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The Search for the Most Efficacious Engagement Point in the Campaign Against HIV: A Closer Look at the Life Cycle of the Human Immunodeficiency Virus

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Abstract

The measurement of mortality and morbidity regarding HIV/AIDS are useful parameters in determining the efficacy of the current treatment methods regarding HIV/AIDS. Since there is no cure to HIV/AIDS, A reduction in morbidity and mortality remains the primary goal of treatment.

The human immunodeficiency virus belongs to a family of human retroviruses containing a non-segmental, linear RNA genome with a double-stranded DNA intermediate. HIV is transmitted through hematogenous spread by unprotected sex, mother to child (perinatal transmission), blood transfusions, or contaminated hypodermic needles. It is no longer considered an exclusively sexual disease. The human immunodeficiency virus specifically targets T-lymphocytes cells, causing damage and apoptosis of T-lymphocytes. The virus causes a decline in the total number of T-lymphocytes (CD4) within the body, increasing the infected individual's viral load. As the CD4 count decreases, the viral load increases.

The human immunodeficiency virus is challenging to treat and currently impossible to eradicate due to its vast number of mutations and molecular heterogeneity. No one mechanism of action by a particular medication can, in and of itself, decrease or eliminate replication or mutations. Thus, the goal in treatment is to control the virus's replication within the host and limit or suppress opportunistic infections.

Three leading organizations provide guidelines for the treatment of HIV: the World Health Organization, Center for Disease Control and Prevention, and the U.S. Department of Health and Human Services. Although each organization has distinct criteria, the primary goal of all is to limit the spread of and eventually eradicate HIV infections. Thus, the objective of antiretroviral therapy is to suppress viral load to preserve immunologic function, improve quality of life, reduce HIV-related morbidity and mortality, and control transmission. Viral load testing is an essential measurement in determining the effectiveness of any antiretroviral regimen or treatment.

One of the most effective ways to decrease replication and viral load is to limit the virus' protease enzyme system. Medications that inhibit the HIV protease enzyme system have been shown to decrease mortality. Protease inhibitors attack a vital point within the viral replication cycle. Medical research has shown that this class of drugs increases survival and decreases morbidity in the HIV-infected population. However, protease inhibitors have specific side effects that adversely affect patient compliance. Overall, protease inhibitors—although not a cure for HIV/AIDS—are valuable in controlling the spread of HIV and decreasing associated mortality and morbidity.

Keywords: *Gag Gene; Immunodeficiency; Integrase, Reverse Transcriptase; Zoonotic Infections*

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; ART: Antiretroviral Treatment; ARV: Antiretroviral; CDC: The Centers for Disease Control and Prevention; CYP450: Cytochrome P450; DNA: Deoxyribonucleic Acid; FDA: United States Food and Drug Administration; GP: Glycoprotein; GRID: Gay-Related Immune-Deficiency Disease; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; INSTI: Integrase Inhibitor; NRTI: Nucleoside Reverse Transcriptase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; OI: Opportunistic Infection; PI: Protease Inhibitor; RNA: Ribonucleic Acid; RT: Reverse Transcriptase; USDHHS: U.S. Department of Health and Human Services; UNAIDS: United Nations AIDS Organization; WHO: World Health Organization

Preface

Research has shown an improvement in survival for persons living with HIV infection. This improvement is partly due to better medication compliance, improvements in medical care, and drug development. Although mortality in the HIV population has decreased over the years, it remains higher than the general non-HIV population. A foundation for this study involved evaluating antiretroviral medication regimens that contain protease inhibitors to determine if survival rates increased.

Data was sourced from eligible studies (Africa, China, France, Malaysia, Rwanda, United Kingdom, and United States). Eleven studies met eligibility criteria (total n = 459,703). The random model was used for effect size. The risk ratio (RR) for death was 0.116 (95% CI: 0.115 - 0.117, p = 0.04). Out of a total of 459,703 people, 39,912 (8.7%) died, and 419,791 (91.3%) survived while on antiretroviral therapy. The Kaplan-Meier survival probability range was 0.61 to 0.95. Heterogeneity for the analysis was determined by Tau-squared and I-squared (0.727 and 99.95, respectively). The Odd ratio was 0.008 (95% CI: 0.003 to 0.020).

The foundational research indicated an increased survival rate with antiretroviral therapy that included a protease inhibitor for people living with HIV. Protease inhibitors seem to have fewer adverse effects, resulting in enhanced patient compliance, which may contribute to HIV-infected individuals' survival rate.

Introduction

The origin and history of the human immunodeficiency virus (HIV)

The exact year and country of the origin for the first HIV case remains ambiguous. The first documented cases in the United States occurred in 1981, as reported in the Morbidity and Mortality Weekly Report from the Centers for Disease Control and Prevention (CDC). The CDC report documented gay young men from Los Angeles, California, presenting with immunodeficiency after being diagnosed with pneumocystis carinii pneumonia [1,2].

The human immunodeficiency virus is thought to be of simian origin. However, scientists cannot agree upon how it was transferred to humans. Various theories have been posited in the scientific community. None of the theories, however, have been substantiated.

The CDC documented five cases of pneumocystis carinii pneumonia in gay men from Los Angeles, California, in 1981. According to the report, none of the men were related or had any relationship with one another. At that time, pneumocystis carinii pneumonia was uncommon in the United States, except in immunocompromised individuals. Moreover, those patients consisted mainly of cancer, genetic immunodeficiency, and chemotherapy treatment individuals [3,4].

After the 1981 CDC report was released, the medical community began to notice other healthy individuals succumbing to immunocompromised optimistic infections, such as cytomegalovirus and Kaposi sarcoma [5]. Cities with a diverse homosexual population started to experience most of the cases. Thus, it was believed that the spread of this medical phenomenon was related to the homosexual activity [2]. At that time, pentamidine isethionate was the sole medicinal treatment for pneumocystis carinii pneumonia. This drug is a protozocidal aromatic molecule that interferes with nuclear metabolism by inhibiting DNA, RNA, phospholipid, and protein synthesis. Consequently, for many patients, this treatment proved ineffective for curing pneumonia. Many afflicted people died, even with treatment [6].

Thirty-six years after this first CDC report, AIDS has been responsible for millions of deaths worldwide [7]. With no definitive origin or knowledge of how the virus was transmitted, early researchers mistakenly believed homosexual activity was the primary cause. Some of these early misconceptions arose as only a small group of individuals (homosexual population) appeared to be susceptible to the disease. Thus, the focus was on sexual behavior, not cellular dysfunction.

Two organizations maintain statistical analysis on AIDS/HIV infections. The United Nations AIDS Organization (UNAIDS) and the CDC. UNAIDS keeps track of HIV transmission, mortality, and people living with the condition worldwide. UNAIDS reported that globally, 36.7 million people were living with HIV as of 2016, while 1.8 million people become newly infected annually. One million people died from AIDS-related illnesses. It is now estimated that 76.1 million people have become infected with HIV since the start of the epidemic, while 35.0 million people have died from AIDS-related illnesses since the start of the epidemic. The CDC keeps similar statistics for Americans. Newly diagnosed HIV cases were 39,782 as of 2016. An estimated 1.1 million people were living with the disease at the end of 2015. It was also reported that 6,721 deaths resulted from HIV and AIDS in 2014 [8].

By the mid-1980s, the human immunodeficiency virus began to appear in the non-homosexual population [1]. Gradually, people outside of the homosexual sexual community began manifesting similar immune-compromised symptoms. Intravenous drug users, hemophiliacs, and non-homosexual persons began to express the virus's signs and symptoms [9]. These observations modified the "homosexual-activity" hypothesis for the spread of the virus.

Cellular-immune dysfunction became a new focus of research into the spread of the virus. In 1982, the CDC issued a new Morbidity and Mortality Weekly Report accounting for non-homosexual cases of pneumocystis carinii pneumonia. This new report detailed heterosexual males who were hemophiliacs [10]. The CDC reported on the possible transmission of an infectious agent through blood products [11]. Subsequent research established an infectious agent as the underlying cause of immunosuppression. Originally referred to as the gay-related immune-deficiency disease (GRID), Shilts (1982) coined the term "acquired immune deficiency syndrome". The term, acquired immunodeficiency syndrome (AIDS), was adopted in 1982, defining how it affected humans [1,2].

According to a 1982 CDC report, 593 cases of the disease appeared between June 1, 1981 and September 15, 1982, with a mortality rate close to 41% in the United States [12]. The CDC defined AIDS as "a disease, at least moderately predictive of a defect in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to that disease". The CDC definition included diseases like pneumocystis carinii pneumonia or Kaposi sarcoma. Any clinically unexplained opportunistic infections could fit the definition [13].

The medications available at this time were ineffective against opportunistic infections caused by this level of immune deficiency. A new approach had to be found that could target the debilitating progression of the disease. It was not until 1983 that a group of researchers from France determined that lymphadenopathy tissue (isolated from node lymphocytes in a French homosexual patient) was retroviral in nature [14].

The French research team determined that antibodies for this type of lymphadenopathy could serve as a marker in determining who had acquired the virus. In other high-risk groups, the same antibodies were noted, confirming that a virus was the principal etiological agent of AIDS. The pathophysiology of AIDS was postulated to be T4-cell tropism and T4-cell interacting with the virus [14]. Thus, the scientific community now focused on mechanisms to target entry points into the cell to disable or compromise the virus.

Identification and classification of human retroviruses and HIV

The human immunodeficiency virus belongs to a family of human retroviruses known as Retroviridae. This family of viruses contains non-segment, linear ribonucleic acid (RNA) genome with a double-stranded deoxyribonucleic acid (DNA) intermediate. The subfamily of Retroviridae is the non-oncogenic lentivirus [15]. This subfamily can cause disease in other species (non-human). Lentiviruses are known to cause disease in horses, sheep, cattle, and monkeys. It was hypothesized that this zoonotic process was the method of transfer to humans [16].

Four human retroviruses are recognized: two human T-lymphotropic viruses (HTLV-I and HTLV-II) that are transforming retroviruses, and two human retroviruses that are human immunodeficiency viruses (HIV-1 and HIV-2), indirectly or directly causing cytopathic ef-

fects. HIV-1 is the most common retrovirus globally [17]. This strain of retrovirus was first described as acquired immunodeficiency syndrome (AIDS) in 1981. The disease was first identified in homosexual men in New York City and Los Angeles in the United States. HIV-2 was first documented in West Africa in 1986 [18]. HIV-1 and HIV-2 are classified as zoonotic infections. Hence, the pan troglodyte species of chimpanzee may have been the source of HIV-1, while HIV-2 is more closely related to the simian immunodeficiency virus [16]. The HIV-2 strain has been limited to western Africa and southern Asia. Due to the relative rarity of HIV-2, the focus of this research is HIV-1.

HIV has spread across the globe since its early discovery in the 1980s. Since that time, public health efforts in the United States and internationally have been aimed at controlling the spread of the virus in the public sector. Currently, there is no cure; however, controlling the spread, decreasing mortality, and reducing morbidity are the domestic and international goals for coping with the disease [19]. Antiretroviral medications are the most effective means, to date, of achieving these goals [20].

Classifications and mechanisms of action of antiretroviral medications

There are several classifications of antiretroviral medications. The first class of medications used on HIV patients is reverse transcriptase inhibitors (Zidovudine, Didanosine, Zalcitabine, Stavudine, Abacavir, Tenofovir, Lamivudine, and Emtricitabine). These drugs target the viral enzyme, reverse transcriptase, needed for replication intracellularly [21]. The other classes of medications also attack enzymes or entry into the CD4 cell. These medications are classified as follows: entry inhibitors (Enfuvirtide, Ibalizumab, and Maraviroc), integrase inhibitors (Raltegravir, Dolutegravir, and Elvitegravir), non-nucleoside reverse transcriptase inhibitors (Rilpivirine, Etravirine, Delavirdine, Efavirenz, and Doravirine) and protease inhibitors (Tipranavir, Indinavir, Saquinavir, Fosamprenavir, Ritonavir, Darunavir, Atazanavir and Nelfinavir). This class of antiretrovirals shows promise in reducing viral load and decreasing mortality and morbidity.

Life cycle of the human immunodeficiency virus

Modes of transmission

The human immunodeficiency virus is transmitted through hematogenous spread. Its main portals of entry are through unprotected sex, mother to child (perinatal transmission), blood transfusions, or contaminated hypodermic needles [22]. Once inside the body, the virus has a proclivity for lymphocytes (CD4) or T-helper cells [23]. T-lymphocytes are part of the human immune system, helping orchestrate the body's response to foreign antigens. It is not clearly understood why HIV targets these cells primarily. Nevertheless, understanding the steps in the life cycle of this virus has helped researchers develop medications that interfere with replication and entry into healthy cells. HIV is an RNA virus that uses reverse transcription to convert RNA into DNA through the enzyme reverse transcriptase [24].

Viral attachment

Once inside the host, the virus must attach itself to cells. Attachment allows infusion into the cell, which involves binding between proteins on the surface of the virus and receptors on the surface of T-cells [25]. The lifecycle of HIV begins by binding the gp120 protein (via a portion of its V1 region near the N terminus of the virus) to a receptor on the host cell surface of a CD4 molecule [26]. Receptors on the surface of T-cells serve as communicators with other cells, signalers, and directors for the complement system during an immune response. There are two primary receptors targeted for attachment by the virus: CD4 and CCR5/CXCR4 [27]. The proteins used for viral attachment are glycoprotein-120 and glycoprotein-4l [28]. They attach to CD4 and CCR5/CXCR4, respectively [29].

CD4 protein molecules are predominantly a subset of T-lymphocytes that serve as helpers for the immune system. Once the gp120 protein binds to the surface of the CD4 molecule, a conformational change facilitates binding to a group of co-receptors [30]. These co-receptors are CXCR4 and CCR5 [31].

The CXCR4 receptor is located on T-cells. The CCR5 receptor is located on T-cells, macrophages, monocytes, and dendritic cells. Both co-receptors are cellular and allow entry into the cell. Individuals with homozygous mutations of the CCR5 receptor are immune or resistant to HIV because the virus cannot enter the cell [32]. Heterozygous mutations cause a slower progression of the disease due to delayed entry into cells. Binding to these receptors results in the fusion of the virus to cellular membranes, which is essential to the HIV lifecycle. Now, viral penetration and infusion can occur.

Penetration and viral infusion

Penetration into the cell allows for genetic material to be infused into the cell's cytoplasm. Glycoprotein-120 allows glycoprotein-41 to bind to chemokine receptors, resulting in structural changes in the CD4 cell necessary for viral fusion [33]. Once this change occurs, the viral envelope and cell membrane can be taken into direct contact, resulting in genetic exchange. Medications, termed fusion inhibitors (enfuvirtide and Fuzeon), prevent the binding of glycoprotein-41 and the chemokine receptor [34]. This medication delays the infecting of healthy CD4 cells; however, once a cell is infected, the virus life cycle continues to use reverse transcription to produce corrupt genetic material [35].

Reverse transcription

The virus releases its genetic RNA into the cell through reverse transcription. The viral RNA is then converted into DNA. This conversion allows the viral substance to be incorporated into the CD4 T-cell genetic matrix [36]. The virus uses an enzyme, called reverse transcriptase, to achieve transcription. The enzyme assists single-stranded viral RNA in the transcription of double-stranded DNA [37]. Thus, the virus gives the host cell instructions on replicating its viral DNA, mainly through nucleoside and nucleotide interaction.

Integrations

Once translation from RNA to DNA is completed, the new foreign DNA must be inserted into host cell DNA through a process is called integration, which takes place in the nucleus of the host cell. Integration involves transportation across the nuclear membrane once inside the cell [38]. The mechanism that the virus uses to accomplish this step is not fully understood. However, once the viral RNA has successfully entered the nuclear membrane, it uses an enzyme (integrase) to insert double-stranded DNA into the CD4 T-cell DNA [39].

The virus encodes reverse transcriptase to catalyze RNA- and DNA-dependent DNA synthesis. The result is double-stranded DNA copies of the viral genome [40]. Next, the genomic DNA enters the nucleus for integration into the host genome. Transcription of integrated virus DNA is accomplished by RNA polymerase II. This process produces RNA that can be spliced and translated into new virions [41]. HIV-1 contains genes that encode for the structural proteins of the virus. The gag gene encodes for the core of the virion [42], while the pol gene encodes for the protease enzyme responsible for processing viral proteins, reverse transcription, and integration [43]. The env gene encodes for the envelope glycoprotein layer [44]. After the successful integration of the viral DNA, protein synthesis can ensue. (There are three integrase inhibitors used medicinally against HIV: raltegravir, dolutegravir, and elvitegravir).

Protein amalgamation

Protein synthesis represents the virus' ability to replicate using host machinery as the producer of replication. Viral replication offspring are referred to as proviruses [45]. When the CD4 T-cell becomes activated, the provirus instructs intracellular equipment to produce apparatuses of HIV. From the viral DNA, two strands of RNA are constructed. Next, this RNA is transported out of the nucleus. At least one strand of genetic material is rendered into HIV protease, reverse transcriptase, integrase, and other structural proteins [46]. The remaining strand becomes genetic material for new viruses.

Viral assemblage

Once viral proteins are manufactured, they must be quarantined into separate entities before induction into viruses. This quarantine or cleavage is accomplished through viral protease enzymes [47]. (Medications that can prevent or interfere with this process could have a significant impact on limiting the destructive outcome from viral progression).

Replication

The last stage in the HIV life cycle is budding. Viral genetic material, bounded in the nucleocapsid, joins the cell's phospholipid membrane to form a new viral envelope. This envelope can now join and enter the host's circulation [48]. Once in circulation, the entire viral life cycle starts over again.

Specific genes work in tandem during replication. The gag gene can produce noninfectious virus-like molecules. Hence, the necessary gene information (needed for the assembly and production of virions) is contained within this gene [17]. The pol gene (a protease) is responsible for cleaving the gag gene and other polyproteins into their component subunits—a process known as maturation [49]. This maturation process involves the enhancement of the gag gene, required for progeny viruses to become pathologic and occur via a process of chemical inhibition or the mutation of essential amino acid residues [50]. Substitutions in gag gene molecules can wedge processing at sites where cleavage by protease commonly occurs. Thus, the HIV protease enzyme is used to cleave long polypeptide chains into smaller proteins that are assembled into a new virus particle [51]. The replicating virus conveys virion buds from the host cell using host cell glycoproteins to encapsulate foreign viral RNA and DNA. These new proteins serve as ligands for the virus to bind CD4 cells [52].

Summary of the HIV lifecycle

The HIV life cycle specifically targets T-lymphocytes cells (CD4). The virus causes damage to and apoptosis of T-lymphocytes [53]. The exact mechanism of apoptosis is not fully understood; however, several hypotheses have been proposed:

- Once a lymphocyte is infected with the virus, an internal signal may communicate apoptosis, instructing cells to commit suicide [54].
- As HIV elements bud from the cell, the phospholipid membrane may be severely damaged, resulting in loss of the cell [55].
- Infected cells may be recognized by immune system cells, which destroy them [56].

During the early stage of the infection (2–7 weeks), a person may have no symptoms; however, some may experience influenza-like symptoms (fever, generalized weakness, or headaches).

Overall, the virus causes a decline in the total number of T-lymphocytes (CD4) within the body. As the viral load increases, the CD4 count decreases. The viral load is measured as HIV RNA per cubic millimeter of blood. Over time, there is a deficiency in the number of CD4 cells required to defend the body. Once a critical deficit is reached (CD4 ≤ 200 cells/uL or CD4 percentage < 14%), a person is said to have acquired immunodeficiency syndrome [57]. These criteria constitute the CDC's definition of AIDS. The WHO's 2007 revision is based on stages of clinical findings, not requiring a CD4 cell count. Specific clinical conditions and or symptoms define these stages.

Regardless of which system is used to establish AIDS, the individual is now susceptible to infections that a normal, healthy immune system could contest. There is a distinction between HIV and AIDS in a person. HIV is the pathological virus that causes AIDS [58]. Thus, someone cannot have AIDS and not be infected with HIV. Thus, AIDS is the final phase of an HIV infection.

HIV-1 molecular heterogeneity

HIV-1 is known to contain at least six other genes (vpu, tat, nef, vpr, rev, and vif) that code for the proteins responsible for viral growth, regulation, and expression [59]. Several of these genes may also contribute to the pathogenesis of HIV in humans. Unlike HIV-1, HIV-2 lacks the gene vpu but has a vpx gene [59], which may explain the differences in molecular heterogeneity between the two strains of the virus.

HIV-1 has varying levels of diverse regions throughout its viral genome. One of the most diverse areas is the viral envelope that can have up to 50% variability [60]. This variability is due to insertions, deletions, substitutions, recombinations, and gains or losses at glycosylation sites of the virus. This molecular heterogeneity causes the virus to have multiple subtypes throughout the world. For example, subtype B (which is more common in the United States, Canada, and South America) can differ by up to 17% in its env gene coding sequence [6]. In contrast, subtype C is more prevalent worldwide, with many countries having both subtypes present in their respective populations [6].

The global presence and prevalence of HIV subtypes

There are nine or more subtypes worldwide: subtypes A, B, C, D, (F, G, H, J, K), CRF01_AE, CRF02_AG, CRF03_AB, and various other combinations [61] that are generally prevalent in specific geographic regions as follows:

- Subtype A: Central, East Africa, and Eastern European countries, including the Soviet Union.
- Subtype B: West and Central Europe, the Americas, Australia, Southeast Asia, Northern Africa, and the Middle East.
- Subtype C: Sub-Saharan Africa, India, and Brazil.
- Subtype D: North Africa and the Middle East.
- Subtype F: South and Southeast Asia.
- Subtype G: West and Central Africa.
- Subtypes H, J, and K: Africa and the Middle East.

Due to the vast amount of mutations and molecular heterogeneity, HIV is a problematic virus to treat medicinally. Consequently, no one mechanism of action by a particular medication can solely decrease or eliminate replication or mutations. Thus, the goal in treatment is to control replication by decreasing the amount of replication needed to suppress opportunistic infections that are the cause of morbidity and mortality in the HIV population [3,6].

Anti-HIV pharmaceuticals: ARTs

Antiretroviral medications, termed nucleoside reverse transcriptase inhibitors (NRTIs), block HIV's reverse transcriptase from reacting with nucleotides. NRTIs include emtricitabine, lamivudine, zidovudine, didanosine, tenofovir, stavudine, and abacavir. Their mechanism of action is by immitating nucleosides in CD4 cytoplasm. The virus incorporates the imposter nucleoside into its increasing chain of DNA [62]. The drug nucleoside prevents the double strand of DNA from replicating.

Another class of medications, named non-nucleoside reverse transcriptase inhibitors (NNRTIs), work by preventing transcription. Currently, there are five approved medications in the United States from this drug class: etravirine, efavirenz, nevirapine, rilpivirine and delavirdine. They function by inhibiting reverse transcription through the attachment of the enzyme reverse transcriptase [63]. This at-

tachment causes malfunction of the enzyme, and transcription stops.

Thus, there are two pathways to attack replication: 1) at the site of DNA elongation and 2) at the enzyme site [64]. If the virus can overcome these two medicinal roadblocks, it will move on to the next step in replication, known as integration.

Medications classified as protease inhibitors (PIs) attack this pressure point within the viral replication cycle. Medical literature has validated that this class of drugs increases survival and decreases morbidity in the HIV-infected population [65]. The following medications are currently available in the United States market under the category of protease inhibitors: Tipranavir, Indinavir, Saquinavir, Fosamprenavir, Ritonavir, Darunavir, Atazanavir, and Nelfinavir.

The protease inhibitor class has several side effects that may affect compliance [66]. Side effects have caused non-compliance, leading to resistance [67–69]. In individuals with protease inhibitor strains, cleavage can be efficaciously implemented. The viral subunits combine to make new virions. The new virions or viral RNA forms a lattice with the cell's phospholipid membrane [70]. This process allows a nucleocapsid to form. This budding or zinc finger is the last step in replication [71]. Current medical researchers are targeting this step. Chemical entities, called zinc finger inhibitors, are postulated to interfere with the viral RNA packaging into the nucleocapsid.

Protease inhibitors as an effective therapy

The HIV-1 protease enzyme is a member of the aspartic proteinase family. It is a homodimer formed by two 99-amino acid subunits [72]. Each monomer contributes two catalytic aspartic acid residues, located on the substrate-binding cleft of the enzyme [43]. They synchronize water molecules used for the hydrolysis of peptide bonds.

Nine sites are sliced by the HIV protease enzyme [73]. The specificity for these sites is based on exchanges between the amino acids connecting the bond and the subsites of the enzyme's active site. These amino acids are essential to efficient cleavage by the protease enzyme.

To date, the United States Food and Drug Administration (FDA) has approved eight protease inhibitors for the treatment of HIV infections: Tipranavir, Indinavir, Saquinavir, Fosamprenavir, Ritonavir, Darunavir, Atazanavir, and Nelfinavir. These drugs are transition state analogs that mimic substrate peptide after nucleophilic attack by a water molecule [74]. In the transition phase, protease enzymes bind tightly to the ligand; however, protease inhibitors compete for the substrate peptides by binding to the ligand's active site. This specificity for the site is due to hydrophobic moieties that imitate hydrophobic side chains of the protease substrate sequences in gag and gag-pol polyproteins [75].

Protease inhibitors cannot decrease the amount of the virus produced; however, the new viruses produced are typically noninfectious [76], and not all protease activity is inhibited. Thus, an individual can still transmit the virus to others while on these medications. Consequently, even partial inhibition of gag and polyprotein processing appears to have an impact on the ability of HIV to infect new cells [77]. With the half-life of infected cells in HIV-infected individuals being approximately two days, this interruption of the infectious cycle can lead to measurable reductions in viral load over short periods.

The effect of antiretroviral medications on mortality

A 2017 study conducted by The Antiretroviral Therapy Cohort Collaboration group analyzed data from eighteen European and North American HIV-1 cohorts. This study is one of the most extensive studies conducted, revealing a correlation between antiretroviral medications and a decrease in mortality. The study considered the survival rates in patients on antiretroviral medications from 1996 to 2013 [8,78].

Patients included in the study had to be aged ≥ 16 years and who began antiretroviral treatment (ART) with three or more drugs between 1996 and 2010 and had at least three years of proposed follow-up. The researchers adjusted for age, sex, AIDS, risk group, CD4 cell

count, and HIV-1 RNA (at the initiation of ART). The research goal was to identify all-cause and cause-specific mortality hazard ratios for the first, second, and third year after ART initiation in four calendar periods (1996–99, 2000–03, 2004–07, 2008–10) [8,78].

The study included 88,504 patients. In the first year, 2106 patients died; 2302 died during the second or third year of treatment. Patients starting ART in 2008–2010 had lower all-cause mortality in the first year after ART initiation than did patients starting ART in 2000–2003 (adjusted HR 0.71, 95% CI 0.61 – 0.83). All-cause mortality in the second and third years after initiation of ART was also lower in patients who started ART in 2008–2010 than in those who started in 2000–2003 (0.57, 0.49 – 0.67); this decrease was not fully explained by viral load or CD4 cell count at year-1. Rates of deaths were lower in patients who started ART in 2008–2010 (vs. 2000–2003) in the first year (0.48, 0.34 – 0.67) and second and third years (0.29, 0.21 – 0.40) after initiation of ART. Between 1996 and 2010, life expectancy in the 20-year-old patient group was about nine years in women and ten years in men. Even in late ART initiation, survival during the first three years was better due to less toxic antiretroviral drugs, better adherence, preventative medicine interactions, improvements in the management of comorbidity, and enhanced medical guidelines [8,78].

HIV antiretroviral treatment (ART) guidelines

Three primary agencies have guidelines for the treatment of HIV infection in humans: the CDC, WHO, and USDHHS. Although these agencies have similar recommendations, there are differences in the recommended treatment for HIV. The CDC and USDHHS are United States agencies that serve a patient population distinct from other countries. The United States healthcare system and economic resources are diverse regarding patient care [79].

Centers for Disease Control and Prevention (CDC) guidelines

The CDC's treatment goal is the eradication of HIV infection; however, eradication cannot be achieved with the currently available antiretroviral regimens. The latency of infected $CD4^+$ T cells is established during the earliest stages of acute HIV infection and persists with a long half-life, even with prolonged suppression of plasma viremia to < 50 copies/mL [80]. Thus, the primary goal of antiretroviral therapy is to suppress viral load to preserve immunologic function, improve quality of life, reduce HIV-related morbidity and mortality, and control transmission [8]. To accomplish these goals, the CDC recommends HIV screening for high-risk groups (gay, bisexual, and other men who have sex with men, intravenous drug users, sex workers, prisoners, transgender people, and women) at least once in a lifetime. HIV positive patients require more frequent testing [8]. Measurement of a viral load should be taken at the time of diagnosis and every 3–4 months thereafter in the untreated patient. $CD4^+$ T cell counts should be measured at the time of diagnosis, and every 3 – 6 months thereafter [81].

The CDC has established timelines on when to start treatment based on the patient's viral load level. Previously, the CDC recommended withholding the start of treatment until the viral load had fallen to a low level [81]. The current guidelines recommend initiating treatment as soon as an HIV diagnosis is made. It has been determined that antiretroviral medications slow the progression of HIV and facilitate long-term positive outcomes.

Viral load testing is an essential measurement in determining the effectiveness of an antiretroviral regimen. Approximately 70–90% of antiretroviral drug-naïve patients achieve high viral load suppression within 6–12 months of therapy initiation [8]. Predictors of virologic success include low baseline viremia, high baseline $CD4^+$ T cell count, a rapid decline of viremia to < 50 HIV RNA copies/mL, adequate serum levels of antiretroviral medication, and adherence to drug regimens.

The CDC also recommends the rational sequencing of drugs for the preservation of future treatment options. This recommendation allows a backup alternative in case the virus mutates and becomes resistant to the current regimen. Alternative regimens include a protease inhibitor (PI) with two nucleoside reverse transcriptase inhibitors (NRTIs), a non-nucleoside reverse transcriptase inhibitor (NNRTI) with two NRTIs, or a 3-NRTI. A class-sparing regimen aims to preserve or spare > 1 classes of drugs for later use. The protease inhibitor

class is a significant part of the recommended use of antiretroviral medication. According to the CDC, the efficacy of protease inhibitors containing antiretroviral regimens includes the following outcomes: durable viral load suppression, partial immunologic restoration, and decreased incidence of AIDS and death. Viral load suppression and $CD4^+$ T- cell responses have also been detected in PI-sparing regimens (e.g. efavirenz plus two NRTIs or abacavir plus two NRTIs). However, it is unknown whether these PI-sparing regimens provide comparable efficacy in clinical outcomes [8].

World Health Organization (WHO) guidelines

The WHO guidelines differ slightly from those of the CDC, regarding what is monitored after initiation of treatment and in response to therapy. The WHO monitors CD4 count, not viral load. Viral load testing can be cost-prohibitive for some poorer countries [82]. Thus, CD4 has become the global marker for monitoring HIV progression or regression in patients on ART.

In 2015, the WHO changed its recommendations. Previously, it was recommended that HIV patients with a specific CD4 count (> 500 cells/mm^3) withhold initiation of treatment until the immune status or clinical manifestations of the disease were noted. The current guidelines recommend that all people living with HIV start ART irrespective of clinical or immune status. This shift towards earlier initiation of ART, together with improved access to HIV testing and treatment, has led to an overall improvement in health status at the start of ART [83]. Nevertheless, globally, an enormous number of patients still lack access to testing or medication, which necessitated further changes in the WHO guidelines [84].

The WHO uses four clinical stages in its treatment guidelines to differentiate a patient's level of infection and disease, described as follows.

Clinical stage 1

- Asymptomatic
- Persistent generalized lymphadenopathy.

Clinical stage 2

- Moderate and unexplained weight loss (< 10% of presumed or measured body weight)
- Recurrent respiratory tract infections (such as sinusitis, bronchitis, otitis media, or pharyngitis)
- Herpes zoster
- Recurrent oral ulcerations
- Papular pruritic eruptions
- Angular cheilitis
- Seborrhoeic dermatitis
- Onychomycosis (fungal nail infections).

Clinical stage 3

Conditions in which a presumptive diagnosis can be made on the basis of clinical signs or simple investigations.

- Unexplained chronic diarrhea for longer than one month
- Unexplained persistent fever (intermittent or constant, lasting longer than one month)
- Severe weight loss (> 10% of presumed or measured body weight)
- Oral candidiasis
- Oral hairy leukoplakia
- Pulmonary tuberculosis (TB) diagnosed in the last two years
- Severe presumed bacterial infections (e.g. pneumonia, empyema, meningitis, bacteremia, pyomyositis, or bone or joint infection)
- Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis.

Conditions in which confirmatory diagnostic testing is necessary.

- Unexplained anemia (< 80 g/l), and or neutropenia (< 500/µl), and or thrombocytopenia (< 50 000/µl) for longer than one month.

Clinical stage 4

Conditions in which a presumptive diagnosis can be made based on clinical signs or simple investigations.

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital, or anorectal of longer than one month)
- Esophageal candidiasis
- Extrapulmonary tuberculosis
- Kaposi's sarcoma
- Central nervous system toxoplasmosis
- HIV encephalopathy.

Conditions in which confirmatory diagnostic testing is necessary.

- Extrapulmonary cryptococcosis, including meningitis
- Disseminated nontuberculous mycobacterial infection
- Progressive multifocal leukoencephalopathy

- Candida of trachea, bronchi, or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or of an organ other than the liver, spleen, or lymph nodes)
- Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, or penicilliosis)
- Recurrent non-typhoidal salmonella septicaemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Visceral leishmaniasis.

In 2016, the WHO modified its guidelines for antiretroviral drugs in treating and preventing HIV infection. It identified four groups of people with specific risks:

1. Individuals presenting or returning to care with advanced HIV disease (WHO stage 3 or disease and or CD4 < 200 cells/mm^3); such individuals may be ART naive or have interrupted treatment.
2. Individuals presenting or returning to care when clinically well (absence of WHO clinical stage 3 or 4 disease and or CD4 cell count ≥200 cells/mm^3); such individuals may be ART-naive or have interrupted treatment.
3. Individuals who are clinically stable on ART.
4. Individuals receiving an ART regimen that is failing.

The goal is to give these four groups of patients additional attention that is needed to reduce morbidity, transmission, and mortality [84].

U.S. Department of Health and Human Services (USDHHS) guidelines

The USDHHS takes a different approach from the CDC and WHO by determining regimen resistance to tailor treatment. This method aims to start the most efficacious combination of medications, giving the patient the best chance for successful viral suppression and CD4 preservation. Thus, in antiretroviral therapy-naive patients, HIV drug-resistance testing is recommended for persons at the entry of care to direct the selection of the initial ART regimen. Moreover, according to 2017 guideline changes, initiation of ART should not be delayed while awaiting resistance testing results.

Genotypic testing is the preferred method for resistance testing to guide therapy in antiretroviral-naive patients. Nevertheless, in patients with acute or recent HIV infection and pregnant HIV-infected women, ART initiation should not be delayed pending test results. The regimen can be modified once the results are reported. Standard genotypic drug-resistance testing in ART-naive persons involves testing for mutations in the reverse transcriptase and protease genes [85]. If integrase inhibitor (INSTI) mutation is suspected, the inclusion of

this genotype testing may be beneficial [23]. HIV drug-resistance testing should also be performed to assist in selecting active drugs when changing ART regimens in the following patients:

- In patients with virologic failure and HIV RNA levels > 1000 copies/mL.
- In patients with HIV RNA levels > 500 copies/mL but < 1000 copies/mL.

It is important to note that drug-resistance testing may be unsuccessful but should still be considered. Drug-resistance testing should also be performed under the following circumstances:

- When managing suboptimal viral load reduction.
- When a patient experiences virologic failure while receiving an INSTI-based regimen, genotypic testing for INSTI resistance should be performed to determine inclusion of a drug from this class in subsequent regimens.
- Drug-resistance testing in the setting of virologic failure should be performed while the person is taking prescribed ART drugs or, if not possible, within four weeks after discontinuing therapy.
- If more than four weeks have elapsed since the antiretroviral (ARV) drugs were discontinued (resistance testing may still provide useful information to guide therapy; however, it is crucial to recognize that previously selected resistance mutations can be missed).
- Genotypic testing is recommended as the preferred resistance testing to guide therapy in patients with suboptimal virologic response or virologic failure while on first-line or second-line regimens.

The addition of phenotypic to genotypic testing is preferred for persons with known or suspected complex drug-resistant ART. This testing preference is recommended for all HIV-infected individuals irrespective of CD4 cell count to reduce the morbidity and mortality associated with HIV infection [85]. ART should be continued indefinitely to improve and maintain immunologic function and viral suppression,

Conclusion

Currently, there is no cure for HIV. Thus, reducing morbidity and mortality remains the goal of treatment. Measurement of these two parameters serves as viable determinants regarding treatment success, especially considering protease inhibitors and their overall effect on mortality and morbidity.

The human immunodeficiency virus belongs to a family of human retroviruses known as Retroviridae, containing a non-segmental, linear RNA genome with a double-stranded DNA intermediate. HIV is transmitted through hematogenous spread by unprotected sex, mother to child (perinatal) transmission, blood transfusions, or contaminated hypodermic needles. Once inside the cell, the virus encodes reverse transcriptase to catalyze RNA- and DNA-dependent DNA synthesis. The result is double-stranded DNA copies of the viral genome. The HIV life cycle specifically targets T-lymphocytes cells (CD4). The virus causes damage to and apoptosis of T-lymphocytes. Overall, the virus provokes a decline in the total number of T-lymphocytes (CD4) within the body. As the viral load increases, the CD4 count decreases.

Due to the vast number of mutations and molecular heterogeneity, HIV is a problematic virus to treat medicinally. Consequently, no one mechanism of action by a particular medication can solely decrease or eliminate replication or mutations. Thus, the goal in treatment is controlling replication by decreasing the amount of replication needed to suppress opportunistic infections—the primary cause of morbidity and mortality in the HIV population. Viral load testing is an essential measurement in determining the effectiveness of an antiretroviral regimen.

Three leading organizations provide guidelines for the treatment of HIV: the World Health Organization, the Center for Disease Control and Prevention, and the U.S. Department of Health and Human Services. Although each organization has distinct criteria, the primary goal of all organizations is to limit the spread of and eradicate HIV infection. Thus, the purpose of antiretroviral therapy is to suppress viral load to preserve immunologic function, improve quality of life, reduce HIV-related morbidity and mortality, and control transmission.

One of the most effective ways to decrease replication and viral load is to limit the protease enzyme system. Medications that inhibit the HIV protease enzyme system have been shown to decrease mortality. Protease inhibitors attack a vital point in the viral replication cycle. Medical research has demonstrated that this class of drugs increases survival and decreases morbidity in the HIV-infected population. They result in fewer adverse effects than other pharmaceutical treatments. However, they have specific side effects that may disrupt patient compliance. Overall, protease inhibitors—although not a cure for HIV/AIDS—are valuable in controlling the spread of HIV and decreasing associated mortality and morbidity.

Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Supplementary Information

The intention of the authors is to publish three interdependent papers on this topic—this being the first and foundational paper; the second being on ART (pharmacology, compliance, affordability, and adverse effects), highlighting the protease inhibitors; and the third being an extensive meta-analysis of ART in HIV, in particular life expectancy or survival. The three papers will be made available through E-Cronicon of the United Kingdom by the same team of researchers and authors.

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