UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY
PBL SEMINAR
HIV & AIDS: An Overview

What is HIV?

- HIV: Human Immunodeficiency Virus;
- HIV belongs to group of viruses called **Retrovirus**;
    - Re-Tro-Virus: Re = Reverse, Tr = Transcription (Viruses that reversibly transcribe their genetic material, that is from RNA ====➔ DNA)
- HIV causes AIDS; HIV damages the immune system in humans
- Two types of HIV: HIV-1 and HIV-2; both types of HIV cause AIDS

What is AIDS?

- AIDS: Acquired Immune Deficiency Syndrome;
- AIDS is the collection of signs and symptoms (Syndrome) resulting from the many diseases in a patient infected with HIV;
- HIV specifically affect the T-Helper Cells
- HIV breaks down the immune system exposing the patient to "Opportunistic Infections"

How long after HIV infection does an individual develops AIDS?

- HIV is a Lentivirus,
- Patient recently infected with HIV may not immediately develop AIDS;
- In some individuals: Decline in T-cell counts and opportunistic infections that signal AIDS may develop soon after infection with HIV;
- Most individuals may not develop symptoms for 10 to 12 years after infection, and a few remain symptom-free for much longer;

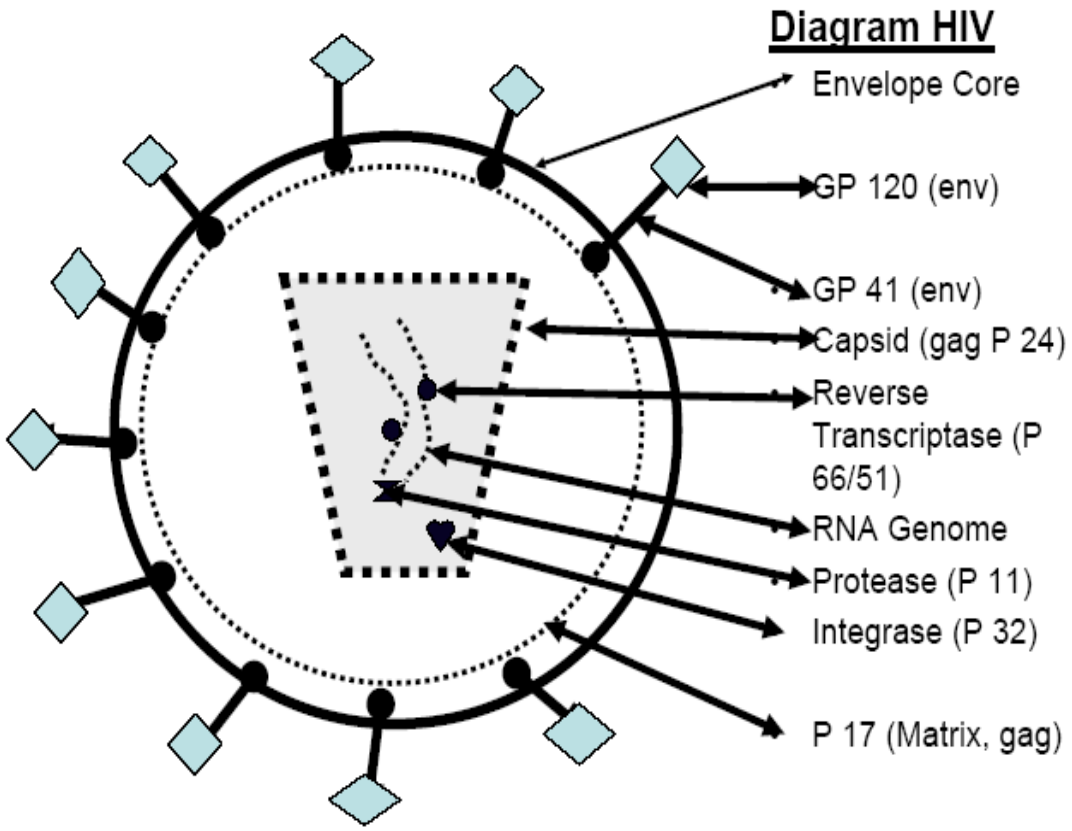
How is HIV related to AIDS?

- HIV infection: HIV mainly enters White blood cells (Immune cells);
- Over time HIV causes progressive damage of T-helper cells (CD4 cells);
    - Reducing the ability of the immune system to protect the body from infections;
- Ultimately the patient becomes vulnerable to various opportunistic infections and other diseases;
- According to WHO and Centers for Disease Control and Prevention (CDC USA) Clinical diagnosis of AIDS may be made if a patient:
    - Tested positive for HIV (using approved confirmatory test methods),
    - Meets one or both of the following conditions:
        - Presence of one or more AIDS-related infections or illnesses;
        - CD4 (T-cell) Count fallen below 200 Cells per cubic millimeter of blood (CD4 count ranges from 450 to 1200 in healthy individuals)
- **Note**: Some common AIDS related infections or illnesses include: Diarrhoea, Fungus infection – Candidiasis (Thrush), Skin infections, TB, Herpes Zoster (Shingles), etc.

What is the basic structure of HIV? (**Fig. 1**)

- Viral Envelope (VE) is lipid bilayer that forms outer membrane of HIV;
- Embedded in Viral Envelope (VE) are complex HIV proteins **Env** (Spikes);
- Each **Env** is made up of:
    - **Glycoprotein 120** (**GP 120**) and
    - **Transmembrane Glycoprotein 41** (**GP 41**);
- Within VE is Matrix consist of Glycosaminoglycans (GAG) and Viral Protein (P 17);
- Within Matrix is Viral Capsid made up of subunits: Capsomeres (Viral Protein, P 24);
- Within Capsid are Two Single Strands (Diploid) HIV RNA (Viral RNA Genome),
- Each Viral RNA has a complete copy of HIV genes;
- Within Capsid are Three Enzymes:
    - Reverse Transcriptase (P 66),
    - Integrase (P32),
    - Protease (P 11);

Fig. 1: Schematic diagram of basic structure of HIV



What is the basic structure of the Genome of HIV?

- Genome of HIV consists of Two (Diploid) RNA Molecules (9.8kilobases in length),
- Genome contains 9 different genes:
    - Three Structural Genes
    - Six Regulatory Genes
- Three Structural Genes encode for major structural proteins:
    - Gag, Pol, and Env; needed to make structural proteins for new virus particles;
        - Example: Env gene codes for a large protein GP 160 that is cleaved by viral enzyme to form GP120 and GP41, which are components of Viral Envelope

- Six Regulatory Genes encode for:
    - Regulatory Proteins: Tat and Rev
    - Accessory Proteins: Nef, Vif, Vpr, Vpu;
        - Examples:
            - Protein encoded by **Nef** (acronym for Negative Factor) is necessary for HIV to replicate efficiently;
            - **Vpu**-encoded protein influences release of new virus particles from infected cells;
            - Protein encoded by **Vif** gene interacts with antiviral defense protein in host cells (APOBEC3G),
                - It causes inactivation of antiviral effect and enhancing HIV replication;
                - **This interaction may serve as a new target for antiviral drugs**
- Both ends of each Strand of diploid RNA genome contain RNA sequence called **Long Terminal Repeat** (**LTR**);
- Regions in LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or host cell;

What receptor on host cells interacts with HIV?

- To infect Host Cells, genetic material of HIV must enter host cell;
- HIV interacts with Specific Cell Surface Receptor and Co-receptors on host cells;
- Major Specific Cell Surface Receptor in host cells is CD4
    - (CD = Clustered Differentiation)
- CD4: Large Glycoprotein located on surface of: Helper T cells, Regulatory T cells, Monocytes, and Dendritic cells;
- CD4 is the receptor that assists T Cell Receptor (TCR) to activate T Cell following interaction with Antigen Presenting Cell (APC);
- CD4: **Primary Receptor** used by HIV to gain entry into Host T Cells

What co-receptors on host cells interact with HIV?

- Co-receptors on host cells are: CCR5 or CXCR4
    - Protein molecules on surface of Lymphocytes or Monocytes that bind to GP120 protein of HIV and facilitate, usually with CD4, entry of diploid Viral RNA and proteins into Host cells

- CCR5 binds Macrophage-Tropic, Non-syncytium-inducing (R5) Viruses, associated with Mucosal and Intravenous Transmission of HIV infection;
- CXCR4 binds T-cell-tropic, Syncytium-inducing (X4) Viruses, which are frequently found during the later stages of AIDS;

TAKE NOTE:

- Naturally occurring deletion of 32 base pairs in the CCR5 gene results in a Mutant CCR5 Co-Receptor;
- Individuals homozygous for this mutation are almost completely resistant to HIV infection;
    - Indicates the role of CCR5 in the spread of HIV and suggests that small molecules that prevent HIV interaction with CCR5 might form a promising new class of antiretroviral drugs;

How does Fusion of the HIV with T-Cells occur? (Figs. 2 & 3)

- GP 120 on HIV envelope attaches to CD4 Receptor on Host Cell;
- Co-receptor (CCR5 or CXCR4) on Host Cell also participate in Fusion of GP 120 with CD4 receptor;
- Fusion separates GP120 from GP 41, thus releasing Spike on GP 41;
- Spike (GP 41) acting like a spring-loaded lancet pierces the membrane of the Host Cell;
- {NOTE: Fusion Inhibitors (T-20 and T-1249) can prevent Fusion by blocking the conformational changes resulting in the release of the spike}

Fig. 2: Interaction of HIV with Host Target Cell

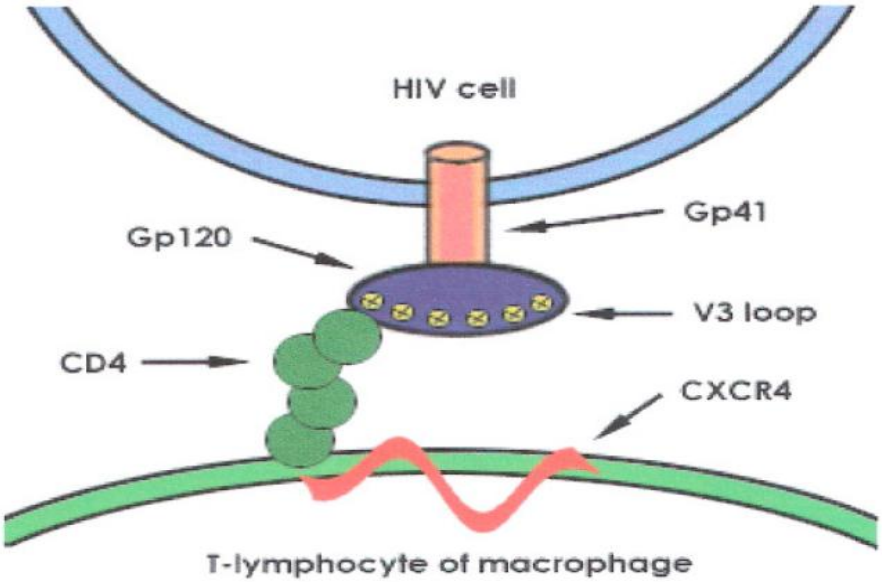
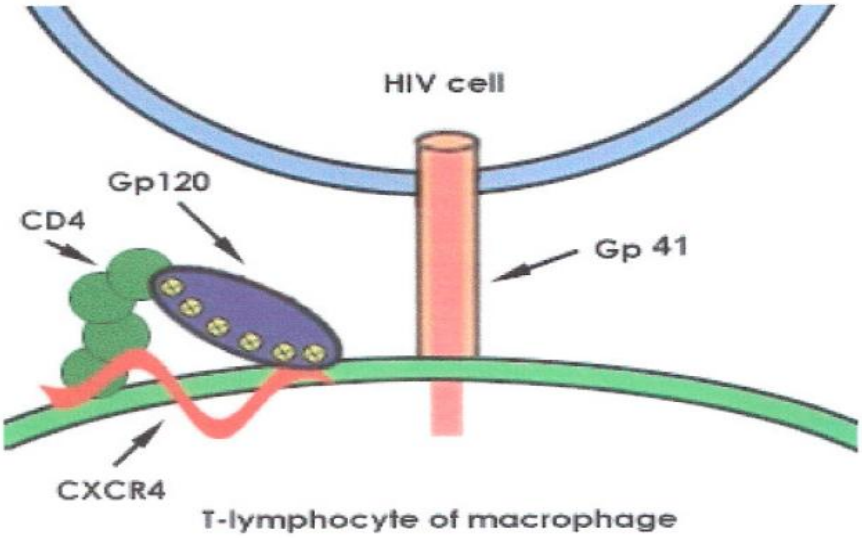


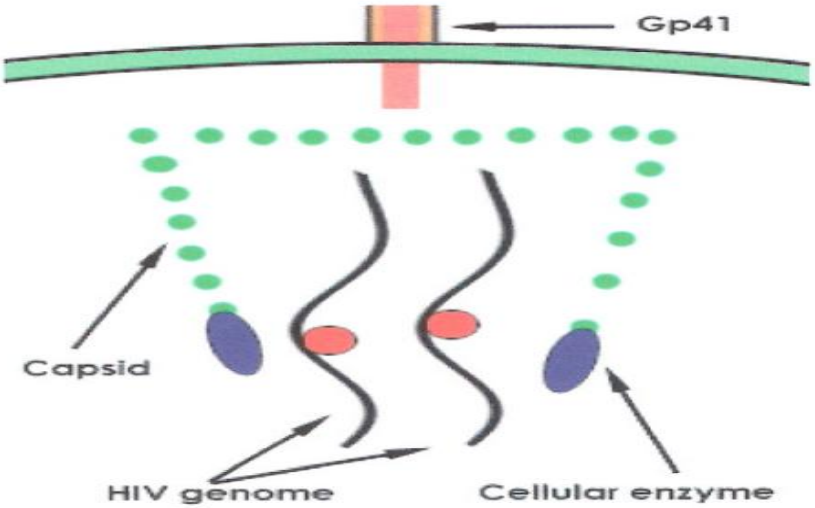
Fig. 3: Fusion and release of Spike



Explain how the viral genome enters the Host cell? (Fig. 4)

- Piercing of Host cell membrane by GP 41 is accompanied by injection of HIV Capsid into Cytoplasm of Host cell,
- Viral Envelope does not enter the Host cell
- Host enzymes hydrolyze Viral Capsid releasing its contents:
    - Diploid Viral RNA genome, Lysine Transfer RNA (tRNALys) that acts as Primer for Reverse Transcription, Viral Reverse Transcriptase (P66), Integrase (P32), Protease (P11), Viral Protein R (Vpr), and Other Viral proteins

Fig. 4: Release of Capsid into Host cell

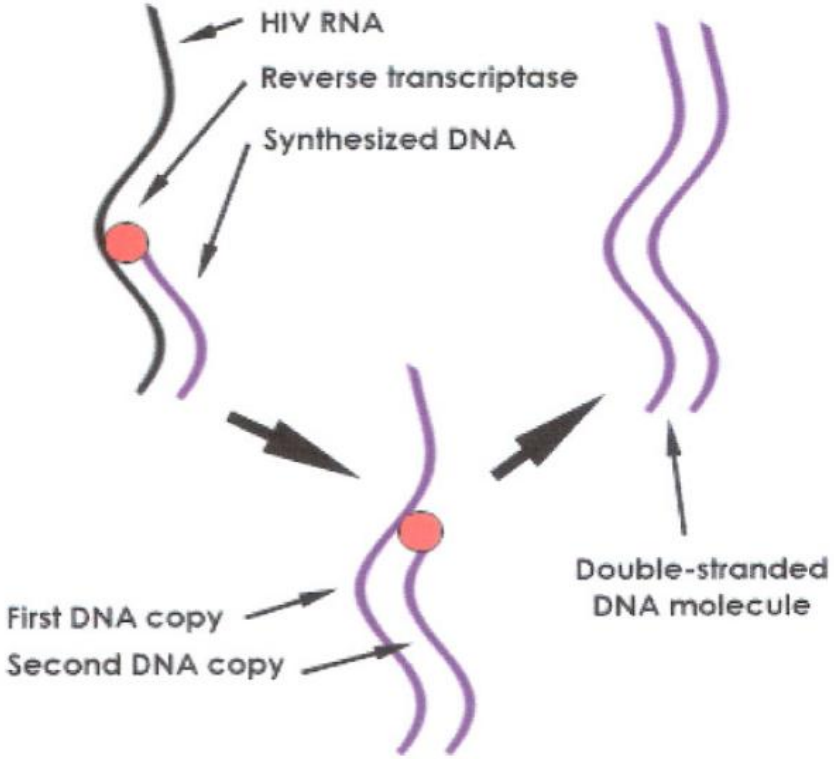


How is HIV genome Reversibly Transcribed? (Fig. 5)

Reverse Transcription: **Viral RNA =====➔double stranded viral DNA**

- Reverse Transcriptase (P66) is a complex viral enzyme that uses one of the Viral RNA strands as template for formation of DNA;
- DNA Polymerase is part of the Reverse Transcriptase complex;
- Lysine Transfer RNA (tRNALys) acts as Primer for the Reverse Transcription;
- DNA Polymerase uses the Primer to synthesize a Single Stranded DNA copy using the Viral RNA as template;
    - Thus forming a DNA/RNA hybrid molecule;
- RNA and DNA are separated;
- RNA template is destroyed by Ribonuclease;
- Single Stranded Viral DNA Replicates forming a Double-Stranded Viral DNA molecule;

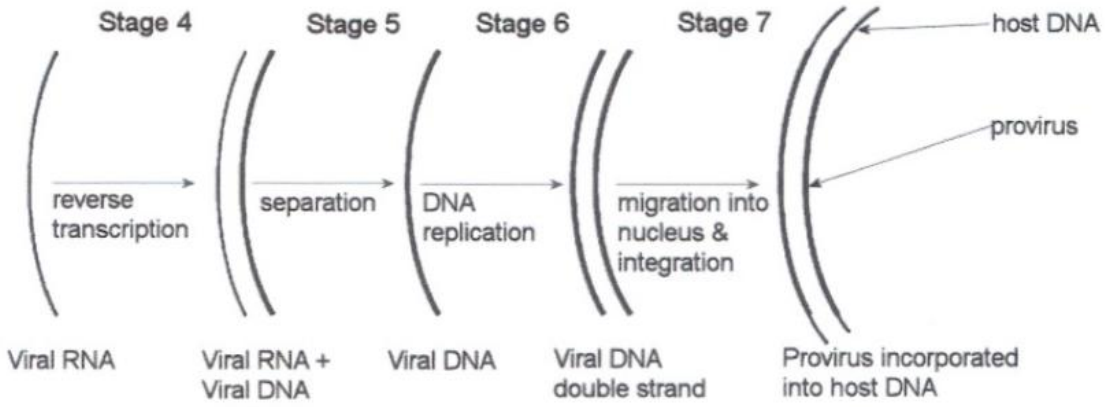
Fig. 5: Reverse Transcription and DNA Replication



How is Viral DNA Integrated into the Host DNA? (Fig. 6)
(Formation of Provirus)

- Integration of newly formed Double-Stranded Viral DNA into Host DNA (Chromosome) is catalyzed by the complex Viral Integrase (P32) made up of three enzymes;
- Process can be separated thus:
  - Exonuclease removes Two Nucleotides from each 3' end of the Viral DNA Duplex;
  - Double-Stranded Endonuclease cleaves Host DNA at integration site;
  - Ligase generates a single covalent linkage at each end of the Proviral DNA, thus forming the HIV Provirus within the Host DNA;

Fig. 6: Stages in Integration and formation of Provirus



What is Dormancy period or Transcriptional Latency?

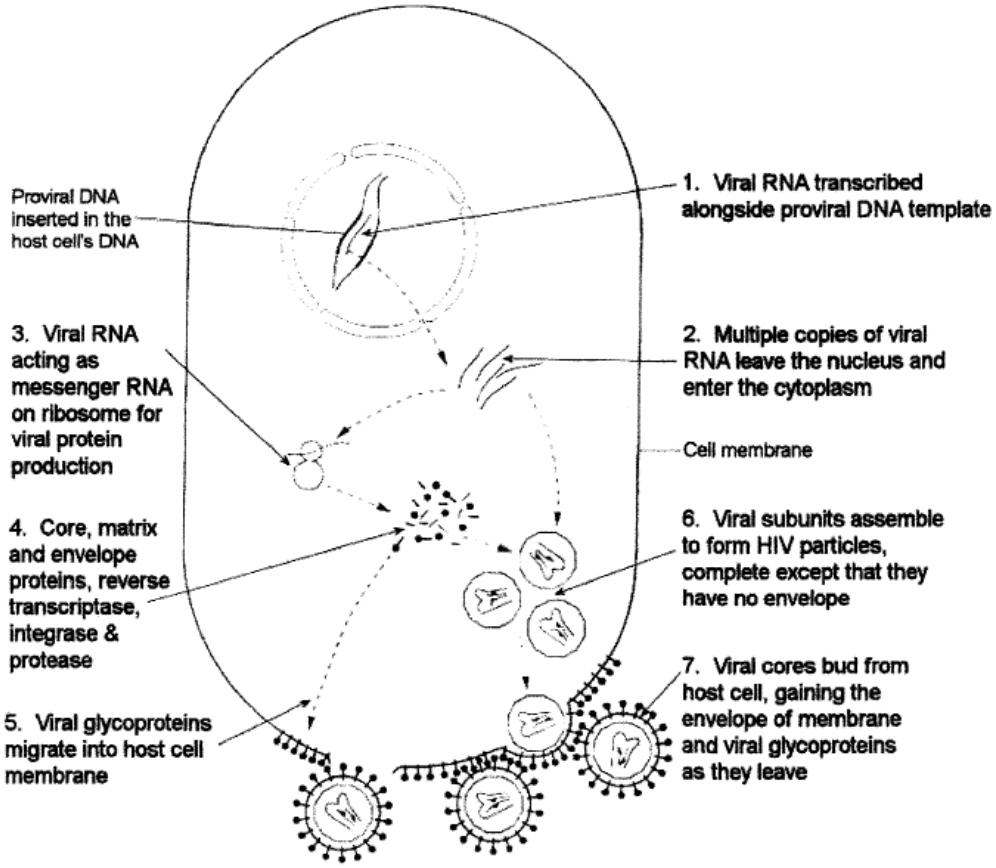
- After formation of Provirus, a relatively long period may occur during which infected individuals show no signs or symptoms and may not be aware of their HIV status;
- Integration leads to latent forms of infection or Transcriptional Latency;
  - Transcriptional latency explains the inability of potent antiviral therapies to eradicate HIV from the body;
- Moreover, despite a vigorous immune response early in infection, these silent pro-viruses are a reservoir that allows re-emergence of HIV when the body's defenses grow weaker;
- Understanding latency and developing approaches to target latent virus are essential goals if eradication of HIV infection is to be achieved;

How are new viruses formed from Proviral DNA? (Fig. 7)

- Activation results in Transcription of Proviral DNA to Viral RNA;
- Multiple copies of Viral RNA are produced and released into the Cytoplasm

- Viral RNA acting as Messenger RNA (m-RNA) attaches to Ribosome in the Host cell for Translation to occur;
- Translation of Viral RNA results in production of the Viral Proteins:
    - Core, Matrix and Envelope proteins, Transmembrane and Control proteins, Reverse Transcriptase, Integrase and Protease;
- Transmembrane Glycoproteins are formed and migrate into host cell membrane;
- Viral particles are assembled via self arrangement of Capsid around Viral RNA and enzymes;
- Each Viral unit buds from host cells, collecting Envelope with GP 120;

Fig. 7: Steps in the formation of new HIV from Proviral DNA of host cell



How is HIV transmitted from infected person?

- HIV is in body fluids (blood, semen, vaginal secretions, breast milk)
- HIV can be transmitted when these fluids enter the bloodstream of another person;
- Sharing needles or syringes with someone who is HIV infected; (this includes needles or syringes used to inject drugs)
    - Other types of needles, such as those used for body piercing and tattoos, can also carry HIV;

- Laboratory studies show that infectious HIV can survive in used syringes for a month or more;
- During childbirth, or breast-feeding;

How is HIV not transmitted?

- HIV is not transmitted through food or air (for instance, by coughing or sneezing);
- Mosquitoes, fleas, and other insects do not transmit HIV
- Sweat, tears, vomit, feces, and urine do contain HIV, but have not been reported to transmit the disease (apart from two cases involving transmission from fecal matter via cut skin)

State briefly the mode of action of drugs for management of HIV/AIDS

- Groups of drugs for management of HIV include the following:
  - Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (**NRTI**):
    - Incorporated into Viral DNA and prevent Reverse Transcriptase from adding Nucleotides to form functional viral DNA;
  - Non-Nucleoside Reverse Transcriptase Inhibitors (**NNRTI**):
    - Attaches to Reverse Transcriptase to prevent HIV from converting RNA into DNA, thus preventing production of New Viruses in infected cells;
  - Protease Inhibitors (PI):
    - Attaches to and inhibit the HIV Enzyme Protease
  - Entry Inhibitors:
    - Prevents HIV from entering healthy CD4 cells by targeting the CCR5 protein;
  - Fusion Inhibitors:
    - Stops the Virus from entering cells by targeting the GP41 on HIV;
  - Integrase Inhibitor:
    - Blocks the action of Integrase,
      - Enzyme in HIV that integrates Viral DNA into DNA of Host cells,
      - It is effective against HIV that has become resistant to other antiretroviral drugs;
  - HIV patients usually take these drugs in combination:
    - Highly Active Antiretroviral Therapy (HAARP);
    - Appropriate adherence to HAART regimens is required for effective management of the AIDS;

Laboratory Test for HIV / AIDS:

- Laboratory methods can be used for:
  - Screen Blood,
  - Diagnose Infection, and
  - Monitor disease progression
- Tests can be used to:
  - Detect Antibody;
  - Identify Antigen;
  - Detect or Monitor Viral RNA, and
  - Estimate T-lymphocyte numbers (cell phenotyping);
- Tests to detect Antibody to HIV are classified as:
  - Screening Assays, which are designed to detect all infected individuals,

    - Confirmatory (supplemental) assays, which are designed to identify individuals who are not infected but who have reactive screening test results;
- Screening tests possess a high degree of Sensitivity,
- Confirmatory Assays have a high Specificity;
- Technical errors do occur, however, and there are biologic factors that can limit the accuracy of HIV tests;
- Effective Quality Assurance Programs are needed in all laboratory;
- Laboratory tests should be used to supplement Clinical diagnosis

Early detection and the Window Period:

- Specific antibody to HIV is produced shortly after infection, but the exact time depends on several factors, including host and viral characteristics
- Importantly, antibody may be present at low levels during early infection but not at the detection limit of some assays;
- Newer-generation assays, including the Third-generation antigen sandwich assays, can detect antibody at about 3-4 weeks after infection
- Window period before the detection of antibody can be shortened by several days using Antigen tests, and by several more days using Nucleic Acid detection methods
- Window period may be only 2-3 weeks if an all-inclusive testing strategy is used
- High analytical sensitivity of third-generation tests is because they detect IgM antibody simultaneously with IgG;

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