Human Immunodeficiency Virus and Latency Reversing Agents
A Path To Cure?

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**Background**

Human immunodeficiency virus (HIV), as its namesake implies, is a virus that ultimately causes a deficient immune system that can lead to Acquired Immune Deficiency Syndrome (AIDS). Since the discovery of this cytopathic virus in 1983, there have been many scientific advances in regards to its identification and treatment. In 1985, a diagnostic serologic test was developed, and shortly after, in 1987, antiretroviral drugs were introduced. Since these breakthroughs, further improvements in diagnosis and management have been made for individuals afflicted with HIV, including the revolutionary development of combination antiretroviral therapy (cART) in 1996. Despite these advances, in 2014, there were an estimated 1.2 million people in the United States living with HIV, and an estimated 44,073 new HIV diagnoses. Treatment with cART does not completely eradicate HIV, and interruption of therapy leads to prompt increase in viral load, therefore lifelong therapy is required for viral suppression. This viral rebound upon therapy cessation indicates the presence of an anatomical reserve where HIV continues to replicate, better known as latent reservoirs. These reservoirs are the main hindrance to complete viral remission, or cure. The purpose of this paper is to explore this problem and address the clinical question: Do latency-reversing agents (LRAs) eradicate human immunodeficiency virus (HIV) in patients with latent HIV on highly active antiretroviral therapy (HAART)? This paper will explore relevant epidemiology, pathophysiology, and innovative research on this topic and then address the question of LRAs' role in the eradication of HIV.
Epidemiology

The major modes of acquiring HIV infection include sexual transmission, parenteral transmission, and perinatal transmission. HIV transmission occurs through certain body fluids from an infected individual, such as blood, semen, pre-seminal fluid, rectal fluid, vaginal fluid, and breast milk. These body fluids must be exposed to a mucous membrane or injured tissue, or be directly injected into the bloodstream for HIV acquisition. The relative importance of these various modes of HIV transmission vary geographically, and are outlined below.

Sexual Transmission

Worldwide, more than 80% of HIV infections occur via heterosexual transmission, and over 50% of all HIV-infected individuals are women. This data reflects HIV infection in sub-Saharan Africa, which contributes to the majority of the world's infected population. In other parts of the world, more men than women are infected with HIV, which sheds light on the men who have sex with men (MSM) population. The MSM population is 19 times more likely than the general population to acquire HIV. In 2013, MSM transmission comprised 68% of new HIV diagnoses in the United States.

Parenteral Transmission

Injection drug use accounts for approximately 30% of new HIV diagnoses, outside of sub-Saharan Africa. In 2014, injection drug use made up 5% of new HIV infections in the United States.
**Perinatal Transmission**

Children are susceptible to HIV transmission in utero, at birth, or through breastmilk.⑦ Annually, greater than 2 million infants are born to HIV infected mothers.⑦

Since this paper will focus on research conducted in the United States, the following epidemiology will reflect statistics within this geographical location. In 2014, the number of deaths of individuals with an HIV diagnosis and AIDS, was 12,333.④ Cumulatively, the estimated number of deaths of individuals with HIV and AIDS through 2014, was 678,509, by any cause.⑤

AIDS is the fourth leading cause of death worldwide.⑦ This fact, along with the alarming statistics above, leads to discussion of the pathophysiology of the virus, and why HIV infection has sustained.

**Pathophysiology**

HIV infects and subsequently undergoes viral replication within T-lymphocytes expressing CD4 antigen. These CD4 positive cells are a critical component of normal cell-mediated immunity. After HIV acquisition, the individual, or host, is predisposed to various opportunistic infections, such as *Pneumocystis jiroveci (carinii)* pneumonia, and neoplasms, such as lymphoma and Kaposi’s sarcoma.⑥ ⑧

HIV also infects brain monocytes and macrophages, producing significant central nervous system symptoms via multinucleated giant cells. HIV may disrupt blood monocyte, tissue macrophage, and B-lymphocyte (humoral immunity) function, predisposing host to infection with encapsulated bacteria. The chief
immune response to HIV infections involves cytotoxic CD8 positive lymphocytes, which response to initial infection, and regulate it for many years. Mutant HIV strains are controlled by proliferation of new clones of cytotoxic T cells. These cells will begin to fail when the majority of CD4 T cells have died, thus diminishing the supply of lymphokines essential to activate the cytotoxic T cells. This failure inevitably leads to AIDS.⁶

Three mechanisms provide explanation why HIV infection persists despite the immune system: (1) integration of viral DNA into host cell DNA, (2) a high mutation rate (3) the production of specific proteins that downregulate class I MHC proteins needed for cytotoxic T cells to recognize and kill cells infected with HIV.⁶

Clinically, HIV infection can be divided into three stages, each of which is outlined below.

**Early, acute stage**

Acute HIV begins one to four weeks after infection, when patients may experience a symptomatic mononucleosis-like syndrome of lethargy, fever, sore throat, and generalized lymphadenopathy. A maculopapular rash of the trunk and extremities may also be seen. At a cellular level, acute HIV is accompanied by an increase in viral replication, and a decrease in CD4 cell count. Infection is established by high-level viremia, in the absence of HIV antibody. Once the acute stage resolves in about two weeks, HIV RNA decreases slightly, and CD8 positive T cells directed against HIV increase.⁶

Infected individuals undergo seroconversion three to four weeks after infection, or have a positive HIV antibody test. This period of time where HIV
antibody is undetectable, may lead to false negative results when attempting to confirm HIV infection. These patients are still able to transmit infection, and if diagnostic suspicion is high, serum should be sent for HIV RNA PCR.\textsuperscript{6}

Approximately 87\% of individuals who become seropositive during acute infection, are symptomatic, and about 13\% will experience an asymptomatic initial infection.\textsuperscript{6} Asymptomatic HIV is accompanied by a gradual decrease in CD4 cell counts, and viremia levels out to a “set point”, or a relatively stable viral load. A viral set point, typically established by approximately 6 months of infection, can vary from person to person, and signifies the amount of virus produced, which remains stable for years. The set point can be an important piece of information when evaluating patients with HIV. After initial infection, the higher the set point, the more likely the individual will progress to symptomatic AIDS.\textsuperscript{6}

\textbf{Chronic HIV infection without AIDS}

This stage is characterized by viral load stability, and gradual decline in CD4 cell count. In untreated patients, this latent period typically lasts 8-10 years.\textsuperscript{3} The majority of patients within in this stage of infection are asymptomatic, although many patients will present with generalized lymphadenopathy on physical exam.\textsuperscript{3}

As the CD4 cell count decreases, a syndrome termed as AIDS-related complex (ARC) can occur during this stage. This syndrome consists of persistent fevers, fatigue, weight loss, and lymphadenopathy. This syndrome is important to recognize, because ARC often progresses to AIDS.\textsuperscript{6}

This stage is of particular interest in untreated patients because cell death and replacement are in close balance. Although HIV is replicating at a high rate, and
CD4 cells are being destroyed, the CD4 cell count decline is slow, and the viral load is relatively stable. This is because a large amount of HIV production is occurring within lymph node cells, but remains sequestered here, thus the lymphoid tissue is a major reservoir for HIV. As the disease progresses, the lymph node tissue is disturbed, and increased amounts of HIV is peripherally released into the bloodstream.\textsuperscript{3,6}

**AIDS and advanced HIV infection**

Chronic HIV infection without treatment transitions to AIDS when the CD4 cell count is below 200 cells/microL, and/or the presence of opportunistic infections. These opportunistic infections are often referred to as AIDS defining conditions, and include *Pneumocystis jiroveci* pneumonia, tuberculosis, cryptococcal meningitis, candidal esophagitis, central nervous system toxoplasmosis, and non-Hodgkin's lymphoma. Most AIDS-related deaths occur when the CD4 cell count falls below 50 cells/microL, and deaths are usually related to late stage opportunistic infections, such as disseminated *Mycobacterium avium* complex (MAC), or cytomegalovirus disease.\textsuperscript{3,6,8}

This complex pathophysiology leads to the discussion of why HIV eradication is difficult, despite medical, and pharmaceutical advances. One barrier to complete HIV suppression is latently infected reservoirs. Latently infected reservoirs are defined as cells that contain the integrated HIV genome, are transcriptionally silent, but able to virally replicate. The main cellular reservoirs of HIV are resting CD4 positive T-cells. Other types of reservoirs include peripheral blood monocytes, dendritic cells, and macrophages, including the microglial cells with are central
nervous system macrophages. The reason why these reservoirs serve as an obstacle to HIV eradication is their location, which is referred to as “sanctuaries”. These sanctuaries, including the male genital tract, adipose tissue, lymph nodes, and the central nervous system, are areas of poor cART penetration, and ultimately efficacy. Various reservoirs other than CD4 T cells, such as macrophages, exist within the immune privileged area of the brain. The central nervous system is considered a sanctuary for many reasons. The blood brain barrier and choroid plexus serve as obstacles for drug penetration of currently used cART. Also, the target reservoirs of the brain are astrocytes and microglial cells, which many existing cART do not target.10

Innovative research on how to, essentially “shock”, latent HIV out of these reservoirs, led to latency reversing agents (LRAs). Originally histone deacetylase (HDAC) inhibitors were developed as oncologic agents, due to HDACs importance in transcriptional regulation, and cell death. Research has led to use of HDAC inhibitors as LRAs, by reactivating HIV secondary to transcriptional induction, and is the main LRA examined in this paper. Other LRAs include histone methyltransferase inhibitors, disulfiram, protein kinase C agonists, proteasome inhibitors, and Toll-like receptor 7 agonists.10

**Methods**

Search strategy was primarily conducted electronically. The databases PubMed and Google Scholar were searched from 2007-2017 for articles in English. The search terms that were utilized in these databases were as follows: eradication,
HIV, latency, reservoir, cure, remission, shock, kill, reversal, vorinostat, and latency reversing agent.

Exclusion criteria applied to the search results included studies conducted outside the United States. Also, studies that evaluated subjects that were not virally suppressed, or were treatment naïve, were excluded. Inclusion criteria were set for abstracts of populations that were HIV infected, aviremic, and cART treated. Further inclusion criteria included trials observing ex vivo response to LRAs.

A bias evaluation was performed for all trials utilizing the Cochrane Risk of Bias Tool. Selection bias for all trials was low, since all patients and samples were de-identified. No bias was present in selection of participants of study, although patients recruited for all trials had to be HIV-infected, and receiving stable standard-of-care cART, with plasma HIV RNA levels below 50 copies/mL. Although participants and trial personnel were aware of assigned intervention for all trials, there were no deviations from intended interventions, producing a low performance and detection bias. Attrition bias was also low for all trials, as patients and their samples were monitored and followed closely, and only excluded from progressing in the trials if a statistically significant response was not produced. Additional epidemiological and pathophysiological data was acquired through search and review of clinical decision resource UpToDate.

**Results**

The data acquisition methods described above yielded a total of 10 abstracts, and 3 were included for discussion in this review.
Archin, et al’s study, “Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency”, is a phase I-II single-center study that will demonstrate how LRAs operate, ex vivo, and in vivo. This study used vorinostat, an HDAC inhibitor, as its LRA, along with the principle that repeated disturbance of latency is required for successful total HIV RNA suppression. The study evaluated effective LRA dosing regimens that would cause repeated HIV RNA expression, and therefore allow treatment of persistent HIV infection.²

The study enrolled 16 HIV infected, aviremic, cART treated subjects to determine the optimal in vivo vorinostat dosing schedule required for effective successive disruption of CD4 positive T cells. The vorinostat dose used, 400mg, was derived from oncology studies, which demonstrated maximal efficacy with minimal drug toxicities, including transient thrombocytopenia, and gastrointestinal symptoms. Leukapheresis evaluation was performed on each subject’s sample, drawing out CD4 T cells, which were then tested with a dose of vorinostat. HIV RNA production was measured to see if the subject’s cells reacted to the LRA, and only those that did respond, were included in the next leg of the study. Further ex vivo analysis was conducted, resulting in data suggesting that daily LRA exposure blunts the viral response, and that paired doses at either 48 or 72 hours should be administered for maximum latency reversal.²

Only participants with a measurable increase in HIV RNA after ex vivo exposure to vorinostat – 12 out of 16 - were administered a single dose of in vivo LRA. Out of the 12 participants that received an in vivo dose of vorinostat, only 7 had a significant increase in HIV RNA isolated from CD4 T cells, with no increase in
the remaining 4 participants. From the ex vivo dosing data, the first participant out of the 7, received a paired dose at a 48 hour interval. This did not result in HIV RNA increase of statistical significance. The participant subsequently received a paired dose of vorinostat at the 72-hour interval, which resulted in a significant increase of HIV RNA produced by CD4 T cells.²

Due to the increased efficacy of the 72-hour interval dosing in vivo in the previous participant, and toxicity concerns related to alternate day dosing of vorinostat, the remaining 6 participants received paired dosing at the 72-hour interval. A significant response was measured in 5 out of 6 participants. Ultimately 3 out of these 5 participants chose to receive 10 consecutive doses of vorinostat at 72 hour intervals, to evaluate if this pulsatile, and serial, LRA exposure would activate persistent HIV infection. Out of these 3 participants, 2 showed a statistically significant prolonged elevation of HIV RNA. See Figure 1 for summarized outline of how subjects progressed through study.²
Throughout the study, latent HIV within the CD4 T cell reservoir was measured using a quantitative viral outgrowth assay (QVOA) at baseline, and following paired, and consecutive vorinostat dosing. From this data, no significant
reduction in latent virus recovered with QVOA was observed, along with no change in HIV RNA after in vivo vorinostat. Therefore the study found that multiple doses of vorinostat minimally impacts reservoir cells in vivo.²

The use of multiple LRAs, with different mechanisms of action, is examined in Albert, Ramani et al’s study “Combinations of isoform-target histone deacetylase inhibitors and bryostatin analogues display remarkable potency to active latent HIV without global T-cell activation.” A critical deficiency of HDAC inhibitors is lack of specificity, resulting in pan-inhibition of HDAC classes, and subsequently unwanted side effects. Side effects include fatigue, nausea, diarrhea, prolonged QT interval, thrombocytopenia, lymphopenia, and neutropenia. This study compared an isoform-targeted compound of an HDAC inhibitor, largazole, with other HDAC inhibitors, including vorinostat. The isoform compound is described to be more specific for HIV latency reversal, than its other HDAC inhibitor counterparts, producing decreased side effects. Comparison was conducted ex vivo on T-cell lines, and using fluorescence activating sorting (FACS) analysis. In short, stronger HIV reactivation – the desired result – will generate a more intense fluorescent signal. Three various isoforms of largazole demonstrated superior latent HIV reactivation, compared to 15 other HDAC inhibitors.¹

Largazole was then combined with a different LRA, bryolog, to evaluate potential synergistic effect. Bryolog was also combined with the other 15 HDAC inhibitors for comparison. This was conducted ex vivo, on T-cell lines. It was found that the largazole combinations with bryolog demonstrated unprecedented synergistic potency has HIV LRAs. This combination was further evaluated for
toxicity related to cytokine release, and it was found that this synergistic combination avoided global T-cell activation. This was done by incubating T cells with the LRA combination, and using ELISA to evaluate cytokine release.¹

These study findings lead to analysis of “On the Way to Find a Cure: Purging Latent HIV-1 Reservoirs”, by Schwartz et. al, to demonstrate limitations of LRAs used as the only mechanism in attempt to clear persistent HIV infection. This study describes how many of the viral reservoirs are located in sanctuaries, or “immune privileged” areas of poor cART efficacy, and drug penetration. As described previously, these sanctuaries include the male genital tract, adipose tissue, lymph nodes, and the central nervous system. This article is highlighted here in order to demonstrate the need to consider the type and dispersal of latently infected cells, in order to most effectively reduce the HIV reservoirs. For example, ex vivo analysis of CD4 T cells in response to LRAs, may provide insight into if latent virus exists and is able to reactivate, but does not shed light on if these reservoirs are located in sanctuaries.¹⁰

Overall, the results from these studies demonstrate that use of a single LRA will not achieve complete latency reversal. A synergistic approach using various mechanisms of action that target HIV reservoirs is required. These results prove that combination LRAs over multiple courses is necessary for persistent HIV infection. Additional in vivo research needs to be explored from this mainly ex vivo analysis, to further evaluate if combination LRAs will penetrate sanctuaries, as well as, produce minimal adverse effects.
Discussion

From this analysis, it is evident that a multifaceted strategy must be utilized in order for complete latent HIV reactivation, and elimination in poor drug delivery sites.

In regards to the study conducted by Archin et. al, the number of participants evaluated was very limited, but the results may be useful in future guidance of vorinostat dosing, and study design, since the idea of LRAs is relatively new. The results of this study, that no significant depletion of viral reservoir was observed, could be due to many factors. One factor is that the number of LRA doses the participants received may not be enough for measurable viral reservoir decline. Another factor, which was discussed in the second article, is that latently infected reservoirs in sanctuaries, are most likely inadequately able to clear virus due to poor cART penetration, and/or immune cell access.

Another limitation of Archin et. al discussed, is that the one participant out of the 3 that received 10 consecutive vorinostat doses, that did not demonstrate a statistically significant increase of HIV RNA from its CD4 T cells, could be due to LRA non-adherence. This participant reported difficulty with compliance to 72 hour interval dosing, which may have contributed to lack of significant response. How this altered dosing regimen affected viral response cannot be completely evaluated.

Due to the high mutation rate of HIV, cell mediated immune responses often fail, leading to another difficulty for complete viral eradication. This along with the presence of sanctuaries, necessitate combination therapies that enhance both cell-mediated and humoral immunity are necessary for effective reactivation of latent
HIV, along with therapies that are able to clear this persistent infection. In regards to humoral immunity, extensive research in vaccine development has been conducted since the discovery of HIV, but all clinical trials have failed to date. Innovative research on broadly neutralizing antibodies proves promising. These antibodies were discovered from HIV infected individuals, and can neutralize many HIV strain variants. These antibodies can be detected in approximately 25% of individuals with treatment naïve HIV infection, which suggests a host immune response to viral replication, and production of numerous viral variants. Cell-mediated immune responses targeting reduced cytotoxic T cell variants, and improved CD8 positive or natural killer cell responses, have to be also considered in the manifold approach of HIV eradication.\textsuperscript{10}

Schwartz et.al, goes on to further explain how LRAs are unable to significantly reduce the size of HIV reservoirs, due to the fact that despite increased latent HIV expression due to LRAs, this does not ultimately lead to the death of these infected cells. HIV also impairs the cytotoxic activity of CD8 positive T cells, which is not remedied by cART. This leads to discussion of a “shock and kill” strategy, where the LRA “shocks” the virus in reservoirs, and the patient’s immune system needs to somehow be boosted to “kill” the remaining virus. The “kill” is employed through both cell-mediated, and humoral immunity, which should be both augmented. This complicated strategy leads to further discussion of use of LRAs in the setting of persistent HIV infection.\textsuperscript{10}

Overall, further research on how to steer immune response to sanctuaries, or immune privileged areas where latently infected reservoirs are located, need to be
explored. The complex molecular mechanisms of dormant HIV require further research in order to create strategies to target sanctuaries, and reservoirs other than CD4 T cells. Boosting humoral immune response through further identification and classification of broadly neutralizing antibodies, and improving virus specific cytotoxic T cell mediated responses, are necessary for the multi-dimensional attack on HIV. Furthermore, use of one LRA which targets a single mechanism involved in latency, is unlikely to be effective in vivo.
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