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HIV Treatment and Cure: A Critical Review of Scientific Advances and Therapeutic Developments

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Abstract

Secondary Immunodeficiency Disorders (SID) such as HIV had claimed to have taken approximately 42.3 million lives. SIDs are defined as a transient or persistent impairment of the function of cells or tissues of the immune system, due to multiple external factors including underlying infections such as HIV, specific medications and medical conditions. HIV, being a retrovirus, specifically targets CD4 T cells, dendritic cells and macrophages. HIV-AIDS has transitioned from being a fatal diagnosis to a manageable chronic condition due to groundbreaking research, drug development and public health interventions. The most common method of treatment for HIV is antiretroviral therapy (ART). Combining drugs from different classes such as Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs), Integrase Inhibitors , were found to suppress viral replication more effectively than monotherapy. Over 95% of individuals who adhered to combination ART (cART) achieve undetectable viral loads making it effective for preventing progression to acquired immunodeficiency syndrome (AIDS) and reducing transmission risk. In the 2000s the focus shifted to improving drug efficacy, reducing toxicity and enhancing accessibility. Around 86% of people under Highly Active Antiretroviral Therapy (HAART) achieved viral suppression preventing disease progression and transmission. INSTIs and HIV entry inhibitors further expanded therapeutic options targeting different stages of the viral life cycle. The last decade has seen further refinement of HIV treatment emphasizing long-term management and prevention including advances in functional cure research such as stem cell transplant and CRISPR/Cas 9 technology. Despite these achievements significant challenges persist. Disparities in treatment access and the emergence of drug resistance strains underscore the need for continued innovation. Continued advancements in HIV research and equitable access to treatment are essential in achieving the goal of ending the HIV epidemic.

Keywords: HIV-AIDS, Treatment, PI, NRTIs/ NNRTIs, Integrase Inhibitors, Stem Cell Transplant, CRISPR/CAS 9 Technology

1. Introduction

Immunodeficiency disorders arise primarily due to impairments in the body's immune system, making it extremely difficult to defend the immune system and overcome pathological states brought upon by foreign or abnormal cells such as bacteria, fungi, viruses and cancer cells. Hence due to the suppression of their immune system, patients are physiologically inclined to develop various disorders, common examples including immune thrombocytopenia, AIDS etc. Immunodeficiency disorders can be classified into two types: Primary Immunodeficiency Disorders and Secondary Immunodeficiency Disorders.

Primary Immunodeficiency Disorders (PID) are relatively rare, persist from birth and

are always genetically inherited. PIDs become more evident during infancy and early childhood, with certain exceptions including common variable immunodeficiency which is detected during adulthood. Alternatively, Secondary Immunodeficiency Disorders (SIDs) are conditions where the immune system is weakened or undergoes severe compromise due to extraneous factors such as underlying medical conditions, infections or due to specific medications. SIDs increase an individual's susceptibility to infections. One of the most common SIDs include human immunodeficiency virus (HIV) infection.[1]

HIV belongs to the family of retroviruses classified under two types, HIV1 and HIV2. They fundamentally target an individual's immune system. HIV invades the body and proceeds to attack T lymphocytes, decreasing their ability to defend the body against infections. HIV tends to have a higher affinity and proceeds to bind to the CD4 receptors of the T lymphocyte cell, hence causing the subsequent disintegration of the T cell. This is a major drawback as the CD4 T cell and its subtypes aid in the coordination of the adaptive immune response by promoting cell-mediated immunity, facilitating humoral immune response by producing antibodies against extracellular pathogens, enhancing inflammation to aid in combating extracellular invaders and maintaining immune tolerance by suppressing the overstimulation of immune responses thus also preventing the development of autoimmune diseases. T helper cells proliferate, resulting in the formation of memory T helper cells that retain memory and thus act as defense upon re-entry of the pathogen. Thus, when the CD4 T cells and their functions are hindered due to HIV, the patient becomes significantly more prone to the development of other diseases ultimately leading to AIDS. If not treated early on can lead to the patient experiencing grave conditions, often fatal.[2]

HIV can be transmitted via the exchange or spread of bodily fluids of the infected individuals including blood, breast milk, rectal fluids, vaginal fluids and semen specifically pre-seminal fluids. This mostly results in cases of unprotected sexual intercourse with the infected patient or due to sharing needles seen in the case of most illicit drug abusers. General symptoms of AIDS include fever, headaches, extreme fatigue, muscle and joint pain, and night sweats, whereas some individuals appear asymptomatic up till the point of diagnosis.[3]

In current-day medicine, a diverse array of tests is used to diagnose AIDS. Methods include antibody tests which can detect HIV antibodies 23 to 90 days after exposure, and rapid antigen/antibody tests which can detect HIV antibodies 18 to 90 days following exposure. Nucleic Acid Test (NAT) can detect HIV 10 to 33 days following initial exposure. In cases of initial screening, Enzyme-Linked Immunosorbent Assay (ELISA) is utilized to detect HIV antigens. Due to its high specificity and sensitivity in detecting p24 antigens, ELISA is advantageous and can be used to detect HIV only 2 weeks after exposure. Though this form of testing appears to be beneficial it cannot be solely used as a form of diagnosis and requires the use of confirmation tests such as Western Blotting, HIV differentiating tests and NAT.[4]

According to the World Health Organization, by 2023, 39.9 million individuals live with HIV globally, approximately 3 million individuals had acquired HIV and between 500, 000 to 820,000 deaths had occurred due to HIV. These figures account for all adults, both women and men as well as children. These figures highlight the significant impact of AIDS globally, affecting a large fraction of the world's existing population.

This review summarizes the mechanism of action of HIV in developing AIDS as well as the evolution and subsequent

effects of various existing and evolving treatment strategies for HIV outlining the milestones and challenges in the development of treatments and their impact on individual and public health.

2. HIV Mode of Action

HIV is a lentivirus, belonging to the retrovirus family, that primarily targets the CD4 T-cells by binding 120 viral glycoprotein to two cell surface proteins of host's immune cells, the CD4 receptor and either the CC-chemokine receptor 5 (CCR5) or the CXC-chemokine receptor 4 (CXCR4). Since the virus is a retrovirus, it can integrate its DNA into the host cell genome, thereby making the eradication of the virus exceedingly difficult with the current therapeutic strategies. HIV enters the target cells via the CD4 receptor and either the CCR5 or CXCR4 receptor through its interaction with the envelope glycoprotein. Once the virus is attached to the cell, the viral membrane fuses with the cell membrane which facilitates the entry of the virus into the cytoplasm of the target cell. Next, the viral DNA (vDNA) is transcribed by the viral reverse transcriptase enzyme (RT) into a double-stranded DNA (dsDNA) molecule. This dsDNA is then translocated to the cell nucleus where it is subsequently integrated into the cell genome as a provirus, a process that is mediated by the virus integrase enzyme. Inside the nucleus, the host enzymes mediate the transcription of the HIV DNA to viral mRNAs. These mRNAs are then exported to the cytoplasm for translation which results in the expression of viral proteins and eventually mature virions.[6] The viral proteins Tat and Nef, in addition to the intrinsic T-cell activation factors, induce the active transcription and the expression of the viral RNA and proteins to produce new virus particles. These new viral particles then exit the host cell and traverse through the tissues to infect other target cells. The CD4-independent viral entry has been demonstrated in astrocytes, B cells, and kidney epithelial cells where the replication of the virus is less likely to occur.[7] **Figure 01** illustrates the life cycle of HIV.

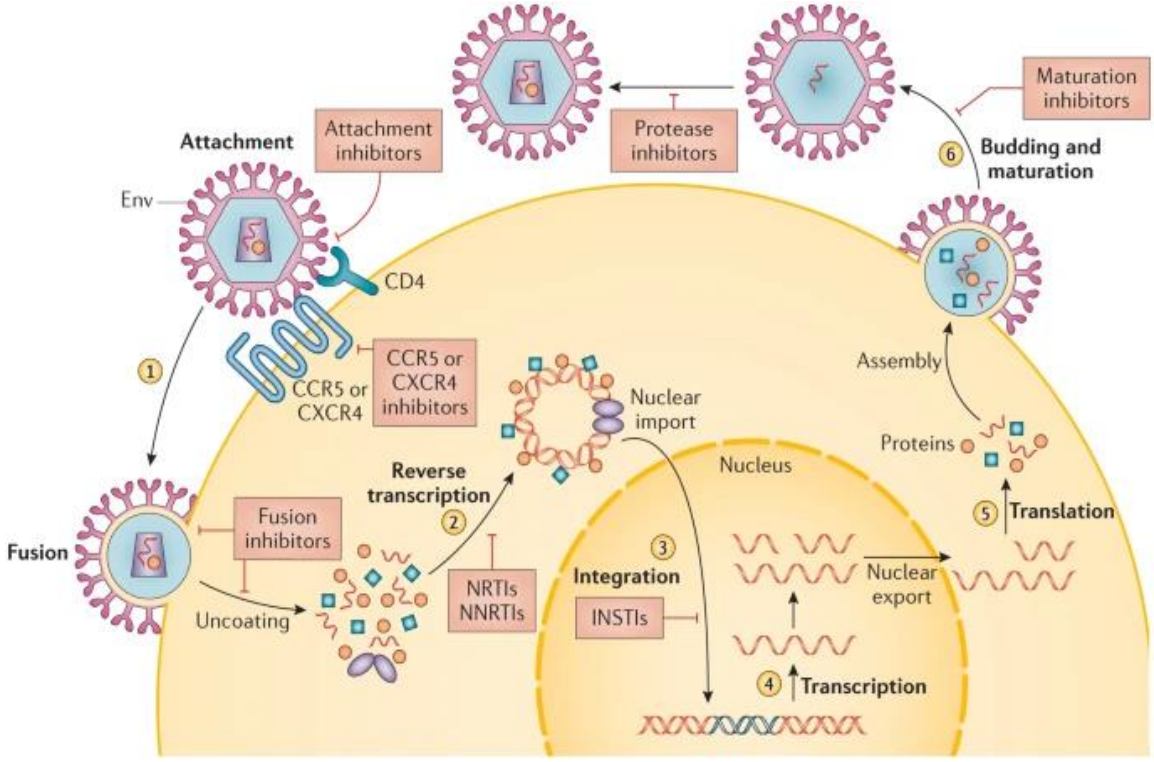

Figure 01: HIV Life Cycle.[6]

3. Treatment strategies of HIV

3.1 HPA 23

HPA-23 (ammonium 5-tungsto-2-antimoniate), a polyoxometalate (POM), was an experimental antiretroviral drug used in the early days of the HIV/AIDS epidemic. While it initially showed promise, further research revealed significant limitations and side effects. HPA-23 exhibits anti-HIV activity primarily by inhibiting critical enzymes and interfering with viral entry mechanisms. HPA-23 effectively suppresses the activity of reverse transcriptase, an enzyme crucial for the conversion of viral RNA into DNA, which is a necessary step in HIV replication. HPA-23 may also affect HIV entry by interacting with the viral envelope glycoproteins, particularly gp120, which is necessary for binding to the CD4 receptor on host cells. This disrupts the virus's ability to attach and fuse with target cells. As a member of the POM class, HPA-23 has demonstrated effectiveness against a range of viruses, but its development for HIV treatment faced challenges due to its toxicity, particularly affecting liver and kidney functions.[8]

In a study conducted in 1985 in Paris, four patients were treated with HPA 23 as part of an experimental therapy for AIDS. The patient cohort ranged from 13 years to 30 years old with underlying conditions such as cerebral toxoplasmosis, pneumocystis pneumonia, paraganglioma, oral thrush and Kaposi sarcoma (KS). HPA 23 was used as a reverse transcriptase inhibitor to reduce HIV (then referred to as LAV) replication. HPA 23 worked by inhibiting reverse transcriptase, thereby suppressing HIV replication in T-cell cultures without killing infected cells. The treatment reduced detectable HIV in peripheral blood lymphocytes during administration, though low levels reappeared in some patients' post-treatment. Side effects included temporary platelet count reductions and mild hepatic transaminase elevations, both of which normalized after treatment, and no renal toxicity was observed.[9]

3.2 Protease Inhibitors

Protease Inhibitors are a form of antiretroviral therapy (ART) and play a crucial role in treating HIV. ART slows down the progression of the virus by blocking the effect of the HIV protease enzyme. The HIV protease enzyme has a pivotal role in the maturation of the virion particles into new HIV particles due to its property of cleaving specific viral protein sites, converting large precursor proteins into smaller proteins that combine with HIV genetic information and proceed to mature into new viral particles. Protease inhibitors mimic the structure of the viral protein and bind to the active site of the enzyme instead, thereby blocking the proteases' ability to cleave the long-chained viral polypeptide by specifically preventing the enzyme from cutting the viral protein chain at the Pol and Gag sites. This is crucial as the Pol gene is necessary to mediate the assembly of the viral particle and the Gag gene plays a significant role when it comes to the replication and proliferation of the mature viral particles. Thus, when these sites are not cleaved, it results in the production of non-infectious viral particles due to them being immature. These not fully formed viral proteins cannot assemble with other viral components and hence fail to produce and release functional proteins that are completely necessary for the replication of HIV and are inhibited from infecting other healthy host cells. Therefore, protease inhibitors reduce the viral load in the patient's body and thus act as a beneficial treatment method for HIV.[10]

Protease Inhibitors can be classified according to four types. First Generation Protease Inhibitors are the earliest formulations of protease inhibitors to ever be used as a form of treatment for HIV. Examples include Ritonavir (RTV), Saquinavir (SQV) and Indinavir (IDV). These were highly associated with severe side effects and very low levels of

bioavailability and were deemed not highly effective. Second Generation Protease Inhibitors were capable of fewer side effects and comparatively more bioavailability and were predominantly designed to pharmacokinetic parameters to prevent resistance. Examples include Darunavir (DRV), Fosamprenavir (FPV), Lopinavir (LPV) and Atazanavir (ATV). For better tolerability and to obtain a very high resistance barrier, a third generation of Protease inhibitors was developed, an example includes Tipranavir (TPV).[11] In a study done by Serafino *et al*., combination ART therapy of Atazanavir and Ritonavir was administered to two patients with HIV. The male patient experienced a reduction in his viral load from 102,900 copies/mL to undetectable amounts, while the female patient also experienced a reduction in her viral load from 1,302,000 copies/mL to lowered levels post-treatment.[12]

Another study done by Vornicu *et al*. discusses a 29-year-old woman with a well-controlled HIV infection who presented with acute kidney injury and nephrotic syndrome. The protease inhibitors given to the patient included Darunavir and Ritonavir. The patient had undergone effective suppression of their HIV viral load as a response to treatment.[13]

3.3 Nucleoside and Non-nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRIs) and nucleoside reverse transcriptase inhibitors (NRTIs) are two distinct classes of viral reverse transcriptase inhibitors (vRTI), which are involved in the antiretroviral therapy (ART) regimen of HIV-AIDS patients. NRTIs are nucleoside analogues that compete with normal 2′-deoxynucleoside triphosphates (dNTPs) during DNA synthesis. Once the NRTIs are incorporated into the growing DNA chain, they cause the chain termination of vDNA transcription due to their lack of a 3′-hydroxyl group. After that, they interact directly with the catalytic site of the reverse transcriptase enzyme, specifically with residues Asp110, Asp185, and Asp186. In contrast, NNRTIs are structurally diverse compounds that bind to a specific hydrophobic pocket in the p66 subunit of reverse transcriptase, consisting of residues Tyr181 and Tyr188. This binding site is spatially distinct from the catalytic site and acts allosterically, inducing conformational changes that inhibit the enzyme's activity without mimicking nucleosides. Thus, while NRTIs block reverse transcriptase by chain termination, NNRTIs inhibit the enzyme through allosteric interference.[14] NNRTIs are prodrugs that require intracellular anabolic phosphorylation to be converted into their active form of phosphorylated NNRTI metabolites.[15] Hence, most of these drugs possess longer half-lives than their parent drug compounds. NTRIs are also prodrugs that require intracellular activation to the corresponding triphosphate (ddNTP) forms by various cellular pathways. Table 1 outlines the plasma half-life and the cellular transport mechanisms employed by the current clinically approved NRTIs. Here, it can be observed that the plasma half-life of the metabolite is longer than the intracellular half-life of the parent drug. However, NRTIs have setbacks, including the rapid development of resistance and a lack of functionality against HIV-2.

Currently, approved clinical NNRTIs are classified into two generations. The first generation includes the drug Nevirapine which has been known to be severely impacted by the emergence of drug resistance, leading to mutations in the amino acid homolog. Moreover, the first-generation drugs also lead to RT-resistant mutations such as Y181C and K103N. To address these harmful and inefficient effects of the first-generation drugs, second generation drugs such as Efavirenz, Etravirine, and Delavirdine have been discovered. These drugs target the HIV polymerase activity of the RT enzyme through allosteric inhibition and pose higher efficacy along with fewer side effects for HIV patients.[16]

Table 01 Nucleoside Reverse Transcriptase Inhibitors.[15]

NRTI Name	Plasma Half-Life	Intracellular half-life
Lamivudine (3TC)	22 h	15 to 16 h
Emtricitabine (FTC)	37 h	39 h
Tenofovir Disoproxil Fumarate (TDF)	~17h	164 h
Tenofovir Alafenamide (TAF)	~125 h	164 h
Zidovudine (AZT)	~2 h	~9 h

Despite the assumption that all the NNRTIs bind to the same hydrophobic pocket site at the HIV-RT, the different classes of HIV-1 specific RT inhibitors differ concerning which amino acids the drugs interact with at the binding site. This hypothesis is extracted from the discovery that different NNRTIs do not exactly possess cross-resistance to each other, although these drugs seem to overlap when a cocktail of drugs is prescribed.[17]

According to the research conducted by Ard *et al.* a 55-year-old man who was diagnosed with HIV-1 infection was administered with a combination of ART regimen Tenofovir Alafenamide (TAF), Emtricitabine (FTC) and Dolutegravir (DTG), out of which TAF and FTC were NRTI and NNRTI respectively.[18] After the completion of ART at its first week a slow rise in the CD4+ T-cell count was observed. Moreover, the viral load was less than 20 copies per millimeter.

In the case study conducted by Pallangyo *et al*., a 29-year-old HIV positive woman was started on antiretroviral therapy which included a cocktail of drugs namely Tenofovir, Lamivudine, and Efavirenz antiretrovirals.[19] Tenofovir and Lamivudine and NRTIs whereas Efavirenz is a NNRTI. The ART helped decrease her viral load and increase her CD4+ T-cell count which was at a low level of 316 cells/mL. However, the ART resulted in an increase in her lipid levels.

3.4 Integrase Inhibitors

Integrase strand transferase inhibitors (INSTI) are a class of antiretroviral inhibitors that specifically block the activity of the HIV-1 integrase enzyme, which is a key player in the viral replication cycle. This inhibition blocks the integration of viral DNA into the host genome, halting viral replication and infection spread. Four approved INSTIs for HIV treatment include first generation raltegravir, elvitegravir and second-generation dolutegravir and bictegravir.[20] Integrase operates within the large functional nucleoprotein complex called intasome, which includes viral DNA and components from the virus and host cell. Integrase catalyzes two key reactions within the intasome to incorporate the reverse-transcribed HIV DNA into the host DNA.[21] During 3'-processing, integrase removes two or three nucleotides from each 3' end of the viral DNA, promoting strand transfer reaction to occur after import the viral DNA into the nucleus, integrase inserts the 3' ends of the viral DNA into the host DNA. INSTIs are characterized by a metal-chelating core that binds two Mg^{2+} ions, a halogenated benzene side chain that interacts with viral DNA, and a flexible linker connecting the core scaffold to the halobenzyl side chain. INSTIs preferentially target the enzyme's active site in the intasome complex, binding to the divalent metal ions and viral DNA. This displaces the viral DNA from the active site, inactivating the intasome and

preventing the integration of reverse-transcribed viral DNA into the host genome by blocking the strand transfer reaction.[22]

In a study conducted by Peterson *et al.*, a 55-year-old woman from West Africa was diagnosed with HIV-2 in 1990. After extensive combinations of various ART cocktails, in 2010 the patient was prescribed Raltegravir with Zidovudine/Lamivudine and Ritonavir-boosted Darunavir. After 1 year of this treatment, her viral load came down to an undetectable level from 61,000 copies/mL and her CD4+ T-cell count which was 282 cells/μL rose to 428 cells/μL.[23]

The case study conducted by François *et al.,* highlights the first documented instance of Dolutegravir (DTG) resistance in ART-naive, perinatally HIV-infected infant, identified during a national HIV drug resistance (HIVDR) survey in Haiti. The infant, born to a mother on a Tenofovir/Lamivudine/Dolutegravir regimen, displayed resistance mutations affecting INSTIs, NRTIs and NNRTIs. Although INSTI resistance was low (1.1% in 143 infants), a unique case of resistance in DTG-exposed populations emphasizes the need for vigilance and further research to improve treatment strategies in low- and middle-income countries.[24]

3.5 Stem Cell Transplant

Stem cell therapy is an approach in contemporary medicine that utilizes the regenerative ability of stem cells to treat a wide range of diseases and injuries. Stem cells can develop into various specialized cell types, facilitating the repairing and restoring of damaged tissues, and organ function and combating illnesses like cancer. Stem cells can be categorized into three main types. Common characteristics include self-renewal and differentiation into other cell types. Adult Stem Cells can give rise to many specialized cell types of tissues and organs, an example includes hematopoietic stem cells (HSCs). HSCs are located in bone marrow forming all mature blood cells in the body like erythrocytes and lymphocytes cells and platelets.[25,26]

A remarkable rise in stem cell therapy in HIV occurred after the two real-life incidents of Berlin and Geneva patients. Timothy Ray Brown was the first person ever cured of HIV, commonly known as the Berlin Patient. Initially, he had a CD4+ T-cell count of 316 cells/mL which indicated a weakened immune response.[27-28] Timothy was treated with standard ART before his stem cell transplant. After receiving a stem cell transplant from a donor with the CCR5-Δ32 mutation resistant to HIV, Timothy did show improvements regarding his immune system as HIV could no longer infect his cells. In response to ART he experienced a reduction in viral load and an increase in his CD4+ T-cell count to a value of 415/mm2.[27]

According to the information analyzed by Asier Sáez-Cirión *et al.,* the Geneva patient, who was already diagnosed with HIV-1 was again diagnosed with myeloid sarcoma in 2015. The individual underwent a stem cell transplant which did not involve a donor with the CCR5-Δ32 mutation. The CCR5 receptor, present on the surface of immune cells serves as a critical pathway for HIV entry. The patient continued ART to maintain HIV suppression, as the virus remained in latent reservoirs and could rebound without treatment.[29] Finally, in 2021, all antiretrovirals were stopped following a consensual decision between the patient and his physician to evaluate the possibility of HIV remission and there was no evidence recorded of remission of HIV for 32 months despite the recurrent testing.[30] In response to the allogenic haematopoietic stem cell transplant the viral load of the patient decreased to 2.22 RNA copies / mL and was eventually undetectable.

3.6 CRISPR/Cas 9 Technology

A promising gene-editing technique for HIV/AIDS treatment is the clustered regularly

interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) systems. It can be used to target the HIV-1 genome or cellular co-factors to decrease HIV-1 infection and eradicate the provirus. It can also be utilized to trigger transcriptional activation of dormant virus in dormant viral reservoirs to eradicate the virus. In the area of gene therapy in human CD34+ hematopoietic stem and progenitor cells (HSPCs), CRISPR/Cas9 technology advanced quickly. With the help of RNA spacers, a transcript from brief segments of host DNA obtained from additional chromosomal elements, the Cas9 helicase, which is a component of the CRISPR-Cas9 machinery, can attach to RNA generated from the palindromic repeats of host DNA and cleave invasive DNA.[31] In 2013, the CRISPR/Cas9-based method was initially investigated for HIV/AIDS treatment. The target sites were the TAR sequences in the R region and the NF-κB binding cassettes in the U3 region of LTR, respectively. As a result, HIV-1 provirus transcription and replication were effectively inhibited.[32] More significantly, it is demonstrated that CRISPR/Cas9 could remove internal integrated viral genes from the chromosome of the infected host cell, indicating that it could be a useful therapy tool for HIV/AIDS. Soon after, studies on using CRISPR/Cas9 to remove the HIV-1 genome were carried out. In an HIV-1 latently infected T cell line, pro-monocytic cell line, and microglial cell line, they employed Cas9/gRNA to target conserved sites in the HIV-1 LTR U3 region.[33] This resulted in the inactivation of viral gene expression and the restriction of virus replication with minimal genotoxicity and no detectable off-target editing. It also showed that targeting multiple sites of the HIV-1 genome could increase the efficiency of excision and disruption of the non-integrated proviral genome. Furthermore, combining two potent single guide RNAs (sgRNAs) that target distinct areas of the HIV genome may stop the virus from replicating and escaping. Additionally, compared to single sgRNA-mediated SaCas9 editing, the combined SaCas9/gRNAs demonstrated greater efficacy in altering the HIV-1 genome.[34] However, a single, guide RNA (gRNA) mediated cleavage can allow the virus to escape. A combinatorial CRISPR/Cas9 gene-editing strategy can mitigate this viral breakthrough. CRISPR/Cas9 technology can be used to alter co-receptors to prevent the entry of HIV-1 via the CCR5/CXCR4 co-receptors and the CD4 receptor. Since CD4 is essential for a healthy immune system, blocking CD4 is not an ideal way to treat HIV-1 infection.[35]

According to the study done by Xu and Deng, CRISPR-edited cells were used in an HIV-positive patient by the Department of Hematopoietic Stem Cell Transplantation at the Hospital of the People's Liberation Army in Beijing.[36] The 27-year-old patient received a diagnosis of both HIV/AIDS and acute lymphocytic leukaemia (ALL). After a year, the HIV infection was under control due to antiretroviral treatment, and the virus was no longer detected in serum RNA. Once the researchers established that the HIV was CCR5-tropic, they found a male donor who had the unmutated CCR5 gene. The CCR5 locus was then edited using CRISPR. The patient was given both unedited CD34-depleted cells and newly modified CCR5 CD34-positive cells. When his CD4+ cell count rose to within the normal range and HIV RNA copies were still undetectable, the patient's antiretroviral treatment was discontinued seven months after alloHCT. The researchers report a successful allogeneic transplantation.[36]

In another study conducted by Khamaikaiwan *et al.,* patient with acute lymphoblastic leukaemia and HIV-1 infection was treated with a transplant of HSPCs with the CRISPR/Cas9-ablated CCR5 gene.[37] The sgRNAs were constructed with high cleavage efficiency to the couple at the start of the first exon of the human CCR5 gene at the Δ32 mutant location after being screened to exclude off-target potentials. After engrafting, the CCR5-knockout HSPCs showed the donor

cells' CCR5 ablation, which lasted for almost 19 months in peripheral blood. This could lead to the discovery of new sources of CCR5Δ32/Δ32 HSC donors. Hence, it can be observed that CRISPR-Cas9 technologies have shown promising potential as a treatment for HIV by offering the ability to directly edit the virus's DNA or modify human cells to resist infection.[37]

4. HIV Treatment regimen in Sri Lanka

Sri Lanka maintains a low-level HIV epidemic, with an estimated prevalence of less than 0.1%, making it one of the lowest in South Asia. By the end of 2018, approximately 3,500 individuals (range: 3,100–4,000) were living with HIV, with 350 newly reported cases that year, the highest annual increase since the first case was identified in 1987. Among key populations, such as female sex workers (FSW), HIV prevalence was reported as 0.1% in Colombo, 1.0% in Galle, and undetectable in Kandy during the 2014/2015 Integrated Bio-Behavioral Survey (IBBS). Despite 92% of FSW reporting the practice of safe sex with clients, only about one-third had undergone HIV testing within the previous year and were aware of their results, highlighting gaps in testing uptake and knowledge.[38]

In Sri Lanka, HIV treatment guidelines recommend ART initiation at a CD4 count of <500 cells/mm³, consistent with the 2013 WHO guidelines. For children aged below 1 year, ART is recommended irrespective of CD4 count. Additionally, Option B+, a program that provides lifelong ART. is implemented for the prevention of mother-to-child transmission (PMTCT), where all HIV-positive pregnant women are eligible regardless of their CD4 count. While treatment coverage and viral load testing are limited, efforts align with WHO guidelines to improve ART access and outcomes.[39]

A 34-year-old woman was diagnosed with HIV in 2011 in Sri Lanka and underwent antiretroviral therapy (ART). She had a baseline CD4 count of 295 cells/μL. Her initial ART regimen consisted of Zidovudine (AZT), Lamivudine (3TC), and Nevirapine (NVP). Her adherence to ART was inconsistent, and after a virological failure in 2016, a high viral load of 26,679 copies/mL and intermediate resistance to Efavirenz (EFV) was recorded. Subsequently, her treatment was switched to a second-line regimen comprising Tenofovir (TDF), Emtricitabine (FTC), and Atazanavir/Ritonavir (ATV/r) which gradually reduced the viral copies to 4200 copies/mL.[40]

According to the annual report by National STDs/AIDs Control Programme (NSACP) of 2023, nearly 96% of patients will be on DTG-based regimens in Sri Lanka. The most prescribed regimen for adults was Tenofovir Disoproxil Fumarate (TDF)+ Emtricitabine (FTC)+ Dolutegravir, and 86.4% of PLHIV were on that regimen. For the pediatric patients DTG 10 mg dispersible tablet was available from Global Fund (GF). PI was mostly restricted as second-line regiments and only 0.8% of patients were on PI-based regimens in 2023.[41]

5. Discussion

Although numerous treatment strategies have been implemented for treatment and management of HIV infection, they also present with their own advantages and limitations.

Protease inhibitors (PI) have multiple advantages including being able to reduce viral load and having high efficacy and bioavailability. Nonetheless, protease inhibitors also come with their set of disadvantages. One major limitation of PI is that cause a series of adverse effects, one being lipodystrophy syndrome – this is caused due to the inhibition of two proteins that play a crucial role in lipid metabolism. PI may bind to proteins, disrupting lipid metabolism, leading to fat redistribution and hence changes in the body's appearance.[42] Another adverse effect is that it can cause

hyperlipidemia, which involves a drastic increase in the patients' triglycerides and LDL cholesterol levels. The use of protease inhibitors also causes insulin resistance. This is because the drugs like Indinavir for example can lead to the disruption of the GLUT4 transport activity of glucose hindering glucose metabolism which leads to increased levels of glucose in the blood, and hence the development of exacerbate diabetes.[43] Another drawback is that they alter the cytochrome p450 enzyme system in the liver, specifically CYP3A. This presents itself as a disadvantage, due to them being able to increase or decrease the levels of co-administered drugs, like blood thinners, statins and antiarrhythmics leading to potential toxicity and low efficacy. Prolonged liver damage, with patients co-infected with Hepatitis B or C is a severe side effect.[44] Prevalent risks associated with the frequent use of PIs include- complexity when it comes to dosing regimens as not all protease inhibitors have been simplified into simple pill consumption, with some having to be still taken with food, which hinders the systemic absorption of the drug, reducing its efficacy, due to the action of GI resident enzymes.[45] Another risk includes the development of resistance to PIs - caused due to prolonged use, albeit slow resistance gradually builds over time. Another disadvantage is that protease inhibitors are a costlier means of treatment making it hard to access in resource-poor countries. PIs also impact the patient's quality of life due to the possibility of addiction.

Despite the significant findings, the limitations of the treatment methods have been increasing awareness in the medical industry. From the therapeutic standpoint, NRTIs used to treat HIV infection like Zidovudine (AZT), Zalcitabine (ddC), Didanosine, Stavudine and Lamivudine (3TC) serve as crucial medications with features that include significant long-term patient use of therapy, a growing patient population and absence of competition from preventive or curative vaccines soon. However, some reports report profound clinical side effects like mitochondrial dysfunction. Studies depict that mitochondrial toxicity (MT) is linked to impaired mitochondrial DNA (mtDNA) replication. Biochemically, this is associated with reduced levels of mtDNA, mitochondrial RNA (mtRNA), mitochondrial polypeptides, and abnormalities in mitochondrial ultrastructure, which align with micromolar mixed Kis values for dideoxy-NRTI triphosphates in various experimental models. Furthermore, NRTIs are associated with liver toxicity, hepatomegaly, steatosis and adult Reytts syndrome. Stavudine has proven links to painful peripheral neuropathy with acetyl-L-carnitine, lipodystrophy and adipocyte apoptosis. Among the frequently administered NRTIs, Lamivudine and Emtricitabine are notable for their comparatively good safety profile which is used in combination with other drugs in HAART regimens to prevent the emergence of HIV resistance mutations. While muscle toxicity associated with Lamivudine has been observed in clinical settings, there is currently no evidence of its toxicity in in vivo monotherapy studies. Emitracibine remains in observation to see if long-term toxicity is to occur in the future. Additionally, the toxicity caused by other NRTIs includes the inhibition of adenylate kinase, adenine nucleotide translocator, NADH oxidase activity, protein glycosylation, and the occurrence of a bystander effect. Nevertheless, clinical studies are not clear in extreme cases where the mortality and morbidity were severe.[46,14]

Referring to integrase inhibitors, both RAL and DTG are generally well tolerated, with low incidences of adverse effects.[47] RAL is associated with mild side effects such as headache and gastrointestinal symptoms but has minimal long-term safety concerns. DTG has shown excellent tolerability but is occasionally linked to weight gain and potential neural tube defects when used during early pregnancy, requiring careful consideration in specific populations. DTG has demonstrated superior viral suppression in clinical trials compared to RAL, particularly in patients with

prior treatment failure or integrase resistance. In addition, DTG's higher genetic barrier to resistance and pharmacokinetic profile contributes to its durability as a long-term treatment option.[48]

The Berlin and Geneva Patient case studies highlighted the importance of stem cell therapy in eliminating cancer cells and HIV viral load. The transplanted healthy stem cells establish themselves in the bone marrow, generating new blood cells.[27,29] However, stem cell therapy in HIV patients has several drawbacks that limit its widespread application. A major concern is the risk of graft-versus-host disease (GVHD) in allogeneic transplants, where donor cells may attack the patient's tissues, leading to organ damage as experienced by the Geneva patient. Additionally, there is always a possibility of cancer relapse in patients with both HIV and cancer, as the therapy might not eliminate all cancer cells. The procedure is also highly expensive and requires specialized facilities, making it inaccessible to many patients, particularly in low-resource settings. Another limitation is the intensive preconditioning required, such as high-dose chemotherapy or radiation, which can severely weaken the immune system, making the body more prone to infections. Recovery from stem cell therapy is often prolonged, with patients remaining vulnerable to complications for months or even years. There are challenging circumstances in finding compatible donors for allogeneic transplants like in Timothy's case study which required a CCR5-Δ32 mutation. The latent HIV reservoirs in the body remain a significant barrier to a complete cure. These reservoirs are unaffected by ART and can be reactivate if treatment is stopped. Ultimately, these cases provide valuable insights into the complexity of stem cell therapy for HIV cure which is believed to be resolved with advancements in future research.

Despite the cutting-edge technology of CRISP/Cas 9 systems, it poses various disadvantages to the recipients. A major concern of CRISPR/Cas 9 technology is its tendency to cause unintended gene mutations and chromosomal translocations. Significant off-target cleavage has been primarily observed in single-guide RNAs containing six or more mismatches. Strategies that have been developed to enhance the specificity and reduce the off-target cleavage include the development of dimerization-dependent RNA-guided FokI-dCas9 nucleases, limiting Cas9 expression in HIV-infected cells via a minimal HIV-1 promoter activated by Tat or delivering Cas9 as ribonucleoproteins (RNPs). However, the ribonucleoproteins delivered are known to trigger innate immune responses causing cytotoxicity. Furthermore, the escape mechanisms of HIV are conducive to reducing the long-term efficacy of CRISP/Cas 9 systems. Although CRISP/Cas 9 inhibits the viral replication of HIV, the mutations induced by non-homologous end joining repair around the cleavage sites facilitate the escape of the viral proteins[32].

6. Conclusion

Over the years the treatment for HIV has evolved to address the specific etiologies of the life cycle of the HIV. This review article discussed case studies of patients diagnosed with HIV-AIDS and their respective treatments. Amongst the treatment discussed above it's evident that so far, the most successful treatment has been the transplantation of stem cells with the CCR5Δ-32 mutation. However, medicinal drugs such as PIs, NNRTIs/NRTIs and integrase inhibitors are still prescribed during the early stages of HIV-AIDS as part of ART. Although the employment of CRISPR/Cas 9 technologies has been rare due to their complications and inaccessibility, these systems have been successful in most cases. While the complete eradication of HIV appears to be a formidable challenge, the use of proper treatment and management since the early stages of diagnosis ensures a higher quality of life and better overall well-being for the patients.

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