancet. 2009 Apr 18;373(9672):1352-63.

[32] Bertrand L, Nair M, Toborek M. Solving the blood-brain barrier challenge for the effective treatment of HIV replication in the central nervous system. Current pharmaceutical design. 2016 Oct 1;22(35):5477-86.

[33] Gupta S, Kesarla R, Omri A. Approaches for CNS delivery of drugs–nose to brain targeting of antiretroviral agents as a potential attempt for complete elimination of major reservoir site of HIV to aid AIDS treatment. Expert Opinion on Drug Delivery. 2019 Mar4;16(3):287-300.

[34] Pomerantz RJ. Reservoirs of human immunodeficiency virus type 1: the main obstacles to viral eradication. Clinical infectious diseases. 2002 Jan 1;34(1):91-7.

[35] Shan L, Siliciano RF. From reactivation of latent HIV-1 to elimination of the latent

[36] reservoir: the presence of multiple barriers to viral eradication. Bioessays. 2013 Jun;35(6):544-52.

[37] Petito CK, Cash KS. Blood-brain barrier abnormalities in acquired immunodeficiency

[38] syndrome: Immunohistochemical localization of serum proteins in postmortem brain. Annals of neurology. 1992 Nov;32(5):658-66.

[39] Strazza M, Pirrone V, Wigdahl B, Nonnemacher MR. Breaking down the barrier: the effects of HIV-1 on the blood–brain barrier. Brain research. 2011 Jul 5;1399:96-115.

[40] Eugenin EA, Clements JE, Zink MC, Berman JW. Human immunodeficiency virusinfection of human astrocytes disrupts blood–brain barrier integrity by a gap junction-dependent mechanism. Journal of Neuroscience. 2011 Jun 29;31(26):9456-65.

[41] Castro-Gonzalez S, Colomer-Lluch M, Serra-Moreno R. Barriers for HIV cure: the latentreservoir. AIDS research and human retroviruses. 2018 Sep 1;34(9):739-59.

[42] Corsi F, Sorrentino L, Mazzucchelli S, Truffi M, Capetti A, Rizzardini G, Fiandra L. Antiretroviral therapy through barriers: a prominent role for nanotechnology in HIV-1 eradication from sanctuaries. Journal of Pharmacy and Pharmacology. 2016;2016(4):328-40.

[43] Spudich S, Peterson J, Fuchs D, Price RW, Gisslen M. Potential for early antiretroviral therapy to reduce central nervous system HIV-1 persistence. Aids. 2019 Dec 1;33:S135-44.

[44] Dahm T, Rudolph H, Schwerk C, Schroten H, Tenenbaum T. Neuroinvasion and inflammation in viral central nervous system infections. Mediators of inflammation. 2016Oct;2016.

[45] Dallasta LM, Pisarov LA, Esplen JE, Werley JV, Moses AV, Nelson JA, Achim CL. Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. The American journal of pathology. 1999 Dec 1;155(6):1915-27.

[46] Thomas SA. Anti-HIV drug distribution to the central nervous system. Current pharmaceutical design. 2004 May 1;10(12):1313-24.

[47] Cao S, Woodrow KA. Nanotechnology approaches to eradicating HIV reservoirs. European Journal of

Pharmaceutics and Biopharmaceutics. 2019 May 1;138:48-63.

[48] Kapoor A, Tan CS. Immunotherapeutics to treat HIV in the central nervous system. Current HIV/AIDS Reports. 2020 Oct;17:499-506.

[49] Gazzard BG, Anderson J, Babiker A, Boffito M, Brook G, Brough G, Churchill D, Cromarty B, Das S, Fisher M, Freedman A. British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. HIV medicine. 2008 Oct;9(8):563-608.

[50] Riddler SA, Haubrich R, DiRienzo AG, Peeples L, Powderly WG, Klingman KL, Garren KW, George T, Rooney JF, Brizz B, Lalloo UG. Class-sparing regimens for initial treatment of HIV-1 infection. New England Journal of Medicine. 2008 May 15;358(20):2095-106.

[51] Elzi L, Marzolini C, Furrer H, Ledergerber B, Cavassini M, Hirschel B, Vernazza P, Bernasconi E, Weber R, Battegay M, Swiss HIV Cohort Study. Treatment modification in human immunodeficiency virus–infected individuals starting combination antiretroviral therapy between 2005 and 2008. Archives of internal medicine. 2010 Jan 1;170(1):57-65.

[52] Di Biagio A, Cozzi-Lepri A, Prinapori R, Angarano G, Gori A, Quirino T, De Luca A, Costantini A, Mussini C, Rizzardini G, Castagna A. Discontinuation of initialantiretroviral therapy in clinical practice: moving toward individualized therapy. Journal of acquired immune deficiency syndromes (1999). 2016 Mar 3;71(3):263.

[53] Shubber Z, Calmy A, Andrieux-Meyer I, Vitoria M, Renaud-Thery F, Shaffer N, Hargreaves S, Mills EJ, Ford N. Adverse events associated with nevirapine and efavirenz-based first-line antiretroviral therapy: a systematic review and meta-analysis. Aids. 2013 Jun 1;27(9):1403-12.

[54] Anlay DZ, Alemayehu ZA, Dachew BA. Rate of initial highly active anti-retroviral therapy regimen change and its predictors among adult HIV patients at University of

[55] Gondar Referral Hospital, Northwest Ethiopia: a retrospective follow up study. AIDS research and therapy. 2016 Dec;13:1-8.

[56] Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, Asher AI, Deeks SG. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. The Journal of infectious diseases. 2009 Apr 15;199(8):1177-85.

[57] Mannheimer SB, Matts J, Telzak E, Chesney M, Child C, Wu AW, Friedland G, Terry Beirn Community Programs for Clinical Research on AIDS. Quality of life in HIV-infected individuals receiving antiretroviral therapy is related to adherence. AIDS care. 2005 Jan 1;17(1):10-22.

[58] Schacker TW, Reilly C, Beilman GJ, Taylor J, Skarda D, Krason D, Larson M, Haase AT. Amount of lymphatic tissue fibrosis in HIV infection predicts magnitude of HAART-associated change in peripheral CD4 cell count. Aids. 2005 Dec 2;19(18):2169-71.

[59] Gulick RM, Lalezari J, Goodrich J, Clumeck N, DeJesus E, Horban A, Nadler J, Clotet B, Karlsson A, Wohlfeiler M, Montana JB. Maraviroc for previously treated patients with R5 HIV-1 infection. New England Journal of Medicine. 2008 Oct 2;359(14):1429-41.

[60] Obel N, Thomsen HF, Kronborg G, Larsen CS, Hildebrandt PR, Sørensen HT, Gerstoft J. Ischemic heart disease in HIV-infected and HIV-uninfected individuals: a population-based cohort study. Clinical Infectious Diseases. 2007 Jun 15;44(12):1625-31.

[61] Lewden C, Chêne G, Morlat P, Raffi F, Dupon M, Dellamonica P, Pellegrin JL, Katlama C, Dabis F, Leport C. HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2007Sep 1;46(1):72-7.

[62] Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large US healthcare system. The Journal of Clinical Endocrinology & Metabolism. 2008 Sep1;93(9):3499-504.

[63] Gandhi RT, Spritzler J, Chan E, Asmuth DM, Rodriguez B, Merigan TC, Hirsch MS, Shafer RW, Robbins GK, Pollard RB. Effect of baseline-and treatment-related factors on

[64] immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from ACTG 384. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2006 Aug 1;42(4):426-34.

[65] Ances BM, Vaida F, Yeh MJ, Liang CL, Buxton RB, Letendre S, McCutchan JA, Ellis RJ, HIV

Neurobehavioral Research Center. HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging. The Journal of infectious diseases. 2010 Feb 1;201(3):336-40.

[66] Weber R, Friis-Moller N, Sabin CA, Reiss P, D'Arminio-Monforte A, Dabis F. Liver-related deaths among HIV-infected persons: data from the D: A: D study. Arch Intern Med. 2006;166(15):1632-41.

[67] Hsue PY, Hunt PW, Schnell A, Kalapus SC, Hoh R, Ganz P, Martin JN, Deeks SG. Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. AIDS (London, England). 2009 Jun 6;23(9):1059.

[68] Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nature medicine. 2003 Jun 1;9(6):727-8.

[69] Reiss P. The art of managing human immunodeficiency virus infection: a balancing act. Clinical infectious diseases. 2009 Nov 15;49(10):1602-4.

[70] Chun TW, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, O'Shea MA, Hallahan CW, Daucher M, Ward DJ, Moir S. HIV-infected individuals receiving effectiveantiviral therapy for extended periods of time continually replenish their viral reservoir. The Journal of clinical investigation. 2005 Nov 1;115(11):3250-5.

[71] Woods SP, Moore DJ, Weber E, Grant I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. Neuropsychology review. 2009 Jun;19(2):152-68.

[72] Joos B, Fischer M, Kuster H, Pillai SK, Wong JK, Böni J, Hirschel B, Weber R, Trkola A, Günthard HF, Swiss HIV Cohort Study 2. HIV rebounds from latently infected cells, rather than from continuing low-level replication. Proceedings of the National Academy of Sciences. 2008 Oct 28;105(43):16725-30.

[73] Wood E, Kerr T, Marshall BD, Li K, Zhang R, Hogg RS, Harrigan PR, Montaner JS. Longitudinal community plasma HIV-1 RNA concentrations and incidence of HIV-1 among injecting drug users: prospective cohort study. Bmj. 2009 Apr 30;338.

[74] Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, MeehanMO, Lutalo T, Gray RH. Viral load and heterosexual transmission of humanimmunodeficiency virus type 1. New England journal of medicine. 2000 Mar30;342(13):921-9.

[75] Dieffenbach CW, Fauci AS. Universal voluntary testing and treatment for prevention of HIV transmission. Jama. 2009 Jun 10;301(22):2380-2.

[76] Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, Quinn TC. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nature medicine. 1999 May;5(5):512-7.

[77] Fauci AS, Folkers GK. Investing to meet the scientific challenges of HIV/AIDS. Health Affairs. 2009 Nov;28(6):1629-41.

[78] Hatano H, Lampiris H, Fransen S, Gupta S, Huang W, Hoh R, Martin JN, Lalezari J, Bangsberg D, Petropoulos C, Deeks SG. Evolution of integrase resistance during failure of integrase inhibitor-based antiretroviral therapy. Journal of acquired immune deficiencysyndromes (1999). 2010 Aug 8;54(4):389.

[79] Hawkins C, Chaplin B, Idoko J, Ekong E, Adewole I, Gashau W, Murphy RL, Kanki P, Plus AP, Harvard PEPFAR Team. Clinical and genotypic findings in HIV-infected patients with the K65R mutation failing first line antiretroviral therapy in Nigeria. Journal of acquired immune deficiency syndromes (1999). 2009 Oct 10;52(2):228.

[80] Quiroz Im. Morbilidad, Mortalidad Y Falla Al Tratamiento Antirretroviral En Adolescentes Con Vih/Sida De Acuerdo Al Mecanismo De Adquisición Del Virus (Doctoral Dissertation, Universidad Central De Venezuela).

[81] Wong HL, Chattopadhyay N, Wu XY, Bendayan R. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. Advanced drug delivery reviews. 2010 Mar 18;62(4-5):503-17.

[82] Mahajan SD, Aalinkeel R, Law WC, Reynolds JL, Nair BB, Sykes DE, Yong KT, Roy I, Prasad PN, Schwartz SA. Anti-HIV-1 nanotherapeutics: promises and challenges for the future. International journal of nanomedicine. 2012 Oct 5:5301-14.

[83] Vyas TK, Shahiwala A, Amiji MM. Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. International journal of pharmaceutics. 2008 Jan

22;347(1-2):93-101.

[84] Saiyed ZM, Gandhi NH, Nair MP. Magnetic nanoformulation of azidothymidine 5'-triphosphate for targeted delivery across the blood–brain barrier. International journal of nanomedicine. 2010 Apr 7:157-66.

[85] Chiappetta DA, Hocht C, Opezzo JA, Sosnik A. Intranasal administration of antiretroviral-loaded micelles for anatomical targeting to the brain in HIV. Nanomedicine.2013 Feb;8(2):223-37.

[86] Boissé L, Gill MJ, Power C. HIV infection of the central nervous system: clinical features and neuropathogenesis. Neurologic clinics. 2008 Aug 1;26(3):799-819.

[87] Heaton RK, Franklin DR, Ellis RJ, McCutchan JA, Letendre SL, LeBlanc S, Corkran SH, Duarte NA, Clifford DB, Woods SP, Collier AC. HIV-associated neurocognitive disordersbefore and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. Journal of neurovirology. 2011 Feb;17:3-16.

[88] Kumar AM, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction. Journal of neurovirology. 2007 May;13:210-24.

[89] Haworth SJ, Christofalo B, Anderson RD, Dunkle LM. A single-dose study to assess the penetration of stavudine into human cerebrospinal fluid in adults. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology: Official Publication of the International Retrovirology Association. 1998 Mar 1;17(3):235-8.

[90] Bhaskar S, Tian F, Stoeger T, Kreyling W, de la Fuente JM, Grazú V, Borm P, Estrada G,Ntziachristos V, Razansky D. Multifunctional Nanocarriers for diagnostics, drug deliveryand targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. Particle and fibre toxicology. 2010 Dec;7(1):1-25.

[91] Antinori A, Perno CF, Giancola ML, Forbici F, Ippolito G, Hoetelmans RM, Piscitelli SC. Efficacy of cerebrospinal fluid (CSF)–penetrating antiretroviral drugs against HIV inthe neurological compartment: different patterns of phenotypic resistance in CSF and plasma. Clinical Infectious Diseases. 2005 Dec 15;41(12):1787-93.

[92] Calcagno A, Bonora S, Simiele M, Rostagno R, Tettoni MC, Bonasso M, Romito A, Imperiale D, D'Avolio A, Di Perri G. Tenofovir and emtricitabine cerebrospinal fluid-to-plasma ratios correlate to the extent of blood-brainbarrier damage. Aids. 2011 Jul17;25(11):1437-9.

[93] Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. International journal of nanomedicine. 2007 Dec 1;2(2):129-41.

[94] Nagpal K, Singh SK, Mishra DN. Drug targeting to brain: a systematic approach to study the factors, parameters and approaches for prediction of permeability of drugs across BBB. Expert opinion on drug delivery. 2013 Jul 1;10(7):927-55.

[95] Begley DJ. ABC transporters and the blood-brain barrier. Current pharmaceutical design. 2004 May 1;10(12):1295-312.

[96] Tiraboschi JM, Niubo J, Vila A, Perez-Pujol S, Podzamczer D. Etravirine concentrations in CSF in HIV-infected patients. Journal of antimicrobial chemotherapy. 2012 Jun 1;67(6):1446-8.

[97] Dauchy S, Dutheil F, Weaver RJ, Chassoux F, Daumas-Duport C, Couraud PO,

[98] Scherrmann JM, De Waziers I, Declèves X. ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood–brain barrier. Journal of neurochemistry. 2008 Dec;107(6):1518-28.

[99] Hartz A, Bauer B. Regulation of ABC transporters at the blood-brain barrier: new targets for CNS therapy. Molecular interventions. 2010 Oct 1;10(5):293.

[100] Aweeka F, Jayewardene A, Staprans S, Bellibas ES, Kearney B, Lizak P, Novakovic-Agopian T, Price RW. Failure to detect nelfinavir in the cerebrospinal fluid of HIV-1-infected patients with and without AIDS dementia complex. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1999 Jan 1;20(1):39-43.

[101] Lockman PR, Mumper RJ, Khan MA, Allen DD. Nanoparticle technology for drugdelivery across the blood-brain barrier. Drug development and industrial pharmacy. 2002 Jan 1;28(1):1-3.

[102] Gessner A, Lieske A, Paulke BR, Müller RH. Influence of surface charge density on protein adsorption

on polymeric nanoparticles: analysis by two-dimensional electrophoresis. European journal of pharmaceutics and biopharmaceutics. 2002 Sep1;54(2):165-70.

[103] Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. The Journal of clinical investigation. 1996 Jun 1;97(11):2517-24.

[104] Chen XP, Wang Q, Guan J, Huang ZY, Zhang WG, Zhang BX. Reversing multidrug resistance by RNA interference through the suppression of MDR1 gene in human hepatoma cells. World Journal of Gastroenterology: WJG. 2006 Jun 6;12(21):3332.

[105] Chattopadhyay N, Zastre J, Wong HL, Wu XY, Bendayan R. Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line. Pharmaceutical research. 2008 Oct;25:2262-71.

[106] Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, Alyautdin R.Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. Journal of drug targeting. 2002 Jan 1;10(4):317-25.

[107] Rao KS, Reddy MK, Horning JL, Labhasetwar V. TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. Biomaterials. 2008 Nov 1;29(33):4429-38.

[108] Gaillard PJ, Appeldoorn CC, Rip J, Dorland R, van der Pol SM, Kooij G, de Vries HE, Reijerkerk A. Enhanced brain delivery of liposomal methylprednisolone improved therapeutic efficacy in a model of neuroinflammation. Journal of controlled release. 2012 Dec 28;164(3):364-9.

[109] Mishra V, Mahor S, Rawat A, Gupta PN, Dubey P, Khatri K, Vyas SP. Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles. Journal of drug targeting. 2006 Jan 1;14(1):45-53.

[110] Dehouck B, Fenart L, Dehouck MP, Pierce A, Torpier G, Cecchelli R. A new function for the LDL receptor: transcytosis of LDL across the blood–brain barrier. The Journal of cell biology. 1997 Aug 25;138(4):877-89.

[111] Shegokar R, Jansch M, Singh KK, Müller RH. In vitro protein adsorption studies on nevirapine nanosuspensions for HIV/AIDS chemotherapy. Nanomedicine: Nanotechnology, Biology and Medicine. 2011 Jun 1;7(3):333-40.

[112] Nowacek AS, Miller RL, McMillan J, Kanmogne G, Kanmogne M, Mosley RL, Ma Z, Graham S, Chaubal M, Werling J, Rabinow B. NanoART synthesis, characterization, uptake, release and toxicology for human monocyte–macrophage drug delivery.Nanomedicine. 2009 Dec;4(8):903-17.

[113] Kanmogne GD, Singh S, Roy U, Liu X, McMillan J, Gorantla S, Balkundi S, Smith N, Alnouti Y, Gautam N, Zhou Y. Mononuclear phagocyte intercellular crosstalk facilitates transmission of cell-targeted nanoformulated antiretroviral drugs to human brain endothelial cells. International journal of nanomedicine. 2012 May 8:2373-88.

[114] Dou H, Grotepas CB, McMillan JM, Destache CJ, Chaubal M, Werling J, Kipp J, Rabinow B, Gendelman HE. Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuroAIDS. The Journal of immunology. 2009 Jul 1;183(1):661-9.

[115] Bressani RF, Nowacek AS, Singh S, Balkundi S, Rabinow B, McMillan J, Gendelman HE, Kanmogne GD. Pharmacotoxicology of monocyte-macrophage nanoformulated antiretroviral drug uptake and carriage. Nanotoxicology. 2011 Dec 1;5(4):592-605.

[116] Vilella A, Tosi G, Grabrucker AM, Ruozi B, Belletti D, Vandelli MA, Boeckers TM, Forni F, Zoli M. Insight on the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. Journal of Controlled Release.2014 Jan 28;174:195-201.