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# A Review of the Advances in the Mechanisms used to Mitigate/Reverse HIV Latency

Review Article

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## Abstract

When HIV infects host immune cells, it inserts its genetic material into their DNA enabling the virus to use the cell's machinery to make copies of itself. Antiretroviral therapy for patients living with HIV results in successful viral suppression. Drug therapy doesn't however, lead to full eradication of infection and the virus continues to persist within a latent reservoir in resting memory CD4+ T cells and macrophages where the virus can hide for a long time without their genetic code being read to make protein, thus eluding the immune system's response and antiviral treatments. HIV infected patients must therefore stay on the drugs for life because once taken off therapy or if treatment fails, the virus hiding in the dormant host cells becomes reactivated and massively proliferates leading to disease progression. Overcoming latent virus reservoir remains the most significant obstacle to HIV cure. The focus in HIV/AIDS research is now turned toward the eradication of the virus in the latent reservoirs or silencing it by preventing its reactivation. However, therapeutic strategies to reactivate latent HIV-infected cells have so far remained elusive, because latency reversing agents either appear to lack sufficient potency or could trigger massive immune system activation which itself could be deadly. This review discusses recently researched mechanisms that could be used to reverse HIV latency or silence the latent virus as well as other approaches towards tackling HIV latency including biological control of HIV reservoirs; immunotherapy as well as seeking a better understanding of the science of latency.

**Keywords:** HIV Latency; HIV Reservoirs; Latency Reversal Agents; Antiretroviral Therapy; Shock and Kill.

**Abbreviations:** ART: Antiretroviral Therapy; LRAs: Latency-Reversing Agents; HDAC: Histone Deacetylases; HDACi: Histone Deacetylases Inhibitors; LTR: Long Terminal Repeat; LTNPs: Long Term Nonprogressors; bNAbs: Broadly Neutralizing Antibodies; Q-VOA: Quantitative Viral Outgrowth Assay; mTOR: Mammalian Target of Rapamycin.

## Introduction

Once it enters the human body, HIV inserts its genetic material into the DNA of the host immune cells. Doing this enables HIV to force the cell's machinery to make many copies of the virus. Antiretroviral therapy (ART) for patients living with HIV has resulted in successful suppression of the AIDS virus (HIV) and this medication has turned what was once a death sentence into a chronically managed disease. However, while drug therapy allows people living with HIV to lead a relatively normal life, it is not a cure as this treatment is insufficient to clear persistent infection, it does not lead to the full eradication of infection and the virus continues to persist within a latent reservoir in resting memory CD4+ T cells and macrophages. HIV latency is due to some HIV-infected immune cells going into a dormant or latent state and not making new virus. Latent HIV reservoirs are established during the earliest stage of HIV infection and throughout the course of the disease. The virus can hide in this latent reservoir in infected cells for a long time, even for several decades, without their genetic code being read to make protein or without any viral protein being expressed, and thus without becoming active and causing any noticeable symptoms, thus eluding the immune system's response and antiviral treatments. These HIV latency sanctuaries are seen as a deliberate survival tactic by the virus, since in them the virus goes undetected by the immune system and is also beyond the reach of even the most potent antiretroviral drugs which do not penetrate well to reach the virus. Thus HIV latency makes it nearly impossible for the virus to be targeted with antiretroviral

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drugs while allowing the same virus to largely avoid the immune system typically causing no symptoms. HIV infected patients must therefore stay on the drugs for life because if the people are taken off their antiretroviral therapies or if treatment fails, the virus hiding in the dormant host cells becomes reactivated and massively proliferates with the cell beginning to produce HIV again, leading to disease progression. This reactivation can occur at any time, which is why HIV treatment needs to be maintained for life.

Maintenance and replenishment of HIV reservoirs has been found to be due to an ongoing HIV replication in lymphoid tissues such as the lymph nodes. In a study, researchers sequenced viral DNA from lymph-node and blood cells collected from HIV-infected patients before and during the first six months of ART and found ongoing replication. This suggests that continued HIV replication in lymphoid tissue sanctuaries refills viral reservoirs in patients on ART who have achieved undetectable blood levels of HIV. The study further found that drug-sensitive HIV strains tend to dominate over drug-resistant strains when the effective drug concentration is low as would be the case in lymph nodes. These observations suggest the importance of devising strategies to deliver clinically effective drug concentrations throughout the lymphoid tissue compartment to help keep viral replication in check [1].

Researchers have recently developed a novel way for dating "hibernating" HIV strains, a finding that confirms that dormant HIV strains (latent HIV strains) can persist in the body for decades, to be reactivated many years later. The study confirms that the latent HIV reservoir is genetically diverse and can contain viral strains dating back to transmission. In essence, they created a highly calibrated 'time machine' that gives a specific time stamp for when each dormant HIV strain originally appeared in a person. They have provided a method for better measuring the timeline of virus latency and evolution within an individual living with HIV thus giving the characteristics of HIV in the latent reservoir which is important in order to eradicate HIV from a person's body. In order to "date" dormant HIV strains within the viral reservoir, the researchers compared these strains to those that evolved within an individual living with HIV over the entire history of their infection [2].

Overcoming latent virus reservoir remains the most significant obstacle to HIV cure. Over the years, researchers have dedicated efforts to deciphering the mechanisms that help HIV hide and finding ways to reverse that process. The focus in HIV/AIDS research is now turned toward the eradication of the virus in the latent reservoirs or preventing reactivation of the latent virus. Clearing away the reservoirs of HIV-infected cells might enable eradication of the virus completely, making it possible to cure HIV patients. Researchers are thus searching for reversal agents to induce the latent virus to express viral protein which could make this virus vulnerable to immune cells and antiretroviral drugs. Therapeutic strategies to reactivate latent HIV-infected cells so as to eradicate the viral reservoir have so far remained elusive, because candidate drugs that reawaken the virus (latency reversing agents), appear to lack sufficient potency or could trigger massive immune system activation which itself could be deadly. Previously identified compounds that can reactivate HIV by activating transcription, have not been very effective, in part because of the noisiness of HIV transcription.

This review discusses mechanisms that could be used to reverse HIV latency or silence the latent virus, and other approaches that have been discovered to deal with the HIV latency as well as new discoveries that give more information on HIV latency.

## Mechanisms that could be used to Disrupt/ reverse HIV Latency

Researchers are exploring two main strategies to tackle the problem of latency. One strategy is to reactivate and destroy the latent virus (called "shock and kill" or “kick and kill strategy”) while the other strategy is to find a way to silence the latent virus for good so that it never gets to be reactivated again. Silencing and reactivating HIV could be combined to develop more effective therapies. One could start by reactivating and killing the virus so that it now becomes easy to target, and then use silencing mechanisms to slow the resurfacing of latent virus. These strategies could potentially allow patients to stop taking drugs, and allow for several years to elapse before the virus reactivates by which time, the immune system could be strong enough to eliminate the virus as it surfaces. Other approaches towards tackling HIV latency include biological control of HIV reservoirs by using other biological agents such as other viruses; immunotherapy as well as seeking a better understanding of the science of latency.

## HIV Latency reversal Agents

The goal of HIV latency reversal is to force the virus to be expressed so it is visible to the immune system and can be targeted by the immune system and antiretroviral drugs. This is termed as the 'shock and kill' approach to treating the infection and it involves using latency-reversing agents (LRAs) to force the infected cells to produce HIV again (the 'shock' phase), while maintaining antiretroviral therapy (ART) to prevent the occurrence of new infections. This reactivation of HIV would cause the shocked cells to be killed directly or indirectly by ART (the 'kill' phase). Several latency reversal agents have been tried and some are discussed below.

### Use of SMAC mimetics

A study performed a few years ago, found that treatment with small molecule antagonists drugs known as Smac mimetics may be used to purge pockets of dormant HIV from a patient's body, eliminating the virus once and for all. These drugs enhance HIV-1 transcription, leading to a reversal of latency. Smac mimetics - already proven safe in early-stage clinical trials for cancer - work by inhibiting BIRC2 and related molecules, boosting the activity of HIV and thus reactivate dormant HIV so that it can be detected by the immune system and killed by antiretroviral drugs. These drugs reactivate the HIV, but don't appear to activate the immune system and are thus useful agents for shock-and-kill strategies to eliminate the latent HIV reservoir. BIRC2 (a protein coding gene) is a repressor of the NF-ϰB pathway, which is a potent negative regulator of LTR-dependent HIV-1 transcription [3].

### Use of histone deacetylase inhibitors

Research has shown that a central mechanism for maintaining

HIV-1 latency is the activity of histone deacetylases (HDAC) that repress proviral transcription by promoting histone deacetylation [4, 5]. Previous work has shown that HDAC inhibitors (HDACi) can disrupt HIV latency by activating hidden (dormant) reservoirs of HIV cells in immune cells *in vitro* [6, 7]. Recently, researchers from Aarhus University, Denmark successfully tested a new drug, a histone deacetylase inhibitor (HDACi), romidepsin, on six HIV participants who have been on antiretroviral therapy for a median time on of 10 years. When after several weeks the researchers analyzed blood samples from the participants they saw evidence of HIV transcription and consequently an increase in plasma viremia in these participants. Importantly, romidepsin did not decrease the number of HIV-specific T cells or function of the cytotoxic T cells, essential for the elimination of HIV-infected cells by the immune system. These results have important implications for the use of romidepsin as a latency reversal agent in a multi-component HIV eradication strategy where this drug may be combined with interventions designed to enhance killing of latently infected cells. The study thus demonstrates that significant reversal of HIV-1 latency *in vivo* is possible without blunting T cell-mediated immune responses [8].

**Histone crotonylation**

In a study of its kind, researchers in the United States of America (USA) showed that HIV latency can be reversed by histone crotonylation which is a genetic mechanism that modifies the proteins that package DNA. The research team used an enzyme called ACSS2, which plays an important role in fatty acid metabolism in the gut. They found out that when they activated the ACSS2 enzyme it activated histone crotonylation process which in turn then increased HIV gene expression, increasing HIV transcription manifold leading to more HIV virus being produced. This way the dormant HIV comes out of hiding and becomes susceptible to anti-HIV drugs and the immune system. In this study, immune cells from HIV patients who had been undergoing antiretroviral therapy and had undetectable viral loads were examined. They were able to disrupt the HIV latency in those samples by inducing histone crotonylation and hence increased viral transcription. When the researchers treated the samples with an ACSS2 inhibitor they were able to reduce detectable virus levels, highlighting the important role of decrotonylation in establishing HIV latency. Importantly, the study found that increasing histone crotonylation works synergistically with other known anti-HIV latency molecules, such as the protein kinase C agonist PEP005 and the HDAC inhibitor, vorinostat. These scientists are now looking for synergistic disruption, by combining histone crotonylation with other mechanisms to reactivate HIV, which may be a move much closer to eliminating HIV latency [9].

**Methyltransferase enzyme inhibitors**

Scientists at the Gladstone Institutes tested the "shock and kill" strategy by systematically screened over 50 methyltransferases and discovered that a methyltransferase enzyme called SMYD2, could be a new therapeutic target for flushing out the HIV that hides in infected individuals. They identified SMYD2 as a regulating enzyme, and found that inhibiting it reactivates, or wakes up latent cells and could therefore be used as a therapeutic target in the shock and kill strategy. They found molecules that act as SMYD2 inhibitors and which were able to activate the virus in latently infected T cells isolated from HIV patients [10].

**Use of PD-1 inhibitors**

In dealing with HIV latent virus, researchers found that a cancer drug, Nivolumap, a PD-1 inhibitor, which is used to treat several cancers in their advanced stages (including melanoma, non-small cell lung cancer and kidney cancer); may be able to eradicate HIV -infected cells in humans. The researchers working in France found the first evidence that Nivolumab while being used to treat an HIV-infected lung cancer patient, led to a 'drastic and persistent decrease' in the reservoirs of cells in the body where the HIV is able to hide away from attack by antiretroviral therapy. When the researchers first gave nivolumab to this patient, they observed that by day 120 the reservoirs of HIV-infected cells showed a drastic and persistent decrease. In this patient they observed, both a re-activation of HIV and an increase in CD8 T cell responses against HIV, which resulted in the drastic decrease in the HIV reservoir, thus leading to a sustained reduction of the HIV reservoirs. There was absence of side effects in this patient which is also good news suggesting this could be an optimum treatment for HIV-infected patients with cancer. However, the researchers are also cautious about their results since this is the first case of such a drastic decrease of the HIV reservoir and also there is need to evaluate, in clinical trials and in a larger group of patients under treatment currently the potential toxicities of these drugs in HIV infected people [11].

**Getting exact location of the latent reservoir and then using latency reversing agents**

In order to develop treatments that reverse latency in HIV reservoirs, there is need to find precisely where they are in the CD4+ T lymphocyte populations, which are highly variable. Scientists have found a new, powerful technique that finds dormant virus hiding in rare cells. To tackle this, these researchers from USA and Canada developed an innovative way to locate, identify and quantify the rare cells containing hidden virus and then test drugs to reactivate the latent HIV. Their method can be likened to "taking a photograph" of each individual cell harboring dormant HIV. This method is about 1,000 times more accurate than current technologies and thus represents a significant breakthrough. They found that the location of the hidden virus varied a lot from patient to patient and thus there will be need to adjust the treatment for individual patients since different patients may need different treatments, depending on the specific HIV hiding places in each case. The new technique can wake up the virus and then find the rare cells that have been hiding it at very low numbers, a limit of one cell in a million, an unprecedented level of accuracy, which opens the door to individualized monitoring of HIV-positive patients and could facilitate the development of personalized treatments. Once the HIV hiding places are found, researchers can then use a "shock and kill" strategy to eliminate the virus in two stages by reactivating it from its dormant state in the cells and then using drugs that can eliminate it. Next, the researchers tested two latency reversal drugs to wake up the dormant virus. The drugs, bryostatin and a derivative of ingenol, were developed to fight cancer but the researchers discovered that the two drugs reactivated different populations of CD4+ T lymphocytes in laboratory tests. In particular the ingenol derivative activated central memory cells, which can live for years in patients. The researchers suggest these experiments should be followed by a clinical trial which would involve using such drugs to reactivate

the virus while the patient continues taking ART to ensure that the reactivated virus cannot infect other cells [12].

**Criticism of the 'shock and kill' approach**

The 'shock and kill' approach to eliminating HIV has been challenged, especially the effectiveness of the treatment. One potential concern is that reversing HIV latency may reactivate the virus in anatomical compartments with suboptimal antiretroviral therapy concentrations, leading to de novo infection of susceptible cells in these sites, thus compounding the problem. Another problem with the latency reversal strategy is that the current latency reversing agents (LRAs) have proven ineffective due to the inability to accurately identify the number of cells in the HIV latent reservoir. To address this challenge, researchers from Buck Institute in Canada developed a new dual-fluorescence HIV-1 reporter virus to investigate the ability of various LRAs to reactivate HIV infection in latent cells. Using this reporter, the scientists discovered that a remarkably small number (less than 5%) of latently infected cells are reactivated by LRAs. These results indicate that while 'shock and kill' might be helpful in reactivating and possibly eliminating a small subset of highly reactivatable latent HIV genomes, other approaches will be necessary to control or eliminate the less readily reactivatable population in patients. They suggested that perhaps this cell population should instead be 'blocked and locked' using latency-promoting agents. They added that for a functional cure, having non-reactivatable latent HIV genomes would be more preferable to a lifetime of chronic active infection [13]. Some researchers have also warned that latency reversal strategy may endanger patients' brains as it may cause brain inflammation due to the activation of HIV reservoirs that could pose a danger. This is according to a study on HIV's close cousin, simian immunodeficiency virus (SIV), in macaques which found that this proposed curative strategy could backfire and make things worse if the virus is in fact lurking in the brain. In this study, scientists treated three pig-tailed macaque monkeys infected with SIV with antiretroviral drugs for more than a year. Then two of the macaques were given ingenol-B, a latency-reversing agent and another latency-reversing agent, vorinostat, which is used to make cancer cells more vulnerable to the immune system. The macaques also continued their course of antiretroviral drugs throughout the experiment period. After a 10-day course of the combined treatment, one of the macaques remained healthy, while the other developed symptoms of encephalitis (brain inflammation), and blood tests revealed an active SIV infection. When the animal's illness worsened, the researchers humanely killed it and carefully removed the blood from its body so that blood sources of the virus would not muddle their examination of the brain. Testing revealed SIV was still present in the occipital cortex of brain, which processes visual information. If these results of the study on macaques with SIV apply to humans with HIV, then this signals a need for extra caution in exploring ways to flush out HIV reservoirs [14].

**A combination of the shock and kill strategy, and the silencing strategy**

In an effort to tackle HIV latency both the 'shock and kill' strategy, as well as the silencing strategy, can be combined. To this end, a team of scientists at the Gladstone Institutes discovered how a new drug called JQ1, which is currently in early-phase human cancer trials, as a single drug that could attack the virus on two fronts i.e. it can reactivate latent HIV as well be used in silencing latent virus,. They showed that by targeting and removing the short form of BRD4, which is the key to silencing HIV, the drug JQ1 then allows the virus to make copies of itself. They found that manipulating the BRD4 protein can either help HIV resurface or strengthen the body's capacity to suppress it. The study could also impact a broader range of diseases, given that JQ1 is already being tested as a way to target the BRD4 protein to treat cancer, heart failure, and inflammation [15].

**Use of genetic engineering proteins to eliminate or reverse latency**

Scientists at the National Institutes of Health (NIH) have created a two-headed protein called VRC07-αCD3 to deplete HIV reservoir, that awakens resting immune cells infected with HIV and facilitates their destruction in laboratory studies, hence potentially contributing to a cure for HIV infection by helping deplete the reservoir of long-lived, latently HIV-infected cells. The engineered protein has two ends: one activates T cells by binding to a surface molecule called the CD3 receptor, and the other based on an antibody called VRC07. The VRC07-αCD3 protein powerfully binds to more than 90 percent of HIV strains. It facilitates the killing of latently HIV-infected cells in three steps. First, the CD3-binding end attaches to a resting, HIV-infected helper T cell, activating the cell so it starts making HIV and displaying pieces of virus on its surface. Next, the HIV-binding end of the protein latches onto those pieces of virus while the CD3-binding end attaches to a killer T cell, activating it and bringing it close to the helper T cell. Finally, the activated killer T cell destroys the HIV-infected helper T cell. The researchers found that the protein triggered the activation and killing of latently HIV-infected helper T cells when the cells were taken from patients on antiretroviral therapy and then incubated in the lab with the patients' own killer T cells [16]. Scientists working in mice, in lab animals, a particle developed by NIH scientists reactivates dormant virus causing it to begin replicating so that either the immune system or the virus itself would kill the cell harboring HIV. To test the approach, the researchers gave antiretroviral drugs to mice that had been infected with HIV, and then administered a synthetic compound called SUW133, to activate the mice's dormant HIV. Up to 25 percent of the previously dormant cells that began expressing HIV died within 24 hours of activation. SUW133 is based on bryostatin 1, a natural compound extracted from a marine animal called *Bugula neritina*. With further development, the researchers hope that the technique could lower the viral reservoir enough for people with HIV to be able to discontinue their anti-viral therapy. The findings of this research are significant because several previous attempts to activate latent virus have had only limited success where most studies showed weak activation of the virus, or severe toxicity, with little effect on the reservoir. In further studies, the scientists plan to evaluate SUW133's effectiveness in larger animals, before it could be tested in humans [17]. HIV-1 inserts its genome permanently into its victims' DNA, forcing patients to take a lifelong drug regimen to control the virus and prevent a fresh attack. In the struggle to eliminate latent virus reservoirs, researchers have found a way to eliminate HIV from cultured human cells for first time. In the first successful attempt to eliminate latent HIV-1 virus from human cells, researchers have designed a way to snip out the integrated HIV-1 genes for good. They deployed molecular tools, a combination of a DNA-snipping enzyme called a nuclease and a targeting strand of RNA

called a guide RNA (gRNA) to hunt down the viral genome and excise the HIV-1 DNA and thus to delete the HIV-1 proviral DNA. The researchers engineered a 20-nucleotide strand of gRNA to target the HIV-1 DNA and paired it with Cas9 nuclease. The gRNA targets the long terminal repeat (LTR) and that way the Cas9 nuclease can snip out the nucleotides that comprise the HIV-1 genome. Once the nuclease has edited out the HIV-1 DNA sequence, the loose ends of the genome are reunited by the cell's own DNA repair machinery, resulting in virus-free cells. To avoid any risk of the gRNA accidentally binding with any part of the patient's genome, the researchers selected nucleotide sequences that do not appear in any coding sequences of human DNA, thereby avoiding off-target effects and subsequent cellular DNA damage. The editing process was successful in several cell types that can harbor HIV-1, including microglia and macrophages, as well as in T-lymphocytes. Cells armed with the nuclease-RNA combination proved impervious to HIV infection and thus the research shows that these molecular tools also hold promise as a therapeutic vaccine. This HIV-1 eradication approach, however, faces some significant challenges: First, the researchers must devise a method to deliver the therapeutic agent to every single infected cell. Secondly, because HIV-1 is prone to mutations, treatment may need to be individualized for each patient's unique viral sequences [18]. Scientists at Temple University (LKSOM) and the University of Pittsburgh show that they can excise HIV DNA from the genomes of living animals to eliminate further infection. The team is the first to demonstrate that HIV-1 replication can be completely shut down and the virus eliminated from infected cells in animals with a powerful gene editing technology known as CRISPR/Cas9. The excision efficiency of their strategy reached 96 percent in EcoHIV mice, providing the first evidence for HIV-1 eradication by prophylactic treatment with a CRISPR/Cas9 system. This study confirmed the data from their previous work and they have improved the efficiency of the gene editing strategy. They show that the strategy is effective in two mouse models, one representing acute infection in mouse cells and the other representing chronic or latent infection in human cells. The next stage would be to repeat the study in primates, a more suitable animal model where HIV infection induces disease, in order to further demonstrate elimination of HIV-1 DNA in latently infected T cells and other sanctuary sites for HIV-1, including brain cells. This would eventually be followed by a clinical trial in human patients [19].

**Silencing the latent virus**

The "shock and kill" approach has the problem of not being able to efficiently deactivate the virus after successful activation. To mitigate this, researchers at Kumamoto University used in Japan developed a new approach that they call "Lock-in and apoptosis". In this strategy of silencing the latent virus, the researchers have developed a new compound called L-HIPPO that is key to the destruction of HIV. L-HIPPO binds strongly to the HIV protein Pr55Gag and suppresses viral budding. When L-HIPPO was added to virus-infected cells, viral budding was suppressed thereby the virus became confined within the cell and the cell would then die through natural apoptosis. They believe that this research will help to completely eradicate AIDS. This treatment with L-HIPPO is being used to eradicate HIV in viral reservoir cells, and is hoped to lead to the complete recovery from AIDS in the near future [20].

**Immunotherapy as a mechanism to tackle HIV latency**

In exploring immunotherapy as a mechanism to tack HIV latency, two main methods have been tried: use of specialized killer T-cells called follicular cytotoxic T cells as well as re-educating autologous immune cells (T cells) *ex vivo* and reinfusing them to the same HIV patients. In the use of specialized killer T-cells, researchers have discovered T cells called follicular cytotoxic T cells that can find HIV hidden in tissues and destroy them. This type of killer T cell are naturally found in the body during infection, but their numbers and killing function needs to be boosted to allow them to eradicate chronic infections. The researchers discovered that these specialized killer T cells can enter hiding spots inside lymphoid tissues and could eradicate this hidden virus pool where viruses can hide during treatment and could eventually cure HIV. These hiding spots are called B cell follicles. These specialized super potent killer T cells could be transferred into patients, or patients could be treated with proteins that can drag these specialized killer T-cells into the right spots, specifically to the hot spots where HIV can hide on antiviral treatment [21]. Another study has conducted a proof-of-concept HIV immunotherapy study through a Phase 1 safety trial with the long-term goal of using ex vivo expanded HIV-specific cytotoxic T cells as part of strategies to clear persistent HIV infection in combination with latency reversal drugs. This study was designed to improve immunotherapies against HIV by re-educating the body's immune system to better fight HIV infection. The researchers in this study used the approach of re-educating the immune cells (T cells) and reinfusing them to test safety. This cell therapy involved collecting T cells from a HIV infected patient (whose viral load had been reduced to an undetectable level by ART) and the ex vivo expansion of these T cells in the laboratory to increase their numbers. These expanded T cells were subsequently infused back into same HIV-infected individuals previously treated with antiretroviral therapy (ART) giving the patient a step forward in clearing persistent HIV infection. These autologous, ex vivo expanded, virus-specific, cytotoxic T-lymphocytes derived from HIV-infected patients T cells were shown to be safe and well tolerated, as well as highly effective. This paves the way for the next step, which is to combine this immunotherapy approach with latency-reversal therapy in order to wake up the HIV out of its latent state, and then clear it out with the immunotherapy [22].

**Using other viruses to fight HIV**

Another approach of dealing with latent virus is by using other viruses to fight HIV. To this end, researchers from Ottawa, Canada, have discovered a new approach that eliminates 'dormant' HIV-infected cells by using viruses to fight viruses. In their investigation, they found that the Maraba virus (MG1), can target and destroy latently HIV-infected cells that standard antiretroviral therapies can't reach. These latently HIV-infected cells are hard to target because they are not distinguishable from normal cells. This study tried a new approach of identifying these dormant cells by using the MG1 virus. This virus attacks cancer cells that have defects in their interferon pathway, which makes the cells more vulnerable to viruses. Using a number of laboratory models of latently HIV-infected cells, the researchers found that the MG1 virus targeted and eliminated the HIV-infected cells, and left healthy cells unharmed. When the researchers added MG1 to relevant blood cells taken from HIV-positive individuals, the levels of HIV DNA in the sample dropped indicating that the

HIV-infected cells had been eliminated. The research team's next step is to try the virus in animal models of HIV or move directly to clinical trials depending on the results obtained [23].

**Use of early sustained antiretroviral drug treatment**

The timing and duration of antiretroviral treatment is one factor that determines the size of the HIV reservoir. In this regard, it has been shown that long-term or prolonged early ART reduces the extent of HIV reservoirs and allows for better virological control after antiretroviral drug discontinuation as compared to later treatment initiation. This was shown by a study that compared HIV-1 reservoirs in a cross-sectional study using polymerase chain reaction-based techniques in blood and tissue of early-treated seroconverters, late-treated patients, ART-naïve seroconverters and long-term non-progressors (LTNPs). A decade of early ART reduced the total and integrated HIV-1 DNA levels compared with later treatment initiation, but not reaching the low levels found in LTNPs. Importantly, lower viral transcription and enhanced immune preservation (CD4/CD8), reminiscent of LTNPs, were found in early compared to late-treated patients. People who started treatment earlier also had healthier immune cells. This suggests that early treatment is associated with some immunovirological features of LTNPs that may improve the outcome of future interventions aimed at a functional cure. Together, the experiments support the benefits of starting drug treatments as soon as possible after a person is infected with HIV-1 and that early sustained antiretroviral drug treatment helps to reduce the size of the HIV reservoirs to contend with [24].

**Use of combination therapy of immunostimulants with broad spectrum antibodies**

Researchers at Beth Israel Deaconess Medical Center in Boston, USA, demonstrated that administering broadly neutralizing antibodies (bNAb) designed to target HIV in combination with agents that stimulate the innate immune system delayed viral rebound following discontinuation of ART in monkeys. This two-pronged approach represents a potential strategy for targeting the viral reservoir. This study involved 44 rhesus monkeys infected with an HIV-like virus and treated with ART for two and a half years, starting one week after infection. After 96 weeks, the animals were divided into four groups of 11 monkeys each. One group, the control group received just ART with no additional investigational treatments. The second group was given only an immune stimulating agent while the third group was given only broadly neutralizing antibodies. The fourth group was given the immune stimulant (Toll-like receptor 7 agonist vesatolimod (GS-9620) in combination with the antibodies (V3 glycan-dependent bNAb PGT121). From week 96 onwards, all animals continued ART treatment until it was discontinued at week 130, at which point the scientists began monitoring the animals' blood for signs of viral rebound (the virus's return). As expected, 100 percent of animals in the control group rebounded quickly and with high peak viral loads, as did nearly all of those given only the immune stimulant. Animals given only the antibodies demonstrated a detectable but modest delay in rebound. Of note, among those given the combination therapy, five of the 11 monkeys did not rebound within six months. Moreover, those that did rebound showed much lower peak viral loads compared to the control animals. This study showed that the combination of the antibodies and the immune stimulant led to optimal killing of HIV-infected cells suggesting a mechanism by which the combination therapy stimulated innate immunity and rendered infected cells more susceptible to elimination [25].

**Better methods of detecting latent HIV in host cells**

Most tests available for detecting the virus are not very cost effective and take a lot of time. In the bid to eliminate virus reservoirs there is need for a more efficient way of checking whether or not HIV is still hiding in CD4 cells. The most widely available test at the moment is the "quantitative viral outgrowth assay" (Q-VOA) which requires large amounts of blood, a lot of work, is quite expensive and may also underestimate the amount of virus left. In this regard, a sensitive assay that can accurately and rapidly quantify inducible, replication-competent latent HIV-1 from resting CD4+ T cells is essential for HIV-1 eradication studies. To this end, researchers from the University of Pittsburgh's in Pennsylvania have come up with a test dubbed TZA Test which is more accurate, more sensitive, quicker, less labor intensive, and less costly than present technology and efficient test for detecting how much of the virus is hidden in the human body after retroviral therapy. The new test works by detecting a gene that is active only when replication-competent HIV is present. The TZA test produces results in 7 days, compared with the 14 days needed by the Q-VOA, and it costs only a third of the Q-VOA price. Additionally, it requires a much smaller amount of blood and number of cells. The TZA test revealed that in people who seem to be almost fully cured of HIV, asymptomatic patients on antiretroviral therapy carry a much larger HIV reservoir than previous estimates and the amount of latent virus is actually as much as 70 times than was detectable by Q-VOA test. Because it needs fewer cells, the TZA might also be useful for detecting HIV in children as well as in tissues where HIV continues to hide. In summary, this assay is sensitive, requires only a small blood volume, is faster, less labor intensive, and less expensive; and can be readily adapted into a high-throughput format. Using this assay, it was shown that the size of the inducible latent HIV-1 reservoir in aviremic participants on therapy is approximately 70-fold larger than previous estimates [26].

**Better understanding of the HIV reservoirs**

Some important discoveries have recently been made that have increased our knowledge and understanding of the HIV latency so that we can be better placed to find new ways of reducing the reservoirs. French researchers have recently identified an HIV reservoir marker which offers new therapeutic strategies for targeting infected cells and which can provide a new avenue toward eliminating the virus. The marker, a protein called CD32a, makes it possible to differentiate "dormant" HIV-infected cells from healthy cells, which resemble them to a very large degree. After comparing infected cells and healthy cells, they noticed that this protein is present only on the surface of infected cells. This was then confirmed by experiments on clinical samples. By studying blood samples from 12 patients living with HIV and receiving treatment, the researchers isolated the cells expressing the marker and observed that almost all were HIV carriers. In vitro, the activation of these cells induced a production of viruses capable of re-infecting healthy cells whereas their elimination entailed a significant delay in viral production. Viral reservoirs will now be possible to isolate more easily and analyze directly. In the longer term, it will lead to therapeutic strategies aimed at eliminating

the latent virus from the organism and make remission - at least temporary - possible in the absence of antiviral treatments [27]. Researchers at the University of Montreal found a way to reduce/slow replication of the AIDS virus in the gastrointestinal tract of people infected by HIV-AIDS, which is particularly the place where HIV reservoirs form among the various peripheral tissues. CD4 T cells which are a prime targets of HIV infection migrate from the blood to the gut thanks to marker molecules expressed on their surface; particularly CCR6 which act like a "postal code" for the cells, indicating they should direct themselves to the gut. The researchers have now discovered that in the colon, the CD4 T cells which express the CCR6 postal code also contain a large amount of another molecule called mammalian target of rapamycin (mTOR), an important regulator of metabolic mechanisms (i.e. regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription). The molecule stimulates HIV replication in CD4 T cells located in the gut and it is partly responsible for the high vulnerability to HIV of the CD4 T lymphocytes expressing CCR6 and residing in the gut. The researchers found that by interfering with mTOR activity during in-vitro experiments with existing medications, they were able to significantly reduce HIV replication in the cells of HIV-infected patients whose viral load was undetectable. Furthermore, medications inhibiting mTOR activity are used successfully in the treatment of cancer and diabetes and this could thus improve quality of life and increase chances of curing HIV-infected patients by using mTOR inhibitors to supplement antiretroviral treatments. This might supplement antiretroviral therapy (ART) and prevent complications associated with chronic infection. There is therefore hope that this type of treatment will reduce even more the amount of virus persisting in gut reservoirs and be an important strategy to cure HIV, and one that deserves to be tested [28]. Macrophages are found in tissues throughout the body including the liver, lung, bone marrow and brain. These cells have been implicated in HIV pathogenesis and in the trafficking of virus into the brain, but their specific role in HIV persistence during long-term suppressive ART had not been established till recently. HIV cure research has till recently focused on clearing the virus from T cells but new knowledge by investigators in the University of North Carolina have found macrophages in sites where HIV persists despite treatment and this has major implications for cure research. These results are paradigm changing because they demonstrate that cells other than T cells can serve as a reservoir for HIV. This means that any possible therapeutic intervention to eradicate HIV might have to target two very different types of cells (T cells and Macrophages). Using a humanized myeloid-only mouse model devoid of T cells, this team showed that ART strongly suppresses HIV replication in tissue macrophages but when HIV treatment was interrupted, viral rebound was observed in one third (33%) of the animals, a finding that is consistent with the establishment of persistent infection in tissue macrophages. No viral rebound was observed in the plasma of 67% of the ART-treated animals at 7 weeks after ART interruption, and no replication-competent virus was rescued from the tissue macrophages obtained from these animals. These observations represent the first direct evidence of HIV persistence in tissue macrophages in vivo. This research demonstrates that tissue macrophages can be infected, that they respond to antiretroviral therapy, that productively infected macrophages can persist despite antiretroviral therapy and most importantly, that they can reinitiate and sustain infection upon therapy interruption even in the absence of T cells, the major target of HIV infection [29]. These findings suggest thus that in order to have a comprehensive HIV cure, it's important to identify all of the relevant HIV-1 reservoirs in the body, since it's possible that the virus hides in the DNA of numerous cell types and each may require different strategies to get a cure. May be a bigger sample needs to be studied to see if the results are replicated. A recent study by researchers at the George Washington University (GW) on the "kick-and-kill" strategy to eliminate the HIV virus uncovered a potential obstacle in finding a cure. The research team conducted their research using the CD8+ T-cells of people living with HIV, in combination with latency reversing drugs to attack and kill the infected cells. They found that latent HIV reservoirs show inherent resistance to CD8+ T-cells, the cells whose primary function is to kill infected cells. Though these reservoirs have inherent resistance to the T-cells that presents an obstacle on the road to curing HIV, the results of this study will help to improve researchers' understanding of how to approach the virus in future, since they have identified what might be an important barrier in killing HIV-infected cells. There is the need to continue to work with hopes of understanding the reason for the resistance to CD8+ T-cells [30]. Researchers have shown that mechanisms that govern HIV transcription and latency differ in the gut and the blood, findings that could inform new therapies aimed at curing HIV. To compare the mechanisms that inhibit HIV transcription in the gut and blood, the researchers quantified HIV transcripts in cells from the blood and rectum of HIV-infected individuals effectively treated with antiretroviral drugs and found that the rectum may be enriched for cells in a deeper state of latency. They found that different mechanisms block HIV transcription and underlie HIV latency in CD4+ T cells in the blood and gut. These differences in the blocks to HIV transcription are important to consider in designing therapies that aim to disrupt HIV latency in all tissue compartments. In particular, infected cells in the rectum may be less susceptible to agents designed to reverse latency or may require different types of therapies [31].

After following through this review of recent research findings, we can note that it is most likely a multipronged approach that will help deal conclusively with the problem of HIV latency and persistent HIV reservoirs. It may be important for the scientists involved in all these researches having a discussion on all the individual approaches and finding out which method or combination of methods will help deal decisively with the problem of HIV reservoirs.

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