Chapter 1

Mechanism of HIV-1 Reverse Transcriptase Inhibitors

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Abstract

The HIV life cycle involves a series of intricate steps: viral entry, reverse transcription, integration into the host genome, transcription and translation, assembly, budding, maturation, and release. The reverse transcriptase (RT) enzyme, a pivotal player in this cycle, facilitates the conversion of viral RNA into double-stranded DNA during reverse transcription. Comprising polymerase and RNase H domains, RT's structure is crucial for its multifunctional role. The polymerase domain synthesizes a complementary DNA strand, while the RNase H domain degrades the RNA template. This enzymatic process results in the formation of a provirus integrated into the host cell's genome. Inhibitors targeting RT, classified into non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs), disrupt this critical step in the HIV life cycle. NNRTIs act allosterically to inhibit RT's activity, while NRTIs function as chain terminators during DNA synthesis, collectively impeding the virus's replication and offering crucial therapeutic interventions in managing HIV infections. Our book chapter covers the fundamental life cycle of HIV, the working mechanism of the RT enzyme, and the effects of inhibitors on this mechanism. The enzyme structure has been visualized using the UCSF Chimera program .

INTRODUCTION

The human immunodeficiency virus (HIV) is thought to have been transmitted from non-human primates to humans over the course of the 20th century. The emergence of HIV as the causative agent of Acquired Immunodeficiency Syndrome (AIDS) was identified shortly after the initial reports of the disease. Since then, HIV has become a global public health concern, with a significant proportion of the world's population being

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affected by the virus. Current estimates suggest that HIV positve globally exceeds 75 million.

The initial diagnosis of HIV infection occurred in 1981, signaling the start of the global HIV/AIDS pandemic. Since then, substantial advancements have been made in the fields of prevention, diagnosis, care and treatment of HIV/AIDS worldwide. Through the identification of newer, more effective and less toxic drug molecules, a decrease in the cost of therapy, and the implementation of innovative approaches to service delivery and treatment access, the disease has been transformed from a rapidly fatal condition to a manageable chronic illness. According to UNAIDS, global estimates indicate that 37.6 million individuals were living with HIV in 2021, with substantial variation in the numbers of affected individuals among different countries. Additionally, 1.5 million people acquired HIV worldwide in 2020, and an estimated 690,000 deaths occurred due to AIDS-related illnesses in the same year. Despite these successes, it remains a priority to ensure that all individuals living with HIV have access to antiretroviral therapy (ART) in order to reduce mortality and comorbidities and to curb further transmission of the virus (UNAIDS, 2022).

1. Life cycle of HIV

HIV is classified in the lentivirus subfamily of retroviruses, which are known to cause chronic, progressive infections. The term "lentivirus" is derived from the Latin word "lentus", meaning "slow", in reference to the prolonged incubation period of these viruses. $CD4^+$T lymphocytes, which are vital in the immune system, are the primary target of HIV. HIV gets into these cells and then kills these cells. Two types of HIV have been identified: HIV-1 and HIV-2 (National HIV Testing Guidelines, 2015). Lentiviruses are known as enveloped RNA viruses with a positive-sense, single-stranded genome. Once inside the cell, viral RNA is converted into double-stranded DNA by the reverse transcriptase enzyme packaged within the virion. HIV's genetic material has now become compatible with the nucleus of the cell. Viral DNA is transported to the nucleus of the cell and integrated into the host's DNA in cooperation with host cofactors via the integrase enzyme, also encoded by the viral genome. (Smith JA and Daniel R, 2006).

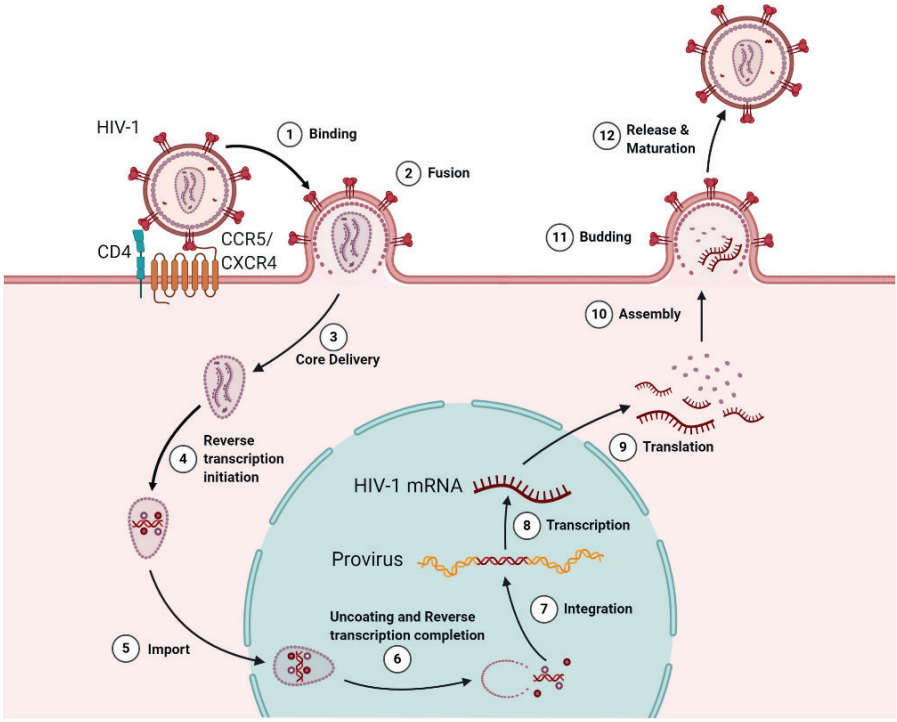


Figure 1. The HIV lifecycle (Ramdas P, et al. 2020)

The basic steps of the HIV life cycle (**Fig 1.**) can be summarized as follows:

1. **Binding:** As the initial stage of HIV infection, HIV must bind to host cell receptors. Host cell receptors (chemokine receptors) of the viral envelope glycoprotein bind with CXCR4 or CCR5 proteins. Specifically, the viral envelope protein binds to the CD4 receptor on the surface of the target cell, while a second viral protein, known as the co-receptor, must bind to one of two chemokine receptors (CXCR4 or CCR5) on the cell surface. These interactions pave the way for the virus to enter the host cell and allow it to initiate the replication process. (**Fig. 1.** Binding 1).
2. **Fusion:** Once binding is achieved, the virus content integrates with the host cell's membrane, causing the viral genetic material to enter the host cell and the "infection" process to begin (**Fig. 1.** Fusion).
3. **Core Delivery:** The virus content is completely transferred to the cell cytoplasm (**Fig. 1.** Core Delivery).

4. **RT Initiation:** The process of reverse transcription, catalyzed by the reverse transcriptase enzyme, initiates within the cytoplasm of the host cell (**Fig. 1.** Reverse transcriptase initiation).
5. **Import:** Thanks to the RT enzyme, the viral genome begins to become compatible with the host cell genome. In other words, DNA begins to be produced from RNA. The resulting DNA fragment passes into the nucleus (**Fig. 1.** Import).
6. **Uncoated and RT completion:** Once inside the host cell nucleus, the viral genome is not packaged like nuclear DNA and the reverse transcription process is completed (**Fig. 1.** Uncoated and Reverse Transcription completion).
7. **Integration:** Viral integrase enzyme is the enzyme that ensures the integration of viral DNA compatible with the host genome into the host genome and controls the process. In this step, the viral DNA is integrated into the host DNA (**Fig.1**. Integration).
8. **Transcription and translation:** The host cell sees the viral DNA as its own DNA and the process of producing viral proteins begins. Like the host's own genes, viral genes undergo transcription and translation (**Fig. 1.** Transcription and translation).
9. **Maturation:** In the final step of the HIV life cycle, all of HIV's proteins have now been produced. Viral RNA and viral proteins are brought together and packaged. As a result, a virion was formed. These virions move towards the cell membrane and are released into the extracellular environment by budding of the cell. The virus progeny begins a maturation process so that it can infect new hosts. (**Fig. 1.** Assembly, Budding and Release & Maturation) (Ramdas P, et al. 2020).

Anti retroviral therapy (ART) method has been adopted for the treatment of HIV infection. This method cannot completely clear the HIV infection from the host cell, but it stops the HIV replication process. Enzymes that play an active role in the HIV life cycle are inhibited, preventing the infection from progressing and causing AIDS. When ART is interrupted, viral replication quickly restarts. For this reason, continuity of ART application is very important. Disruptions in ART application are directly related to ART resistance (WHO, 2022).

2. Reverse Transcriptase

Temin and Baltimore discovered the reverse transcriptase (RT) enzyme with their work in 1970. After it was identified that RNA viruses have RNA-dependent DNA polymerase (RDDP) activity in their virions, studies on this enzyme accelerated. RT function is characterized by facilitating the synthesis of DNA complementary to RNA. While RNA is formed from DNA in cellular processes, the function of this enzyme runs counter to the central dogma of molecular biology. As a result of the discovery of RT, viruses that encode RT and use it as an important step in the virus life cycle were called retroviruses. Later studies revealed that RT also has DNA-dependent DNA polymerase (DDDP) function and can cleave RNA through ribonuclease H (RNase H) activity. With developing technologies, retroviruses and RT studies, especially the function of HIV RT in viral replication, have been shown to be very important and have attracted the attention of scientists.

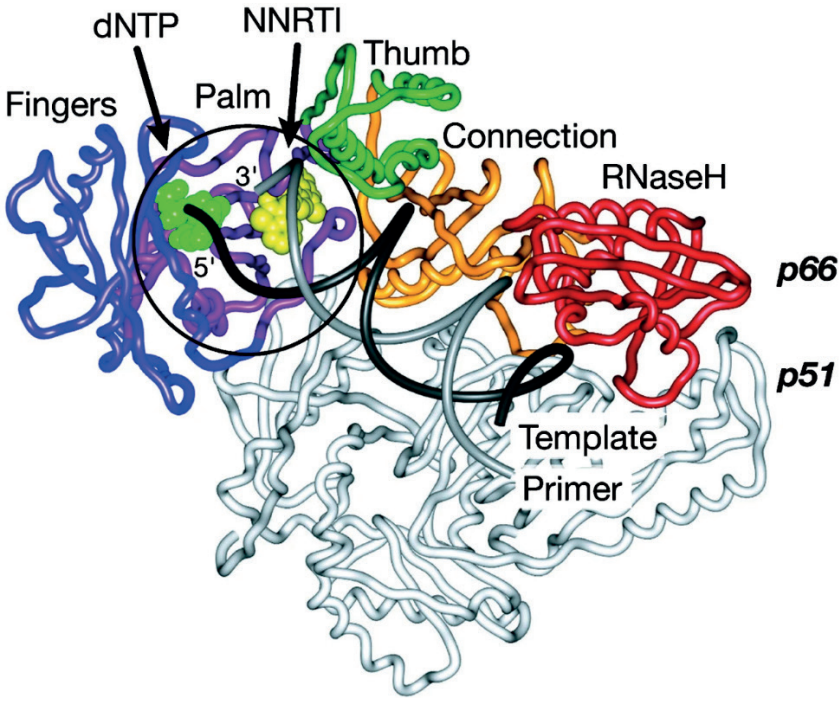


Figure 2. Structure of HIV RT (Pata JD, et al. 2004)

The RT enzyme produced by the Gag-pol gene consists of a 66-kD subunit of 560 amino acids (p66) and a 51-kD subunit of 440 amino acids (p51). When its structure is examined, it is an asymmetric heterodimer (Figure 2.). In these dimers, one p66 subunit is enzymatically active while the other copy

(p66') provides structural support. The RNase H domain, known to be localized at the C-terminus of p66, is disordered. The initial RT structure is cleaved by HIV protease, forming functional RT as a p66/p51 heterodimer; The p51 subunit contains approximately 440 amino acid residues. The first crystallization study of the RT enzyme was resolved in complex with the non-nucleoside reverse transcriptase inhibitor nevirapine (NVP). This structure is in complex with double-stranded DNA. As a result of this study, the RT structure provided insight into the basic properties of the multifunctional enzyme. As with the molecular structure of all other polymerases, the polymerase domain of RT consists of the fingers (1-85 and 118-155), palm (86-117 and 156-236), subdomains (319-426), thumb (237-237) 318) and is characterized by a hand-like conformation with connectivity. When the molecular structure of RT is examined in detail, the inactive p51 subunit and the p66 subunit have the same polymerase subdomains. In addition, it has become clear that the spatial localization of p51 results in an asymmetric organization of the heterodimer. The RT enzyme has been demonstrated to interact with the template/primer in a cleft extending from the polymerase active site containing catalytic aspartates (D110, D185, and D186) to the RNase H active site. The active sites are separated by a duplex length of 18 nucleotides (Ruiz F., etall, 2020).

2.1. Inhibitors of RT

Scientists are working intensively on the treatment of HIV infection. Many RT inhibitors have been developed for clinical use. The development of anti-HIV drugs falls into two main classes: nucleoside/nucleotide RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). These inhibitors have been widely incorporated into HIV treatment regimens and continue to contribute significantly to the management of AIDS (**Fig.3.**).

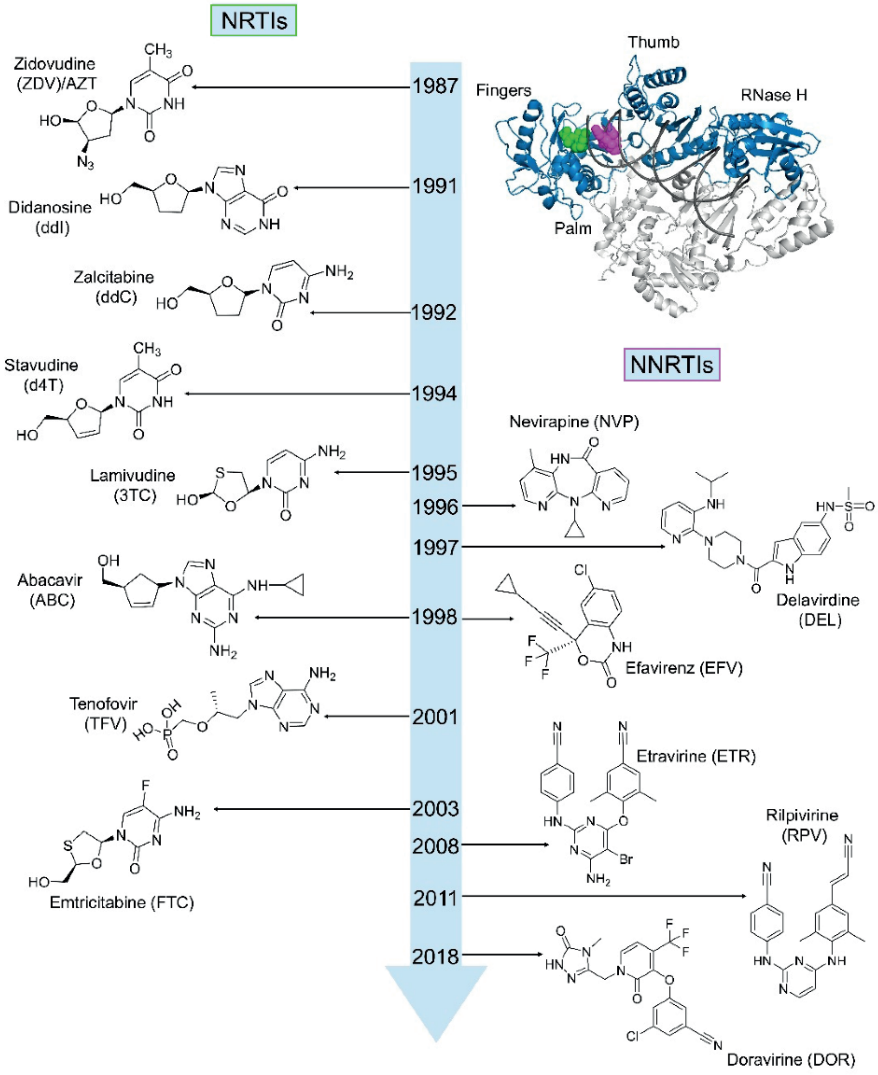


Figure 3. Timeline of HIV-1 RT-inhibiting drugs approved for clinical use (Singh A K. and Das K., 2022).

Despite these advances, structural changes of RT due to accumulation of mutations and the emergence of resistance to inhibitors due to the high rate of viral replication remain a major challenge in the treatment of HIV and the control of AIDS. This infection, which requires life-long treatment, also complicates the toxicity potential associated with long-term drug use. New drugs are periodically added to existing HIV inhibitors in order to control such problematic conditions and overcome resistance and toxicity. In addition to the existing RT drug classes, NRTIs and NNRTIs, alternative

druggable sites and other classes of RT inhibitors are beginning to be developed. This publication focuses on NNRTI and NRTI (Singh A K. and Das K., 2022).

2.1.1. NRTI

NRTIs are known as the first class of antiretroviral drugs to be approved by the FDA. NRTIs appear to be transported into cells by simple diffusion or facilitated diffusion mediated by nucleoside carrier structures. Lipophilic NRTIs such as tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF), as well as azidothymidine (AZT), abacavir (ABC), and stavudine (d4T), have been observed to passively cross cellular membranes with nonfacilitated pathways. This transition is achieved due to its hydrophobic properties. However, NRTIs are also known to cross the cell membrane using various cell surface transporters that induce facilitated diffusion. A series of carrier proteins on cellular surfaces control and regulate the cellular uptake of NRTIs. Many of these transporters are in the class called solute carrier (SLC) family. SLC family members involved in NRTI transport include organic cation transporters (OCTs), organic anion transporters (OATs), concentrated nucleoside transporters (CNTs), and compensatory nucleoside transporters (ENTs). It has been discovered that the types of SLC families involved in NRTI transport vary depending on the type of organ involved. Absorptive cells in the small intestine actively use OCT1, OCT2, CNT1-3, OAT2, ENT1 and ENT2 transporters for NRTI uptake. In lymphocytes, ENT1 and ENT2 proteins are known to regulate the uptake of specific NRTIs such as AZT and didanosine (ddI). Hepatocytes use OAT2 and OCT1 carrier proteins for NRTI transport. In contrast, renal tubule epithelial cells directly uptake NRTIs via CNT1, CNT2, OAT1-4, and OCT2 transporter proteins.

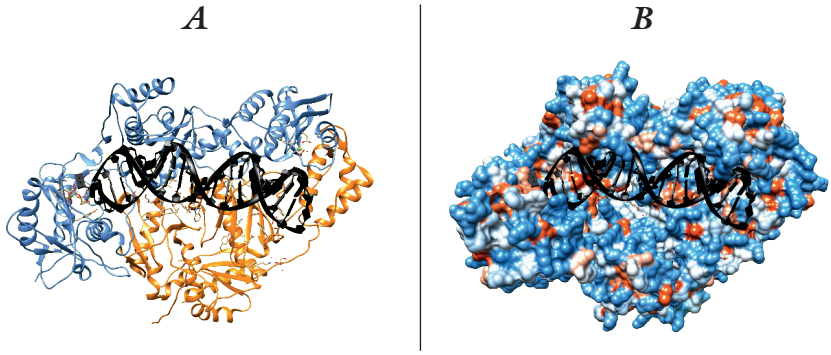


Figure 4. NRTI bind HIV-1 RT with DNA. Ribbon presentation (A), p66 (blue), p51 (orange), DNA (black). Hydropaty presentation (B), hydrophilic (blue), hydrophobic (red), notr (white), DNA (black) (Made with the UCSF Chimera)

NRTIs are known to act in two different ways, as prodrugs or active drugs. Upon cellular uptake, NRTI prodrugs act by being metabolized to the corresponding active drug form. This active form is then phosphorylated to the active diphosphate (DP) or triphosphate (TP) form. Once in its active form, the drug abruptly terminates viral DNA synthesis by first inhibiting the enzymatic activity of RT. In this way, it functions as a functional nucleoside analogue (**Fig. 4.**) (Han HK., 2011, Holec AD, et al., 2017).

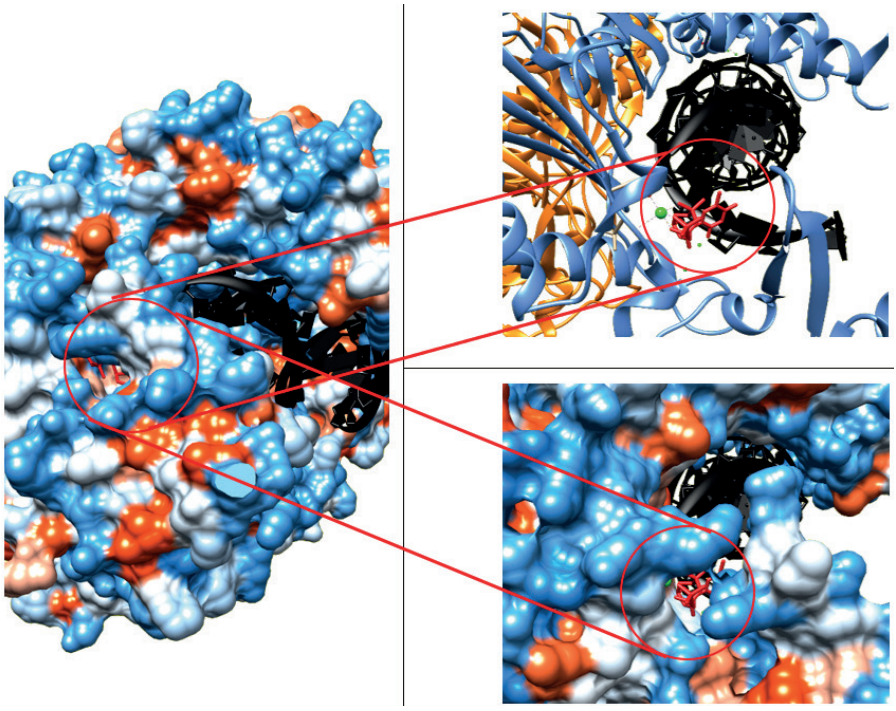


Figure 5. NRTI active site (Made with the UCSF Chimera)

These drugs must be administered in the form of prodrugs, which must be taken up by host cells and phosphorylated before becoming active. The host cell's kinases activate the inhibitor.

NRTIs lack a 3'-1 group on the 2'-deoxyribosyl moiety and have a nucleoside or hydroxyl base. The absence of the 3'-hydroxyl group in NRTI prevents the formation of 3'-5' phosphodiester bonds in growing DNA chains. As a result, viral replication is inhibited. An important feature of these drugs is that they are incorporated into the host cell during its own RNA-dependent DNA synthesis or DNA-dependent DNA synthesis. (Arts EJ and Hazuda DJ, 2012).

2.1.2. NNRTI

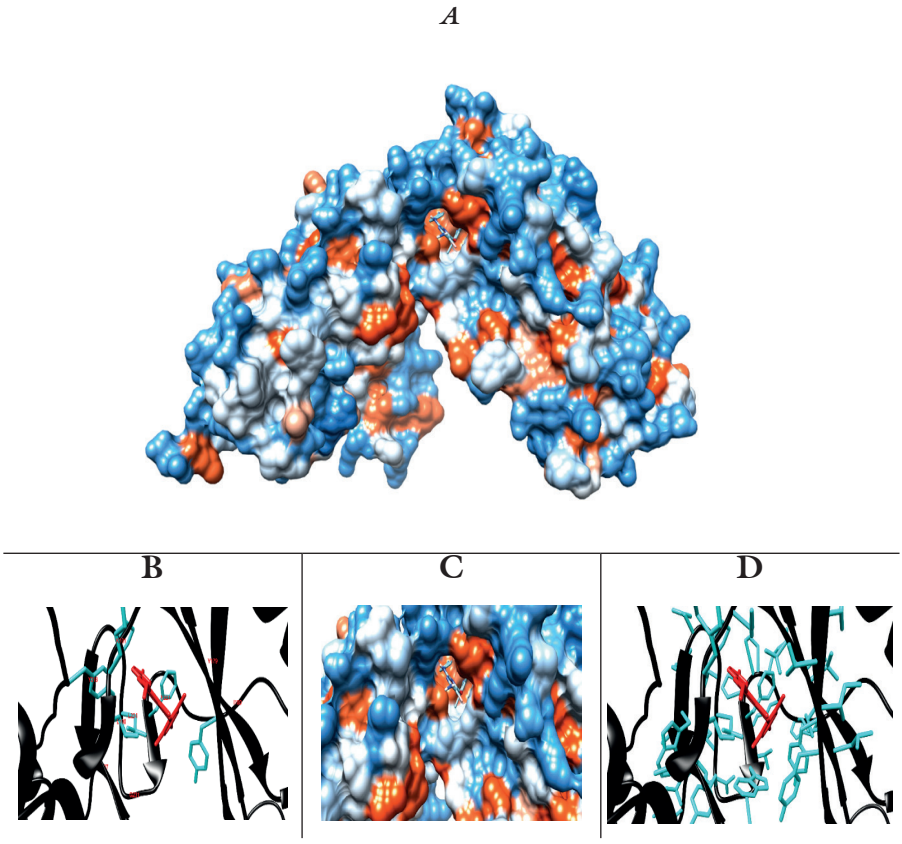
NNRTIs are other RT inhibitors used in the treatment of HIV-1 infection. They do not bind to the region where the NRTIs mentioned in the previous section bind; these inhibitors target the nucleoside non-binding pocket, which is a different region from the active site. By binding to the allosteric site of RT, NNRTIs inhibit the enzyme from efficiently converting viral RNA into DNA, a critical step in the viral life cycle (Sax PE, at all., 2014).

They are prescribed as an important inhibitor of antiretroviral combination therapy (cART) regimen to effectively manage HIV infection and AIDS treatment. It has been reported in the literature that their specific uses vary depending on factors such as drug resistance profiles and potential side effects. These inhibitors; Efavirenz (EFV), Nevirapine (NVP), Etravirine (ETR), Rilpivirine (RPV), Doravirine (DOR), Delavirdine (DLV).

EFV, NVP, and RPV are prominent NNRTIs, each exhibiting unique pharmacological properties. These drugs are highly selective for the HIV-1 reverse transcriptase, minimizing interference with host cellular processes. The efficacy and safety of NNRTIs have been well-documented in clinical trials, making them integral components of highly active antiretroviral therapy (HAART) regimens.

When the side effects of NNRTIs are examined, it is known that there are symptoms such as hepatotoxicity and rash. In such cases, patients need to be carefully monitored and guided. Especially when used as monotherapy or due to virological incompatibility, the development of resistance becomes a critical problem for the patient. After these situations occurred, trial of combination therapy promoted the control of RT resistance (De Clercq E., 2010, Soriano V, et al. 2007).

NNRTIs act through an allosteric mechanism, are noncompetitive, and differ from NRTIs and protease inhibitors. This effect is considered unlike any other, thought to be due to the ability of these inhibitors to bind to a specific hydrophobic pocket within the RT enzyme.



A. Hydrophobic surface visualization of RT (white: neutral, red: hydrophobic, blue: hydrophilic). B. Ribbon representation of the RT (black), etravirine (red), and associated amino acids (blue). C. Hydrophobic pocket of RT D. Ribbon representation of the RT(black), etravirine (red), and surrounding amino acids (blue) (Made with the UCSF Chimera).

Allosteric Inhibition: There is a hydrophobic pocket in a different location than the catalytic active site of the RT enzyme. NNRTIs inhibit HIV-1 reverse transcription by binding to this hydrophobic pocket. During this binding, conformational changes are triggered and inhibit the enzyme from performing its catalytic function.

Specificity: NNRTI inhibitors targeted to the hydrophobic pocket of the RT are specific only to this region. It is thought that it cannot bind to different regions of RT or to a different enzyme.

Non-Competitive Inhibition: NRTIs are known as competitive inhibitors. This is one of the situations that limits the activity of inhibitors.

NNRTs do not have such a situation. They cannot directly interfere with the binding of bases, which are the natural substrates of the RT enzyme.

Resistance Development: Despite the significant activity of NNRTIs, cases leading to the development of resistance mutations in the RT enzyme have been reported. Mutations in the hydrophobic pocket in the allosteric region of RT and that may affect this pocket reduce the binding affinity of NNRTIs by changing the shape of the pocket. This can lead to complete resistance or low-level resistance (Ren J., 2002, Rhee SY, et al., 2021).

3. MECHANISMS OF NNRTI RESISTANCE

NNRTIs) in the context of HIV treatment results from genetic mutations in the HIV-1 reverse transcriptase gene. These mutations (**Table 1.**) can affect the conformation of the NNRTI binding pocket, leading to reduced drug binding and inhibition. Common resistance mutations include K103N, Y181C, and G190A, which can confer varying degrees of resistance to different NNRTIs. The emergence of NNRTI resistance poses a challenge in managing HIV infections, necessitating tailored antiretroviral therapy regimens and adherence to resistance testing for optimized treatment strategies (Paredes and Clotet, 2010; Rhee et al., 2022).

	100 L	101 K	103 K	106 V	138 E	181 Y	188 Y	190 G	230 M
DOR	I	EP		AM		IV	L	SE	L
EFV	I	EP	NS	AM		CIV	L	ASE	L
ETR	I	EP			AGKQ	CIV	L	ASE	L
RPV	I	EP			AGKQ	CIV	L	ASE	L
NVP	I	EP	NS	AM		CIV	L	ASE	L

Table 1. Major Non-Nucleoside RT Inhibitor (NNRTI) Resistance Mutations -Stanford Drug Resistance DataBase

NNRTI resistance generally occurs by changing the amino acids located in this pocket and playing an active role in ligand interaction. Mutations trigger conformational changes. Ligand affinity decreases or disappears completely due to not only conformational changes but also changes in the physico-chemical properties in the region as a result of mutation.

While some mutations in this region may cause resistance to all NNRTIs, some mutations may not cause resistance to all. Additionally, different mutations in the region may have a synergistic effect and lead to resistance (**Table 1.**) (McClung RP, et al. 2022).

CONCLUSION

Today, all inhibitors are used in different combinations in the treatment of HIV infection. RT inhibitors are specifically reported in our book chapter. Additionally, the working mechanism of RT is explained in detail. It is expected that new RT inhibitors will be developed in the near future.

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