

HUMAN IMMUNO DEFICIENCY VIRUS

Human Immuno Deficiency Virus (HIV)

- **First AIDS case recognized in USA, 1981**
- **But, blood obtained in 1959 in the DRC represents the oldest infection**
(Zhu et al., 1998)
- **HIV RNA retrovirus discovered 1983**
- **Family: Retroviridae**
- **Enveloped, ds RNA virus**
- **Are of two types: HIV-1 & HIV-2**

Family *Retroviridae*

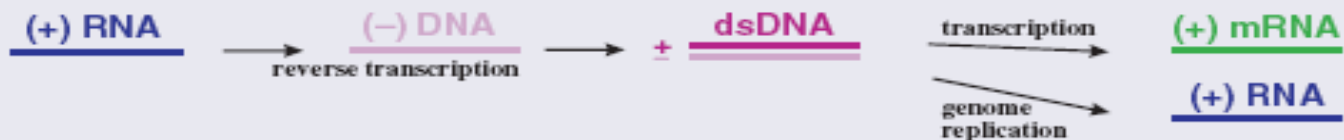
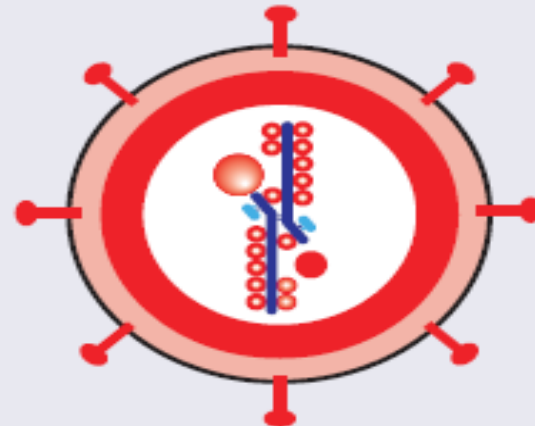
retro (Latin) = backwards

Hosts: mammals
birds
other vertebrate animals

Diseases: immunodeficiency diseases
leukaemias
solid tumours

Virion

- Enveloped
- 80–110 nm diameter
- Genome: single-stranded RNA
plus polarity
9–10 kb
- Contains reverse transcriptase



John et al.,2007

HIV-1 & HIV-2

- **Transmission: same routes**
- **HIV-1 is widely distributed globally**
- **HIV-2:**
 - **found in West Africa**
 - **less easily transmitted**
 - **less pathogenic**
 - **shorter duration of infection**
- **MTCT is relatively rare with HIV-2**

Tab: 1 Enzyme activities present in the retrovirus virion

RNA-dependent DNA polymerase (reverse transcriptase; RT)

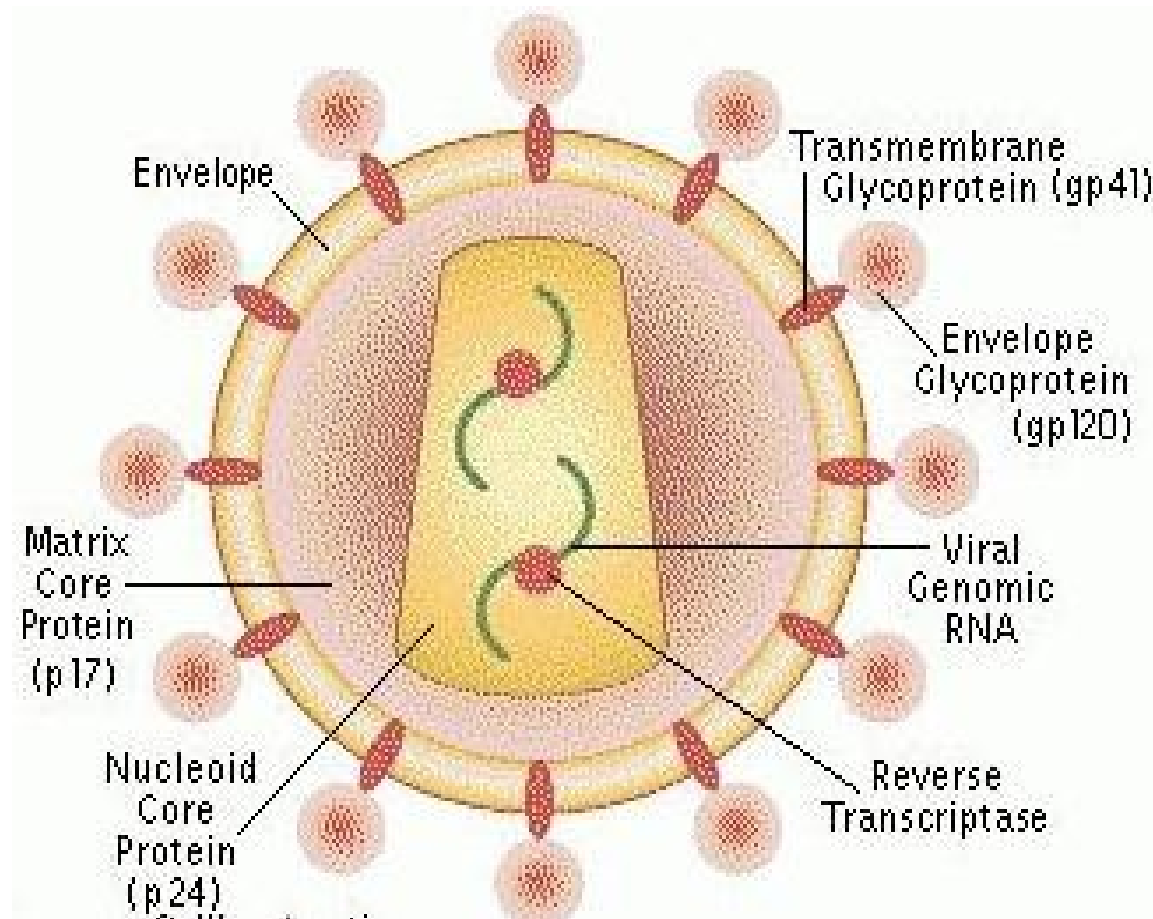
DNA-dependent DNA polymerase

Ribonuclease H (RNase H)

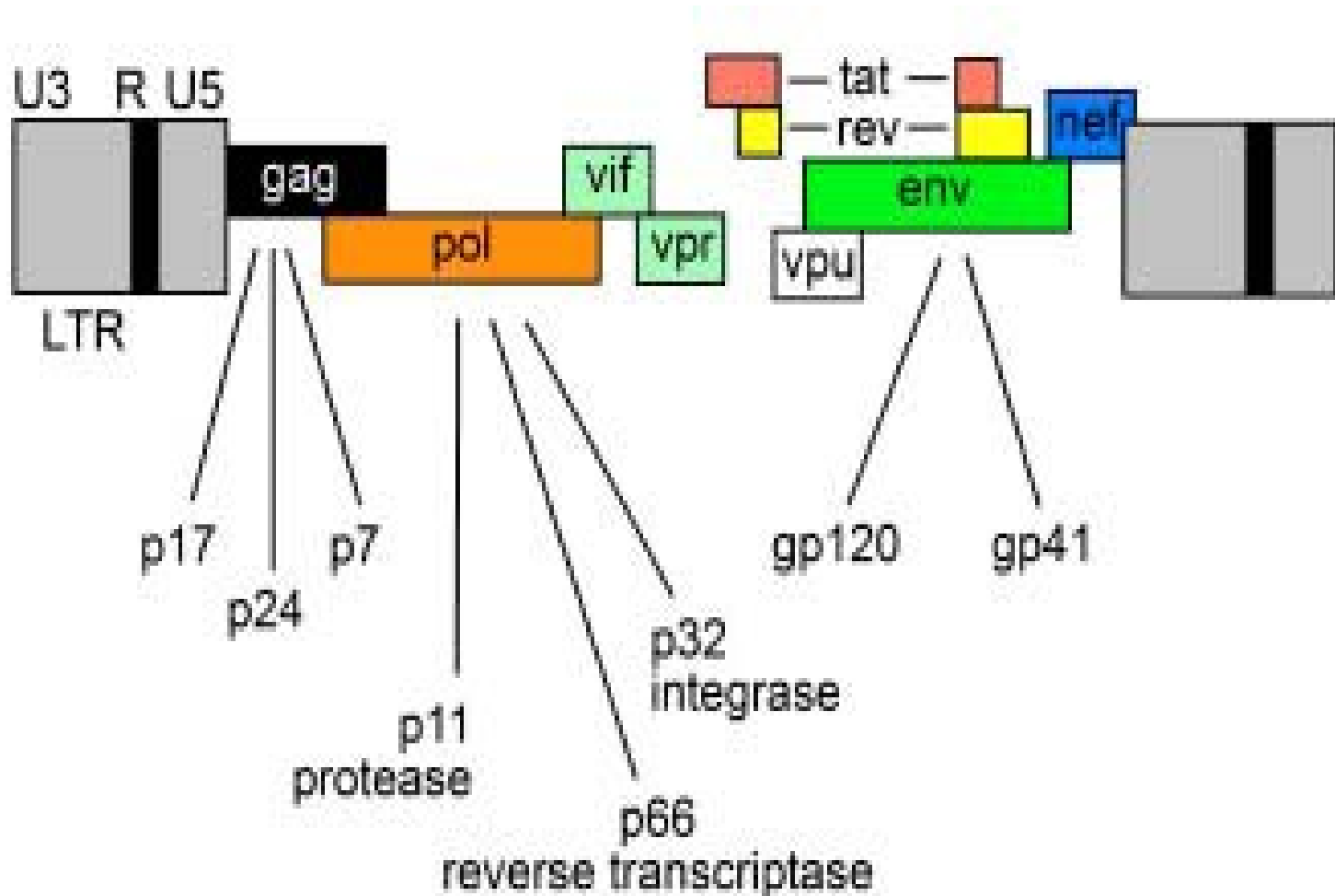
Integrase

Protease

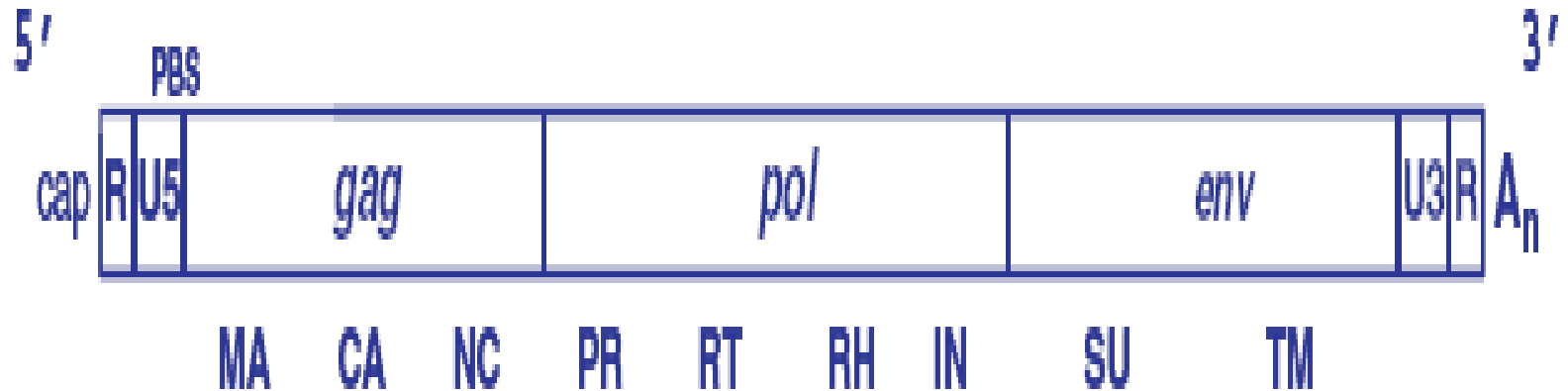
Structure of HIV virion particle



HIV-1 genome organization & gene products



HIV-1 genome Cont'd...



HIV-1 genome Cont'd...

Genes	<i>gag</i>	<i>group-specific antigen</i>
	<i>pol</i>	<i>polymerase</i>
	<i>env</i>	<i>envelope</i>
Proteins	CA	capsid
	IN	integrase
	MA	matrix
	NC	nucleocapsid
	PR	protease
	RH	ribonuclease H
	RT	reverse transcriptase
	SU	surface glycoprotein
Non-coding sequences	TM	transmembrane glycoprotein
	PBS	primer-binding site
	R	repeat sequence
	U3	unique sequence at 3' end of genome
	U5	unique sequence at 5' end of genome

Gene	Gene product	Function
Structural genes:		
<i>gag</i>	p55	p55 Nucleocapsid, precursor of p18, p24, p15
	p18	Matrix protein
	p24	Capsid protein
<i>pol</i>		Polymerase region
	p66/51/10	Reverse transcriptase/RNase/protease
	p31	Integrase
<i>env</i>	gp160	Glycoprotein, precursor of gp120, gp41
	gp120	Surface protein (binds to CD4 molecule of host cell)
	gp41	Transmembrane protein

- **Replication of retroviruses depend on three genes:**
 - **Gag (group specific antigen)**
 - **pol (polymerase)**
 - **Env (envelope)**
- **The classical structural scheme of a retroviral genome is:**

5'LTR-gag-pol-env-LTR 3'

LTR (long terminal repeat regions)

- **Represent the two end parts of the viral genome, that are connected to the cellular DNA of the host cell after integration**
- **Do not encode for any viral proteins**

Gag gene

- **Makes the cone shaped viral capsid**
- **codes for core proteins & structural virion components**

env gene

- **codes for viral esurface glycol -proteins (gp¹²⁰ & gp⁴⁰)**

pol gene

- **codes for viral enzymes (reverse transcriptase, integrase & protease)**

gp¹²⁰

- **Outer envelope protein**
- **Responsible for attaching the virus to CD4 receptor**
- **Has cytotoxic effect on nerve cells**

gp⁴¹

- **Induces the hydrophobic interaction of viral & host cell membrane to fuse**
- **Immuno - suppressive characteristics when released as a peptide**

Replication cycle of HIV-1

1. Attachment of gp¹²⁰ to CD4 molecules is depending on the virus type & chemokine receptors:

- **CXCR4..... for T- cell tropic strains**
- **CCR5.....for macrophage tropic strains**

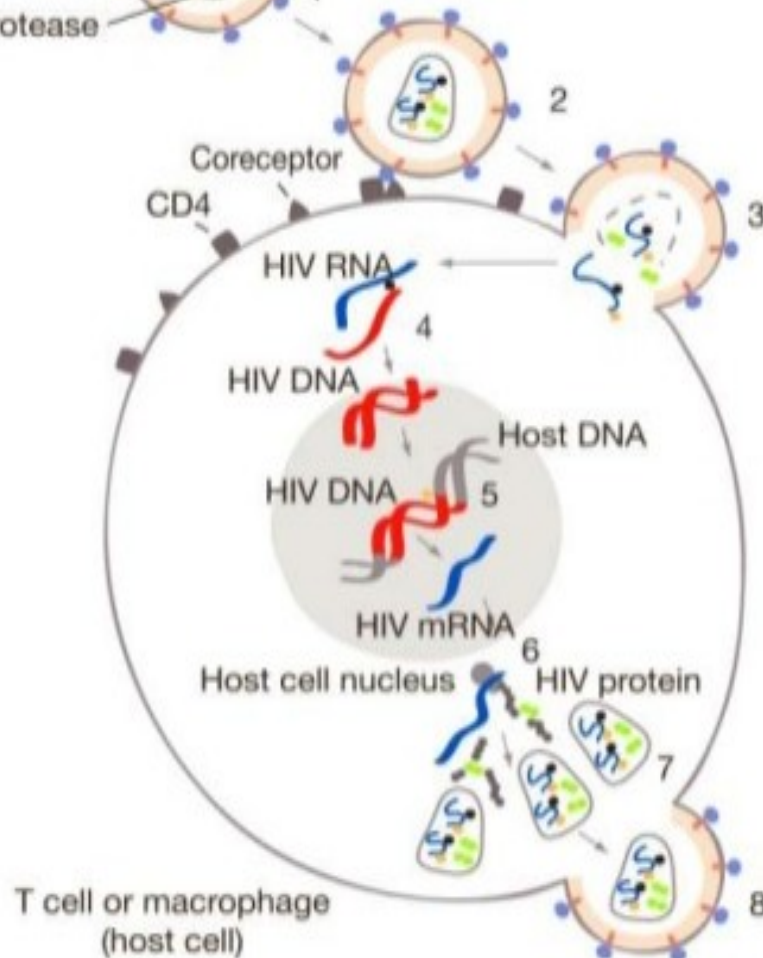
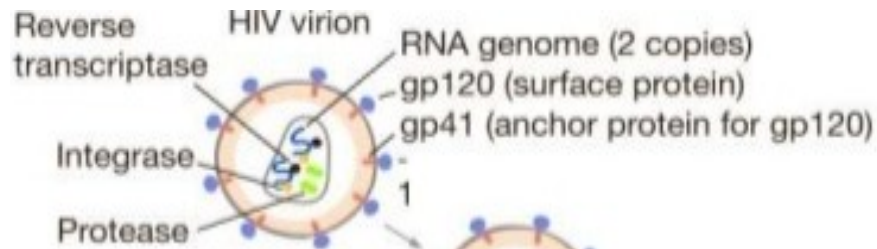
Note: Both macrophage &T-cell tropic viruses replicate in CD4

2. Fusion of the virus to cell memb Via gp⁴¹

Steps of replication

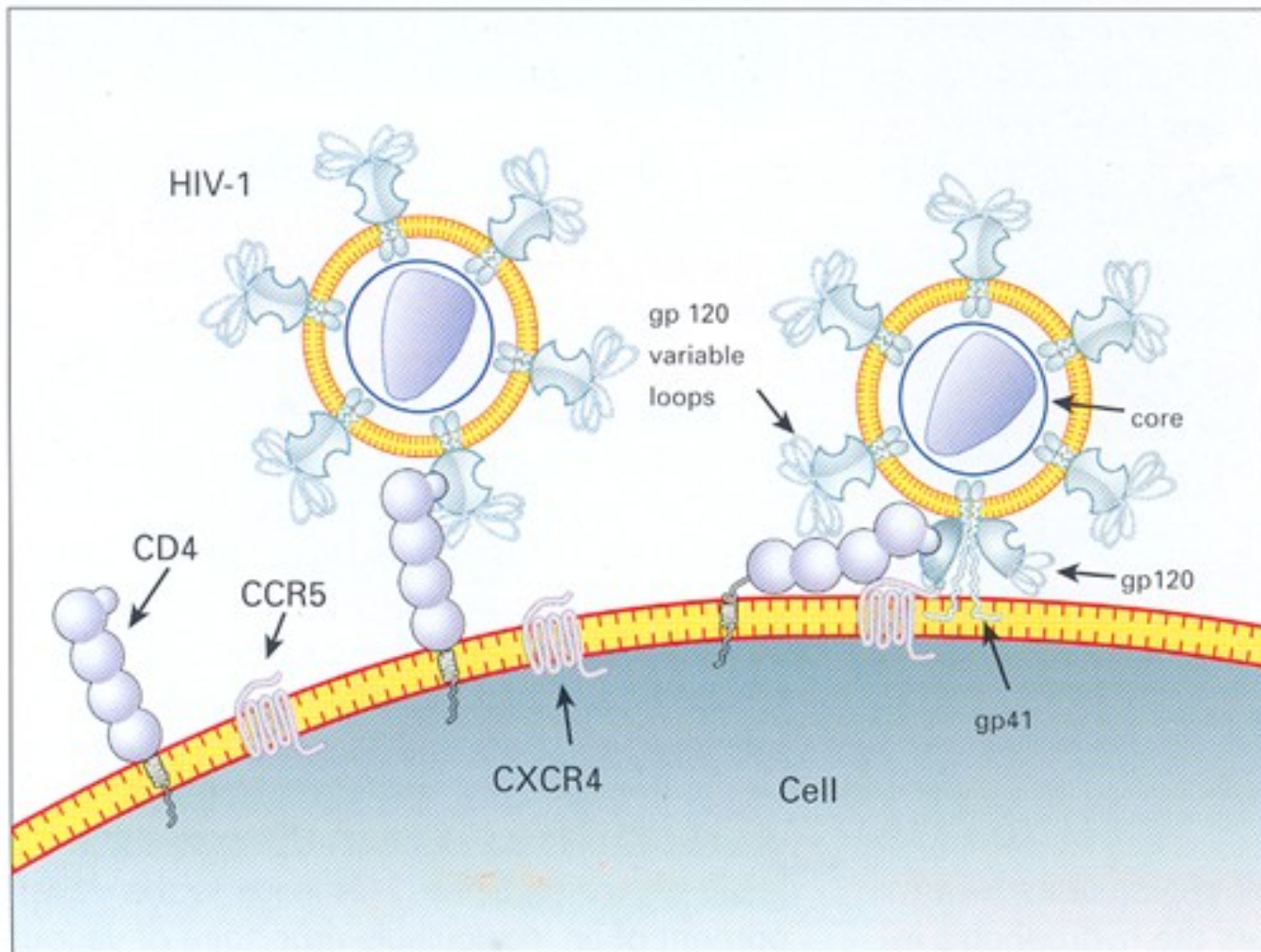
3. Virus penetrates into host cell:

- **Uncoating**
- **Reverse transcription**
- **Pro virus synthesis**
- **Integration**
- **Synthesis**
- **Maturation of virus progeny**
- **Release**



- 1) HIV's extracellular, or virion stage
- 2) HIV's gp120 protein binds to CD4 and coreceptor on host cell
- 3) HIV's RNA genome, reverse transcriptase, integrase, and protease enter host cell
- 4) Reverse transcriptase synthesizes HIV DNA from HIV's RNA template
- 5) Integrase splices HIV DNA into host genome. HIV DNA is transcribed to HIV mRNA by the host cell's RNA polymerase
- 6) HIV mRNA is translated to HIV precursor proteins by host cell's ribosomes. Protease cleaves precursors into mature viral proteins
- 7) New generation of virions assembles inside host cell
- 8) New virions bud from host cell's membrane

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**Mechanism of HIV attachment to its receptors on the host cell.
(Source: NIH reagent program)**

RT & synthesis of viral mRNA

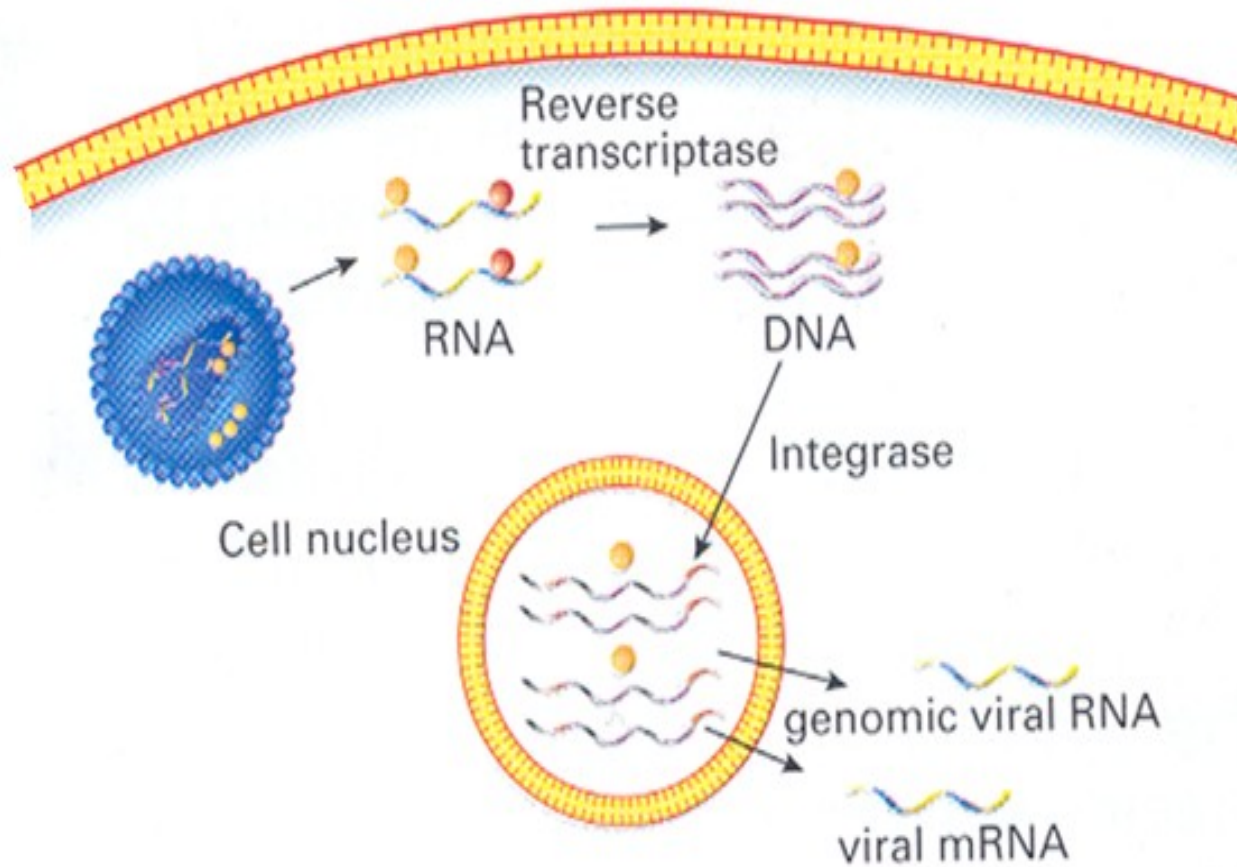
- **Initially, inactive protease is reactivated**
 - ▶ **Inducing dissolution of core structures**
- **Changes in ionic composition & p^H of host cytoplasm**
 - ▶ **Reverse transcriptase (RT) becomes active**
- **RT 1st transcribes one of the RNA strands into DNA**
 - ▶ **Simultaneously RNAase H breaks down the 1st RNA strand**

RT & synthesis of viral mRNA ...

- **RT switches to the 2nd RNA strand & transcribes it into DNA**
- **The dsDNA transported from the cytoplasm to nucleus**
 - ▶ **Integrated into human genome via the action of integrase**
- **Protein synthesis takes place:**
 - **1st proteins.....become regulatory proteins**
 - **2nd proteins.....become structural proteins**

Note: Up to 5 HIV DNA have been detected in one lymphocyte

!



Reverse transcription & synthesis of viral mRNA

RT based transpiration

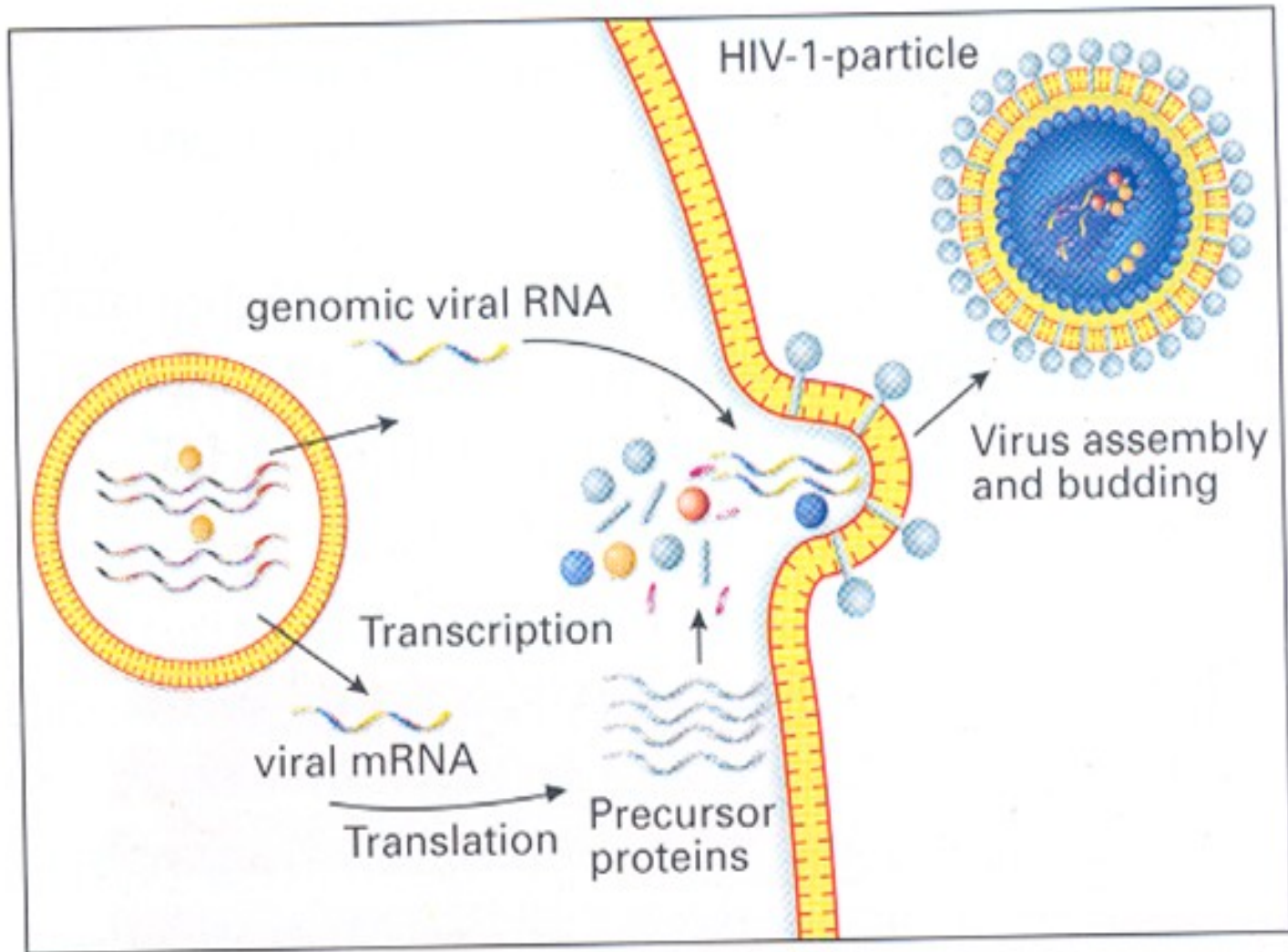
Occurs without proof reading ; hence:

- **Transcription errors are not corrected**
- **Error rate is one in 10,000 transcription**
- **One base is incorporated per transcription**
- **Results in mutation**
 - ▶ **Drug resistance / immune elimination**

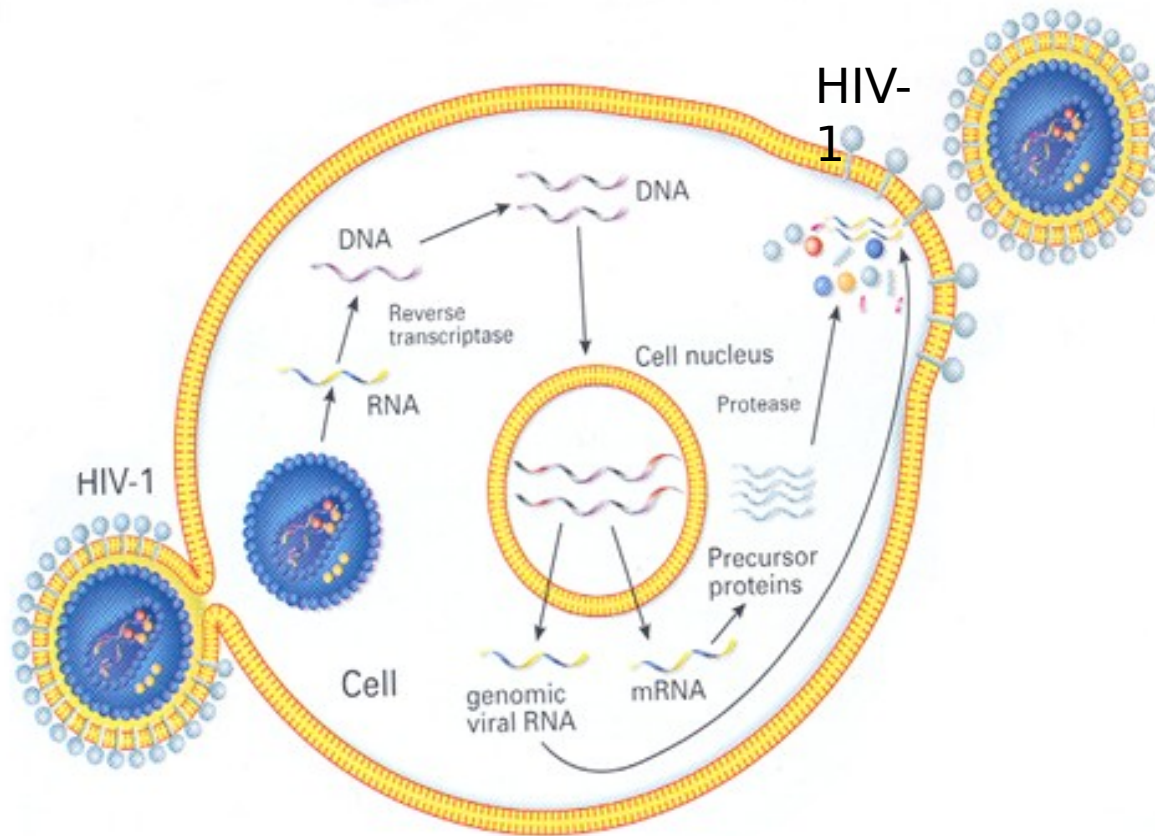
RT based transpiration...

Viruses unable to multiply are generated when:

- **Active center of viral enzyme is mutated**
- **Regularly proteins cannot attach to their binding sites**
- **Gp¹²⁰ cannot bind to CD4 molecules**
- **Interaction b/n gp¹²⁰ and gp⁴¹ lost**
- **Condensed viral core cannot be assembled**



HIV particle assembly & budding from the cell membrane

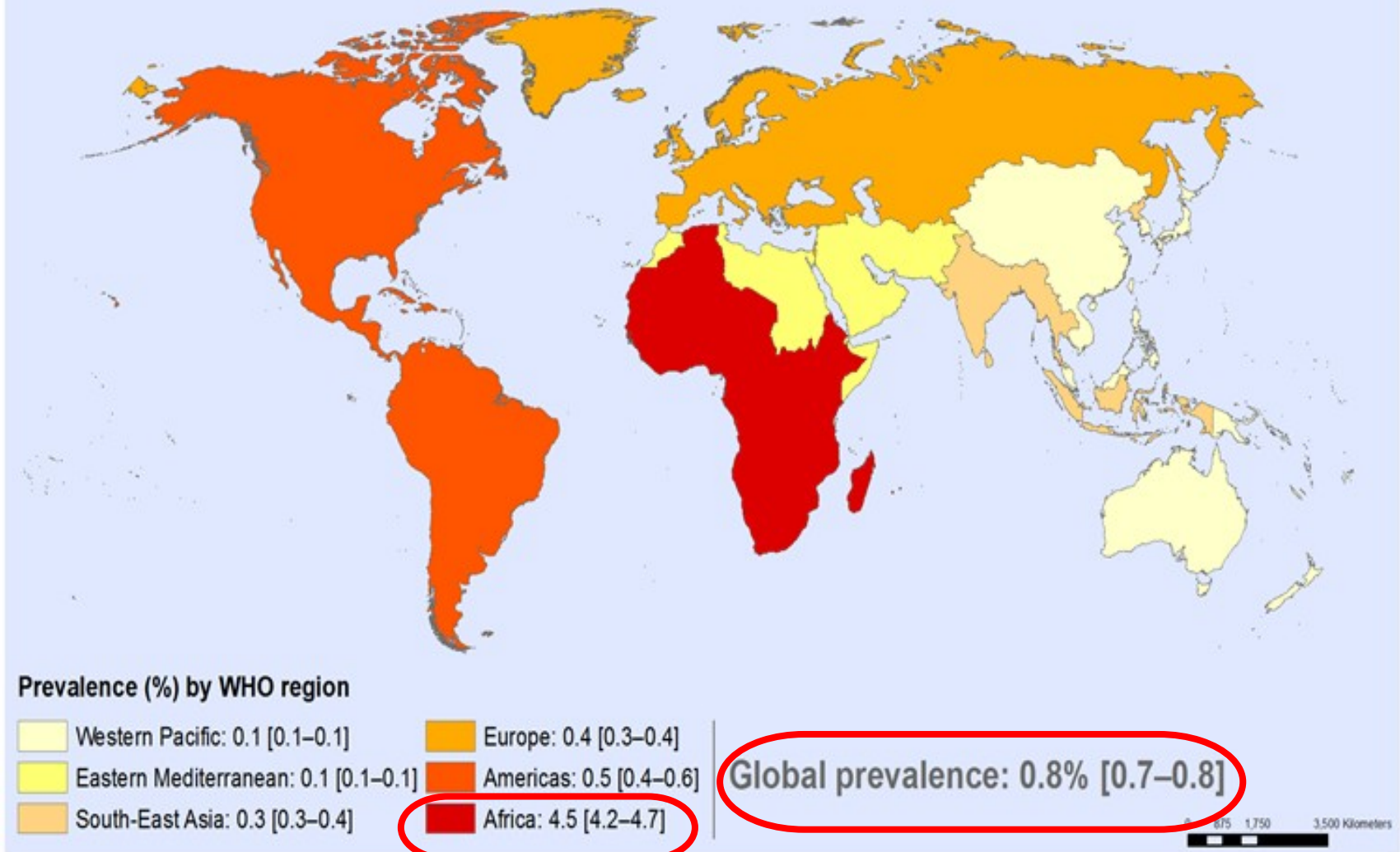


HIV multiplication within the host cell : After viral RNA is transcribed into pro - viral DNA & integration has occurred , mRNA is synthesized resulting in the translation of proteins & the assembly of particles

Epidemiology

Adult HIV prevalence (15–49 years), 2013
By WHO region

(WHO, 2014 report)



Global summary of the AIDS epidemic | 2013

Number of people living with HIV in 2013

Total	35.0 million	[33.1 million – 37.2 million]
Adults	31.8 million	[30.1 million – 33.7 million]
Women	16.0 million	[15.2 million – 16.9 million]
Children (<15 years)	3.2 million	[2.9 million – 3.5 million]

People newly infected with HIV in 2013

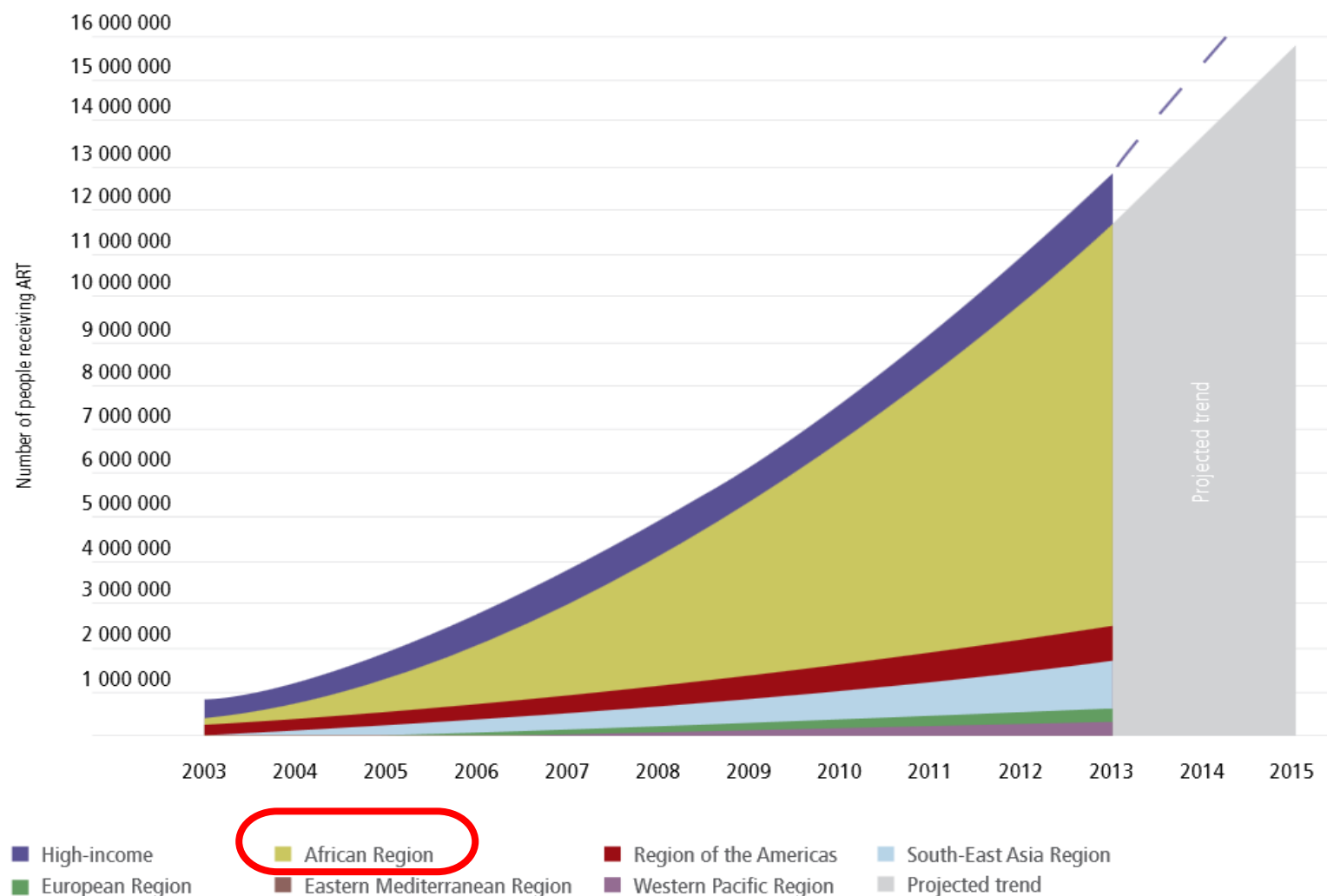
Total	2.1 million	[1.9 million – 2.4 million]
Adults	1.9 million	[1.7 million – 2.1 million]
Children (<15 years)	240 000	[210 000 – 280 000]

AIDS deaths in 2013

Total	1.5 million	[1.4 million – 1.7 million]
Adults	1.3 million	[1.2 million – 1.5 million]
Children (<15 years)	190 000	[170 000 – 220 000]



Actual and projected numbers of people receiving antiretroviral therapy in low- and middle-income countries by WHO region and in high-income countries across WHO regions, 2003–2015^a



^aCountry income classification by the World Bank at the time of the 2011 Political Declaration on HIV and AIDS.

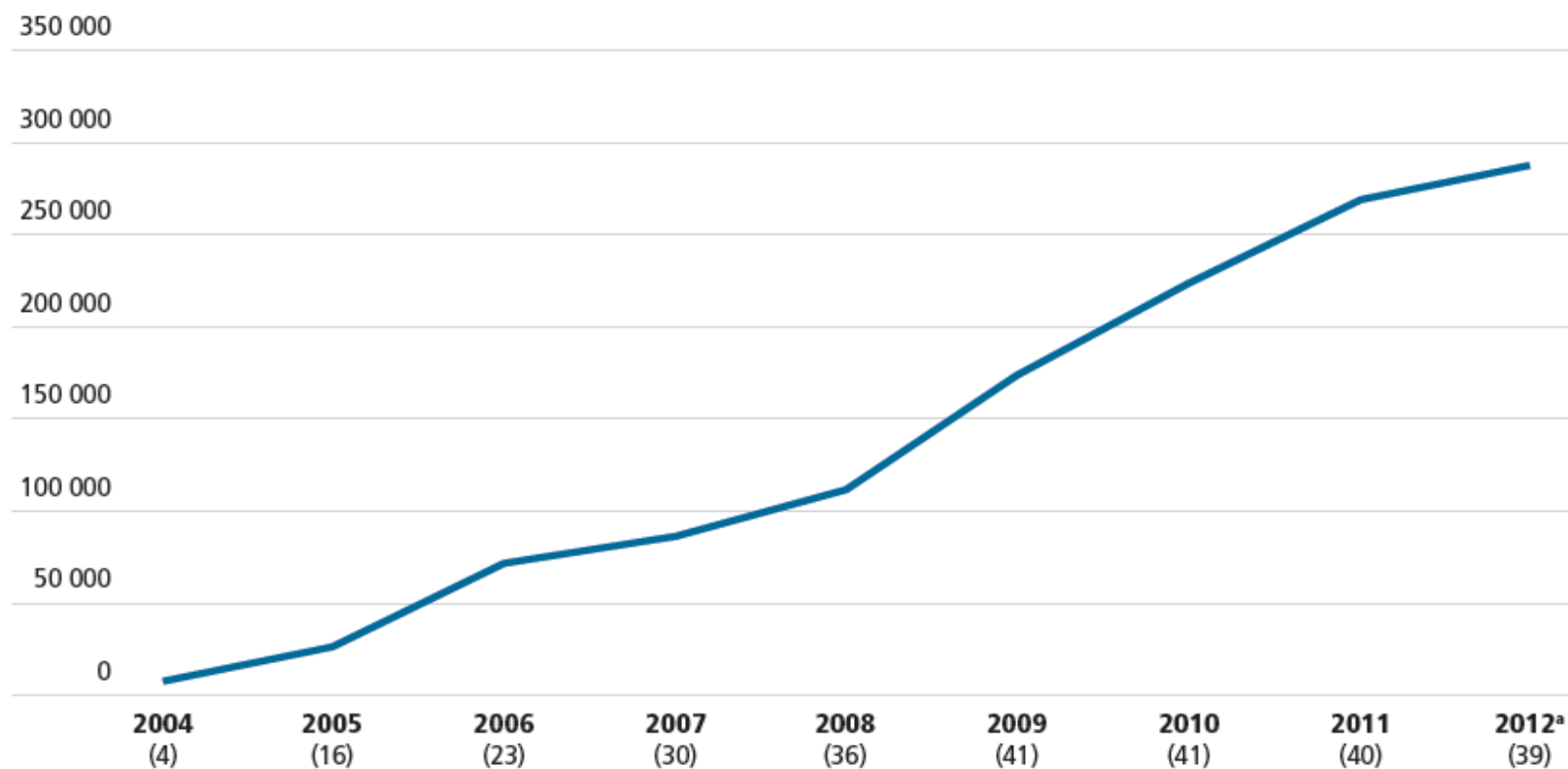
Source: Global AIDS Response Progress Reporting (WHO/UNICEF/UNAIDS)

Summary comparison of WHO antiretroviral guidelines: immunological criteria for initiating antiretroviral therapy, 2010 and 2013

	2010 guidelines (8)	2013 guidelines (7)
Adults and adolescents living with HIV	≤ 350 CD4 cells/mm ³	≤ 500 CD4 cells/mm ³
Children living with HIV	<24 months old: all 2–5 years old: ≤ 750 CD4 cells/mm ³ or 25%	<5 years old: all
Pregnant women living with HIV ^a	No specific provision	All
People coinfectd with TB and HIV	All	All
People coinfectd with HIV and hepatitis B	All with chronic active hepatitis	All with chronic severe liver disease
Serodiscordant couples	No specific provision	All

^a For their own health, excluding other options with the primary purpose of preventing the mother-to-child transmission of HIV.

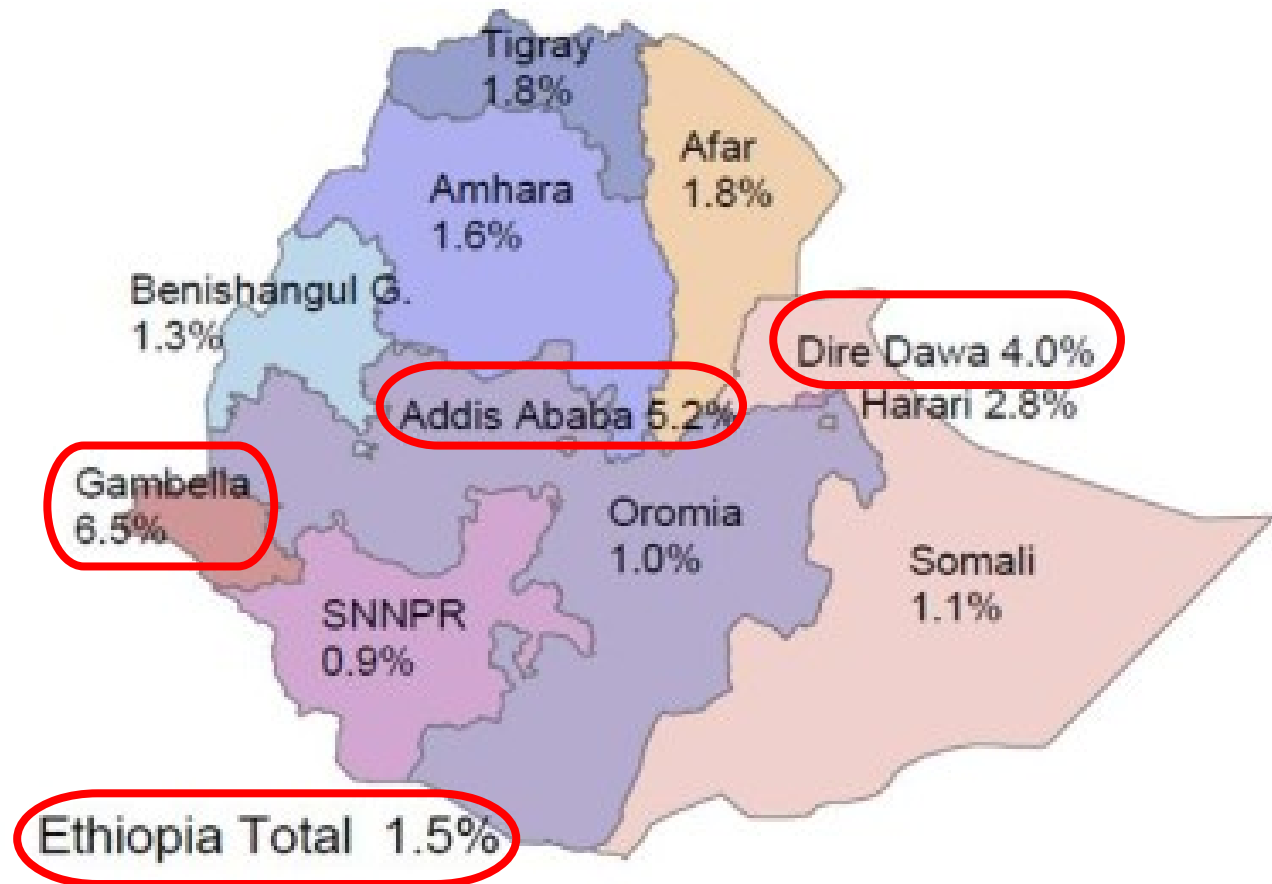
Number of people with TB and HIV receiving antiretroviral therapy in 41 countries with a high burden of TB and HIV, 2004–2012



^a The data for 2012 are provisional, as reported by 17 June 2013.

Note: The numbers in parentheses refer to the number of countries reporting data.

HIV prevalence by region, DHS 2011



Source: EDHS, 2011

Transmission

A) Sexual contact

- **Homo sexual & hetero - sexual exposure**
- **STIs fuels rate of transmission**

B) Blood and blood products

- **Blood transfusions**
- **Intravenous drug abusers**
- **Health care workers**

C) Vertical transmission

10 - 30% of babies can acquire infection

Infection may occur:

- **In utero: vertical transmission**
- **Perinatally: Infected birth canal & umbilical cord**
- **Post natally: via breast feeding**

N.B: > 60% of infection occur during final term & birth

Risk of contracting infection dependent on

- **Viral dose (load)**
- **Integrity of the exposed body site**
- **Type of body fluid**
- **Volume of body fluid**

Risk after a single exposure

- **> 90% blood or blood products**
- **14% vertical transmission**
- **< 2 % injection drug use**
- **< 1 % genital mucous membrane**

Types of immune responses against HIV

**Humoral & Cell mediated IR partially control the infection
(early phase)**

A) Innate immunity

Antigen presenting cells :

- **Macrophages**
- **Dendritic cells**
- **B- cells**

B) Other innate cells:

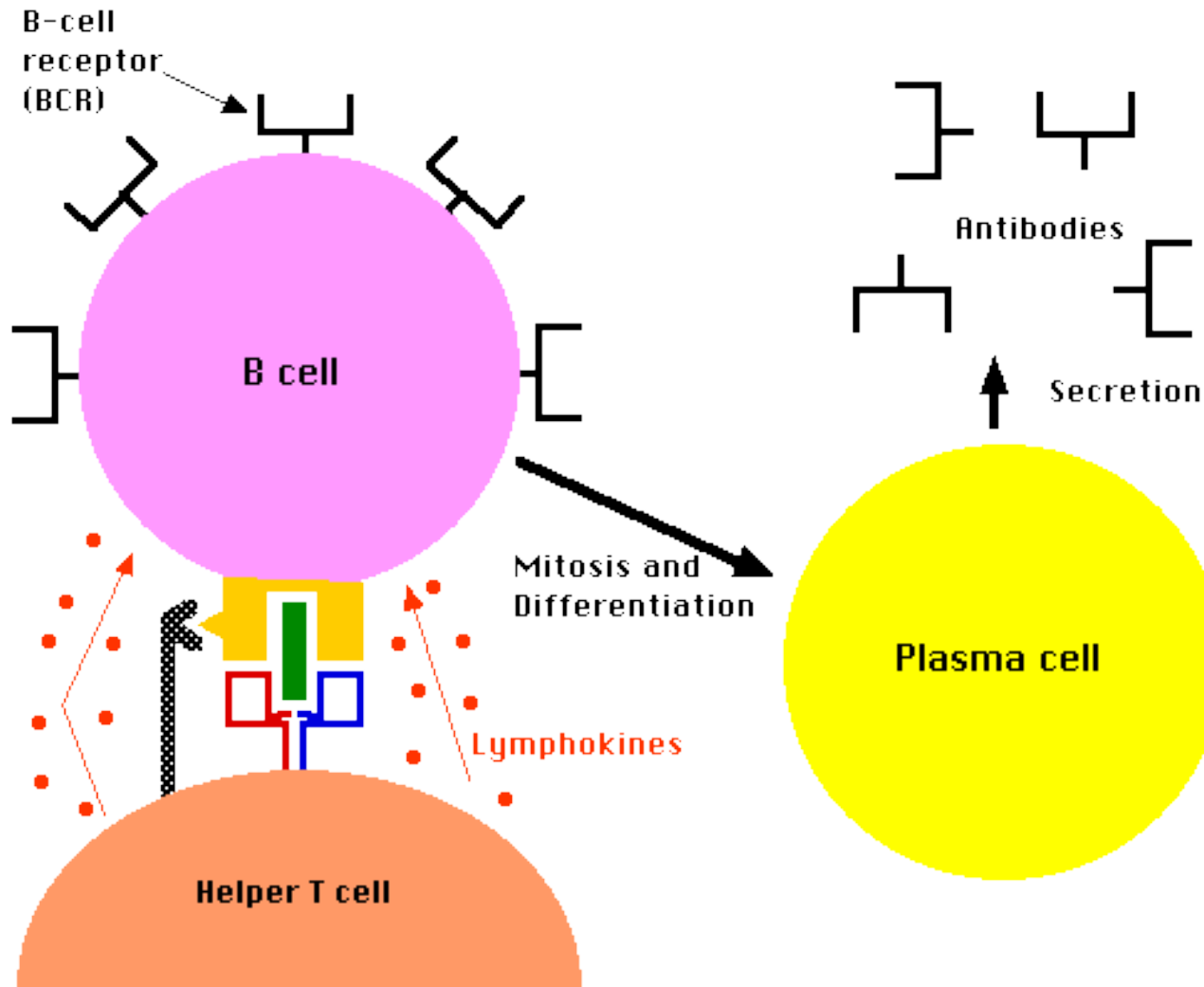
- **NK cells**
- **Gamma delta**
- **Soluble factors (cytokines)**

Adaptive immunity:

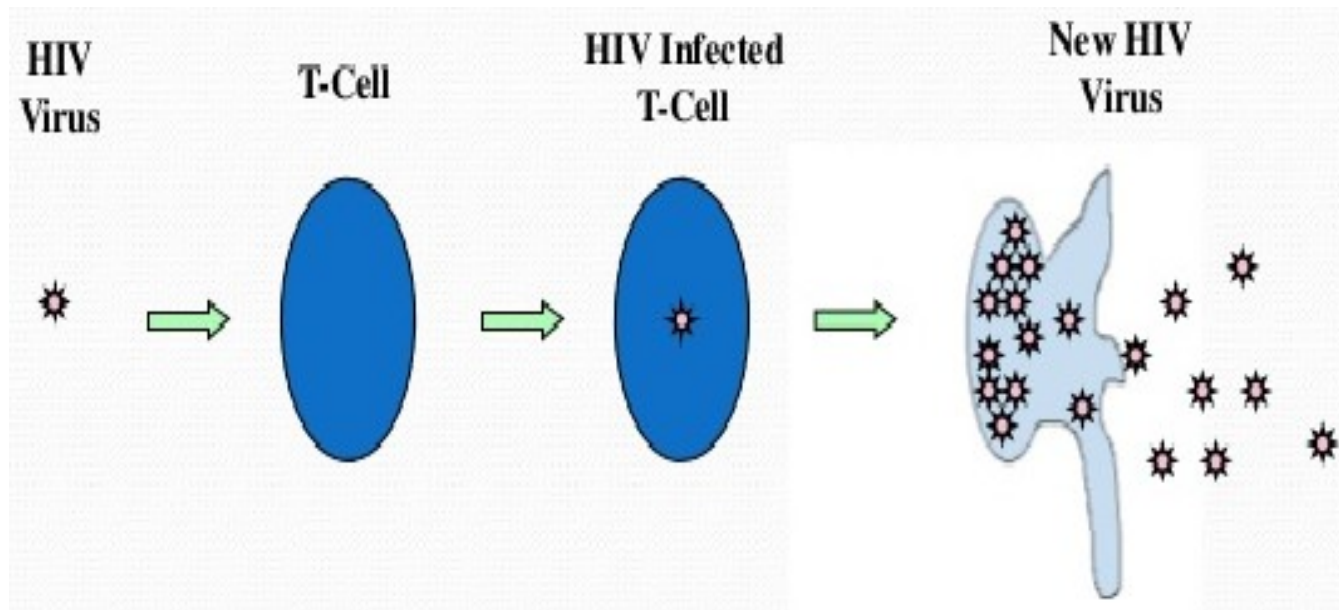
- **CD4 Th cell : conductor of immune orchestra**
- **CD8 T cell (cytotoxic T cell)**
- **B- cell (neutralizing Abs)**

Note: Mechanism of immune response against HIV infection is not fully understood !

Immunology & CD4 (T- helper) cell



Pathogenesis of HIV infection



Immune pathogenesis of HIV infection

Mechanisms

- **Syncytium formation**
- **Apoptosis**
- **Auto immunity**

Table 1. Mechanisms of CD4+ cell depletion

Direct

Single cell killing

Syncytial formation

Indirect

Killing mediated by specific immunity

Autoimmune mechanisms

Superantigen-mediated destruction

Apoptosis

Impact of HIV

- **Economic impact**
- **Overstrained healthcare systems**
- **Decreasing life expectancy**
- **Problem of child survival gains**
- **Increased numbers of orphans**

Role of Lab in HIV Dx

- **Presence of HIV infection in individuals can be ascertained only through the use of lab. tests**
- **Specimens include: Whole blood, plasma , serum**
- **WHO -UNAIDS have established an algorithm for the use of various tests for:**
 - **screening**
 - **surveillance**
 - **diagnostic purposes**

Diagnosis of HIV

Methods:

- **Nucleic acid-based test (RT - PCR)**
- **p24 antigen testing**
- **Culture**
- **Ab to HIV**

Laboratory with DNA PCR setup

Three rooms dedicated to specimen/reagent/control preparation and extraction, amplification and detection procedures



Antibodies to HIV are detectable:

- **Within 4 to 6 weeks of infection by commonly employed tests**
- **In virtually all infected individuals within six months**
- **Once antibodies appear in the blood, they persist for the lifetime**
- **Level of viraemia immediately after the acute primary HIV infection or the viral load set point, is a predictive marker of HIV disease progression**

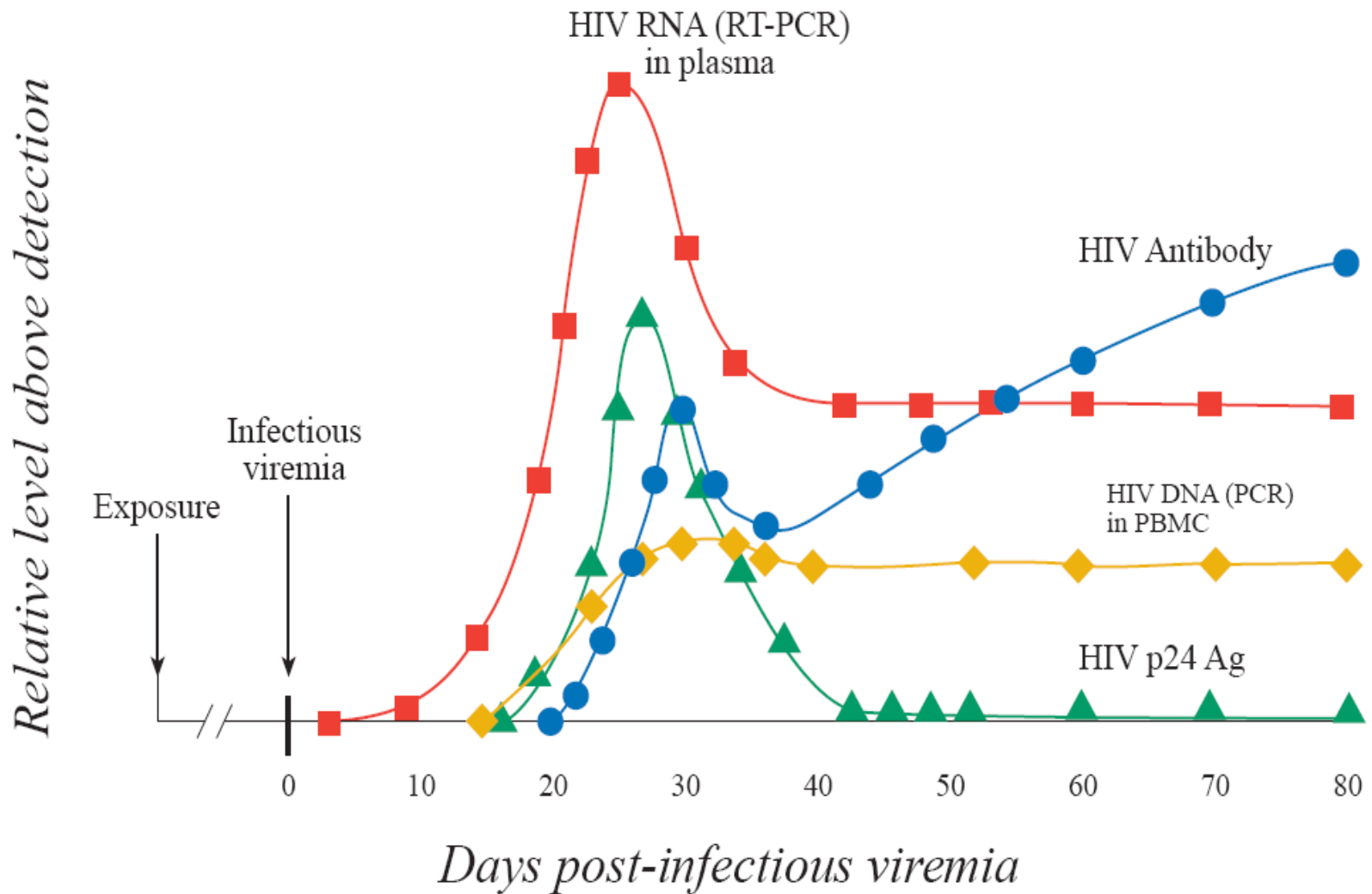


Fig. 2 Sequence & time course of virological and immunological events during HIV-1 infection : *Am J Med* 1997;102 (5B): 117-124

Description of Fig 2:

- **After infection, virus may replicate in mucosal & lymphoid tissue that drains the inoculation site, for variable periods lasting from 1 to 2 weeks but occasionally up to 6 months**
- **Following this period of viral replication, there is hematological dissemination day 0 in the figure**
- **Subsequently, there is a rapid rise in detectable viral RNA (squares) in plasma on about day 10 and HIV-p24 antigen (triangles) on day 15**
- **Anti-HIV antibody (circles) appears between days 20-30 post viremia**

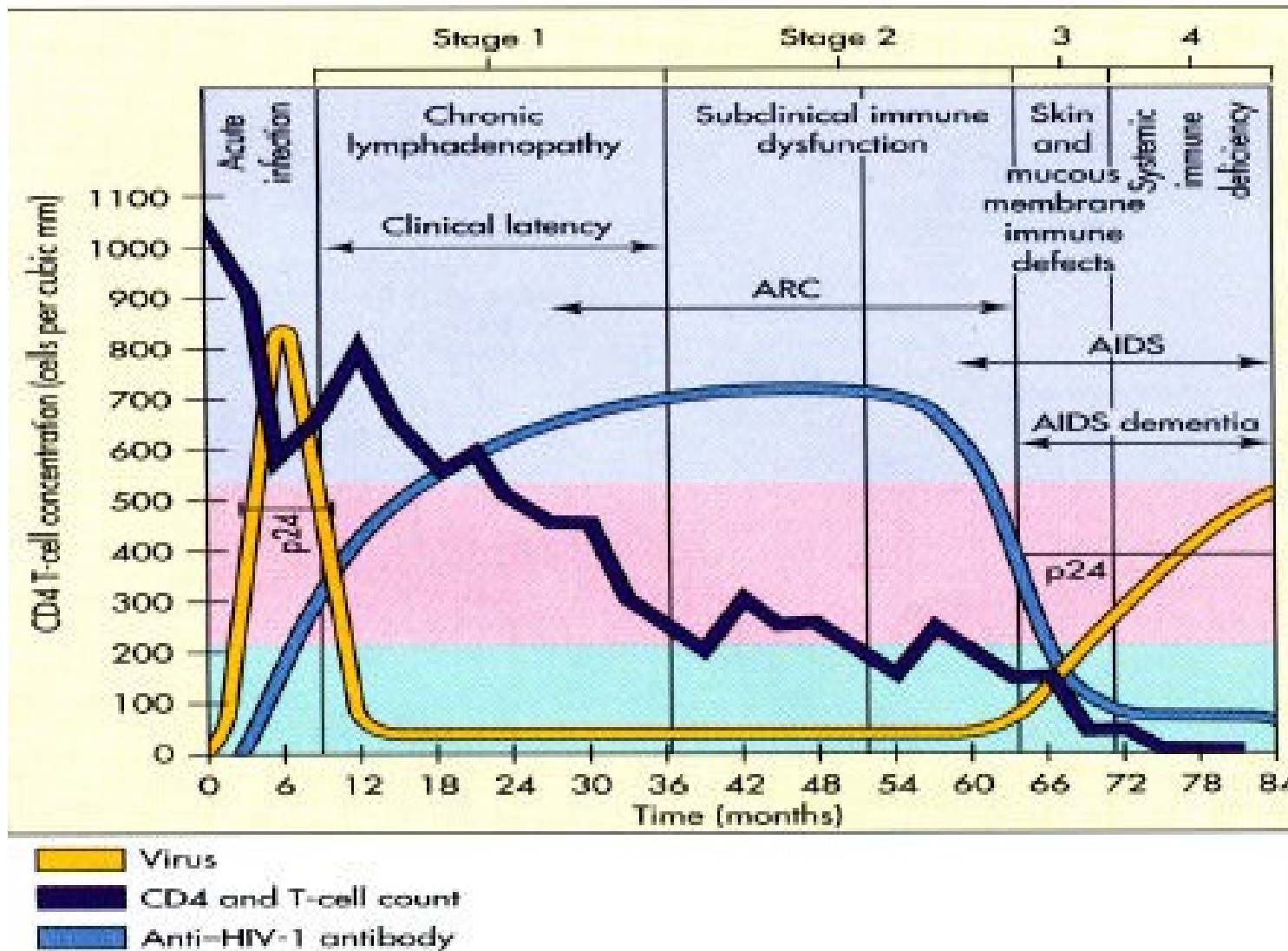


Fig .3 Laboratory markers during HIV-1 disease progression : WHO, 2004

Natural History of HIV Infection

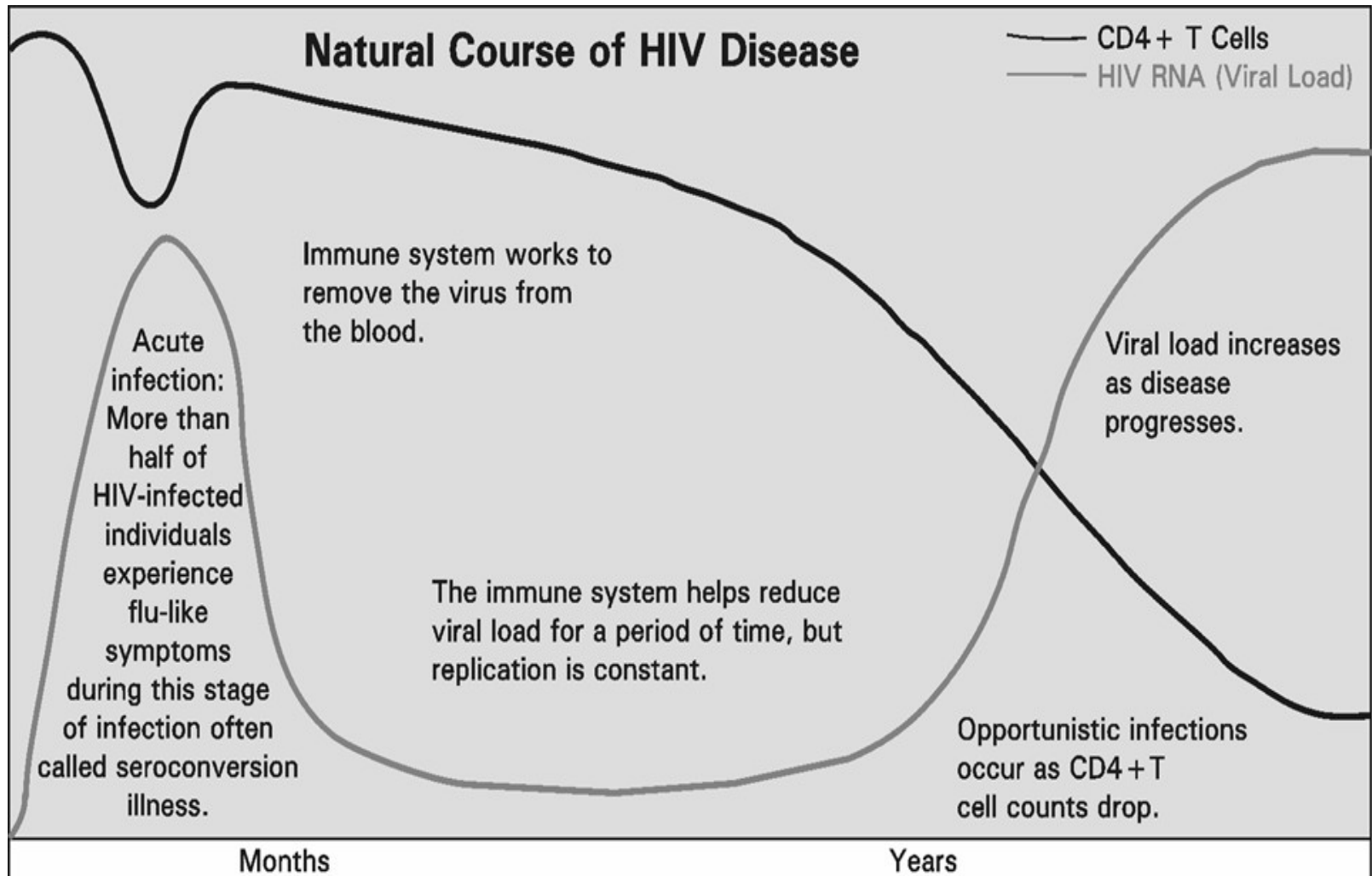
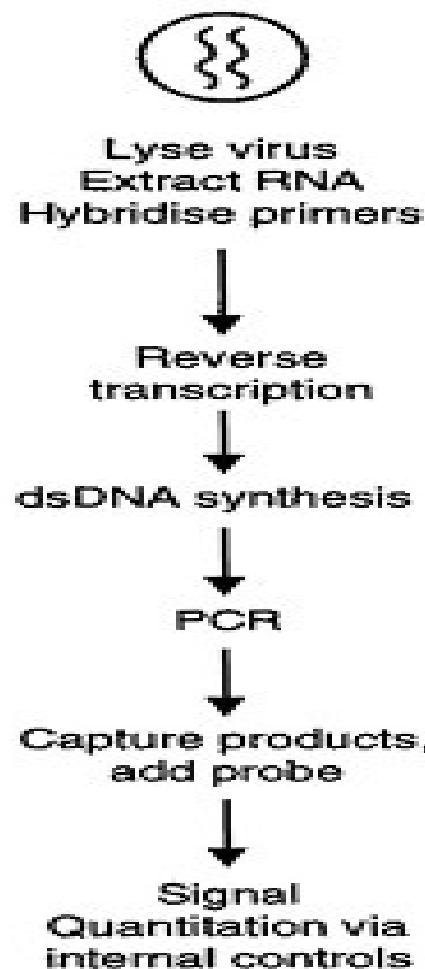
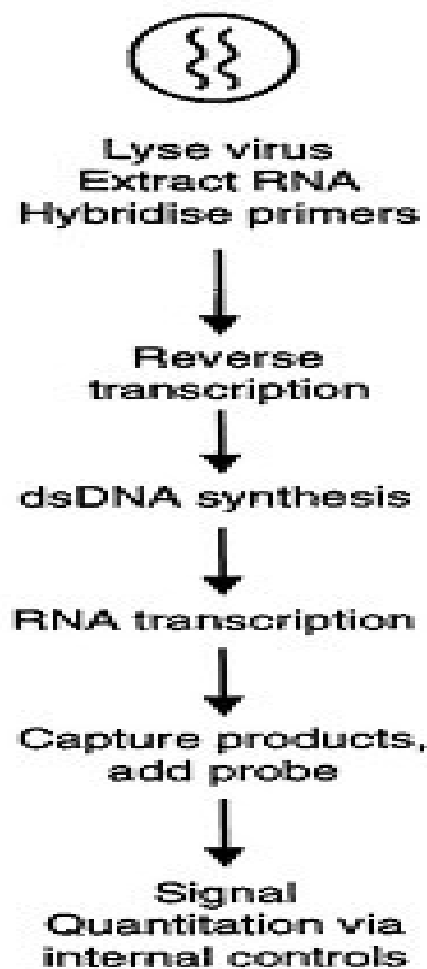


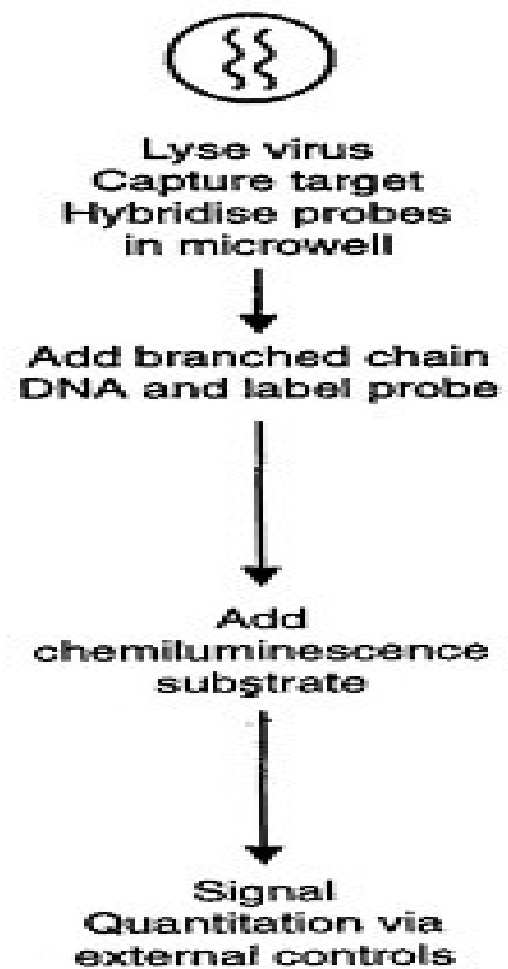
Figure 1. The natural progression of HIV disease. Without intervention, viral load will continue to increase as CD4+ T cell counts decline.



(a)



(b)



(c)

**Quantitative molecular methods for HIV plasma RNA:
(a) PCR (b) NASBA (c) bDNA**

Discordant HIV test results ?

- **Key receptor for HIV: CD4**
- **Co- receptor : CCR5 & CXCR4**
- **At least there are 14 potential chemokine co- receptors have already been determined**
- **However, CCR5 & CXCR4 are the dominant *in vivo* receptors utilized by HIV**

- **CCR5 is expressed on:**
 - **T cells ; macrophages ; dendritic cells & microglia cells**
 - **serve as a co- receptor for macrophages tropic HIV strains**
- **CXCR4**
 - **is highly expressed on naïve Th cells**
 - **mainly serve as a co- receptor for T cell-tropic HIV strains**
- **Besides, a number of other trans-membrane molecules have been described that facilitate infection with certain HIV strains**

- **A naturally occurring mutation, a 32 bp deletion in the gene encoding CCR5 is present up to 13% of Europeans**

► **Deletion in Ethiopian population??**

- **People who are homozygous for this mutation (1 -2%) of Caucasians display almost complete resistance to HIV infection.**

- **Chemokine co receptors are found in sphingolipid and cholesterol rich micro- domains of the plasma membrane of target cells**

⇒ **These micro - domains are referred to as lipid rafts**

- **The lipid rafts serve to preserve the co - receptors' conformation**

Other assumptions causing discordant results

- **HLA.....strength of Ag presentation to T- cells**
- **CTL response.....Effectiveness vs response**
- **Natural killer cells.....Effectiveness vs response**
- **Repeated infections.....shorten life span of T-cells/wbcs**

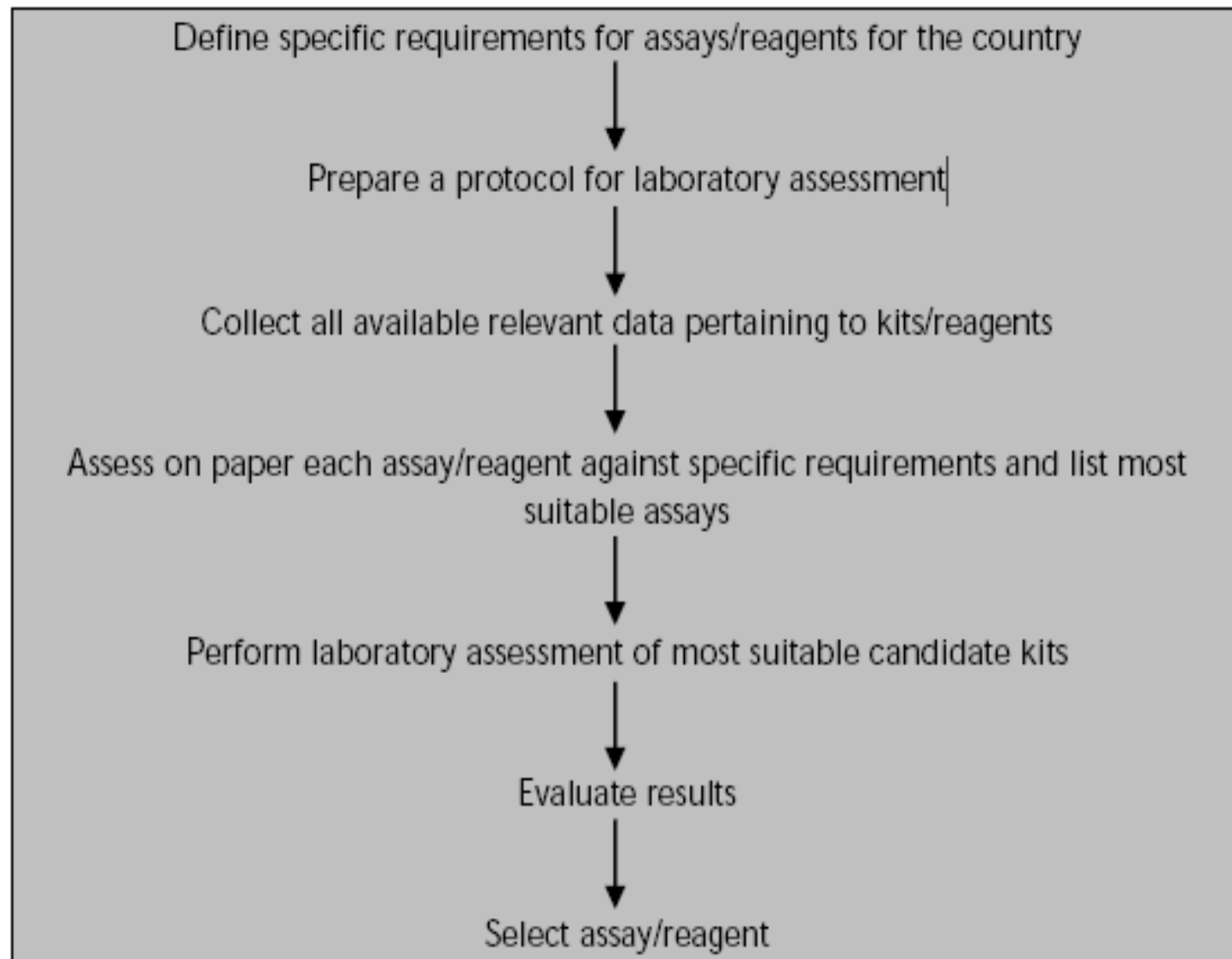
HIV Dx in infants

- **Diagnosis of HIV infection in babies born to HIV-infected mothers cannot be established by conventional antibody tests**
- **The presence of anti-HIV Ab in the newborn may not necessarily indicate primary infection:**
 - **may be due to passive transmission of anti-HIV Ab from mother to uninfected child**
 - **These maternal antibodies may persist even up to 18 months**

- Hence, diagnosis in children **less than 18 months of age** is possible only by the detection of :
 - HIV nucleic acids
 - Viral culture
 - Detection of the p24 antigen
 - Per cent of T-lymphocytes which express CD4
(for HIV infected pediatric population)

- **A wide variety of test kits are now available for HIV diagnosis**
- **Selection of appropriate test kits/assays/reagents is critical to ensure quality in laboratory services**
- **Every country or laboratory must therefore define a policy for selection of the most appropriate test kit**

Evaluation & final selection of kit/reagent/assay



- **Monitoring of patients on chemotherapy is essential in all infectious diseases**
- **It is of greater importance in HIV because of:**
 - **Severity of illness**
 - **Potential of the virus to mutate & become resistant to drugs**
 - **Cost of treatment**

- **Hence, lab. are bound to play a critical role in the successful implementation of ART programme**
- **ART aims at :**
 - ✓ **Reducing the viral load**
 - ✓ **Augmenting the immune response of patients**

HIV viral load measurement

- **Useful in monitoring treatment**
- **Requires for establishment of a baseline plasma viral load before starting ART**
- **The viral load in the case of successful ART becomes undetectable in 4 to 6 months of therapy**
- **A number of assays for virus load are in use, however :**
 - **Need rigorous standardization**
 - **Continued quality assurance ... need resources!**

- **The assessment of viral load, though possible, is :**

- **Very expensive**
- **Complex**
- **Sophisticated procedure**

► **Hence, not recommended by WHO**

Monitoring of ART

Monitoring	Laboratory area
Virological	Viral load Resistance to antiretroviral drugs
Immunological and Haematological	CD4 count Total lymphocyte counts
Microbiological	Occurrence of new opportunistic infections Recurrence of treated opportunistic infections Antimicrobial susceptibility of bacterial pathogens Reactivation of TB
Adverse drug reaction	Liver and kidney function tests Haematological parameters

Immunological monitoring of ART: CD4 counts

CD4 +T Cell

A glycoprotein of the immunoglobulin superfamily expressed on :

- T helper cells (Th)**
- monocytes**
- macrophages**
- dendritic cells**

Function

- increases the activation of Th cells by amplifying the intracellular signal generated upon activation of the T cell receptor**
- Maintain the integrity of the human immune system**
- facilitates attachment of HIV by binding to gp¹²⁰**

Clinical utility of CD4⁺ T cells monitoring:

- **Are primary target cells attacked by HIV**
- **Depleted during the course of the disease**
- **The utility of CD4⁺ T cell measurements**

involves:

- **considerations for HIV disease staging & AIDS definition**
- **Assessment of prognosis**

- **Accurate enumeration of CD4⁺ T cell counts is very crucial for:**

Monitoring the rate of progression to AIDS, both for:

- **initiating prophylaxis for OIs**
- **monitoring the impact /effectiveness of ART**

N.B: The CD4 number does not Dx HIV infection

Re: CD4 + T-cells may be reduced in other :

- **viral infections not associated with HIV**
- **immune deficiency states Eg: Agammaglobulinemia**

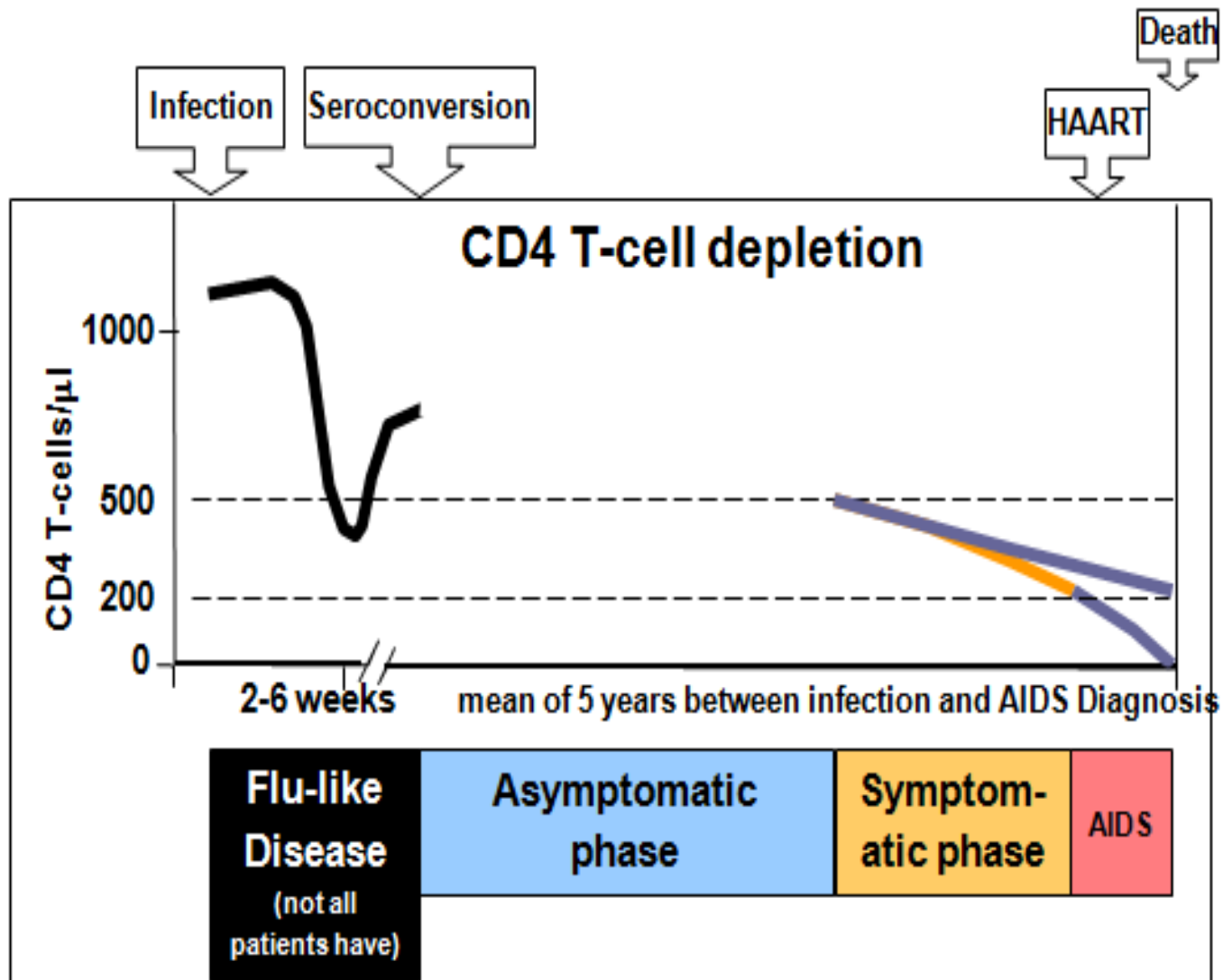
CD4 T- cells & HIV

- **Decrease in CD4 + T-cells is associated with :**
 - **Increased risk for opportunistic infections**
 - **Disease progression**
- **CD4 counts of < 200 cells /ul are associated with greater risk for pneumocystis infections**
- **CD4 counts <100 cells /ul are associated with greater risk for Mycobacterial infections**
- **CD4 +T-cell count is used for :**
- ▶ **Clinical prognosis, monitoring therapeutics & for inclusion criteria for ART**

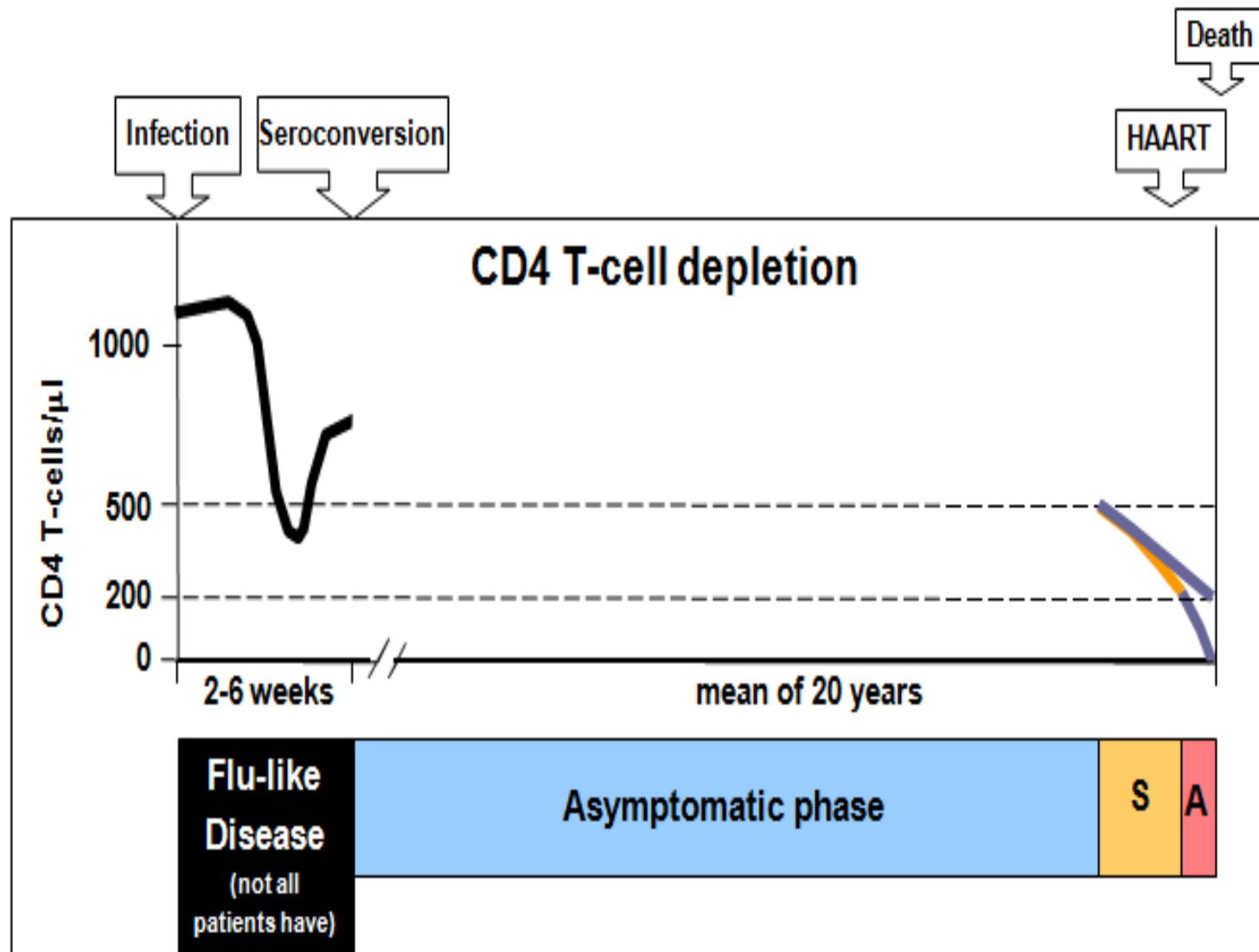
Time course of CD4⁺ T- cell reduction

- **HIV infection will progress to AIDS at different rates in different people Why??**
- **CD4 + T-cell count may be used as an adjunct/tool to monitoring individuals to determine if they are:**
 - ✓ **fast progressors**
 - ✓ **slow progressors**
 - ✓ **non-progressors**

Clinical Consequences of HIV Infection (Fast Progressor)



Clinical Consequences of HIV Infection (Slow Progressors)



Reference range for CD4-positive T-cells

- **Reference range for CD4 + T-cells varies with:**
 - **patient age**
 - **time of specimen collection !!!**
 - **geographic location**
- **Adult: 500 -1500 cells/ul White population**
- **Eth. popn: 350 -1350 cells/ul (*Aster et al;*
1999)**

Clinical spectrum

1.HIV infection

2. ARC include:

- **Immunological, dermatological & neurological
sins**
- **Fever**
- **Weight loss**
- **Night sweating**
- **Chronic diarrhea**

3. AIDS

- ✓ **Final stage of infection**
- ✓ **Complete failure of CMI**
- ✓ **Opportunistic infections & tumors**

4. Death

Opportunistic infections (OIs)

1. Pneumocystis jirovecii..... causes pneumocystis pneumonia

► **Fever, dry cough, weight loss, weakness**

2. Toxoplasmosis of the brain

► **Encephalitis, causing neurological disorders**

3. Viral infection

- **CMV retinitis...Blurred vision, reduced visual acuity**
- **HSV...Herpetic neuropathia of craniospinal ganglia**

4. Esophageal candidiasis

- **Swallowing difficulties**
- **Nausea & vomiting**

N.B: In > 80% of cases, both oral cavity & pharynx are affected

5. Kaposi's sarcoma (KS)

- **About 30 - 50% of HIV infected homosexuals develop KS**
- **HHV 8: Triggers KS**

6. Atypical Mycobacterium infection

- ▶ **Severe OIs in > 90% HIV pts (CD4 cell count < 50 cells / μ l)**

7. Cervical carcinoma

- **Pathological pap smears occur 10 to 15 times greater frequency in sero pos. women than sero neg.**
 - ▶ **HPV infection exacerbates the problem**
- **Regular gynecological examination of HIV infected women !!!**

Treatment (Rx)

1. Non- specific

Provision of Vitamins & minerals are essential

- **Chronic HIV infection exerts persistent metabolic strain on the body**
- **Link b/n serum conc. of vit E & B12 with disease progression**

2. Treatment of OIs

- ▶ **Bacterial, fungal, viral, protozoal & others**

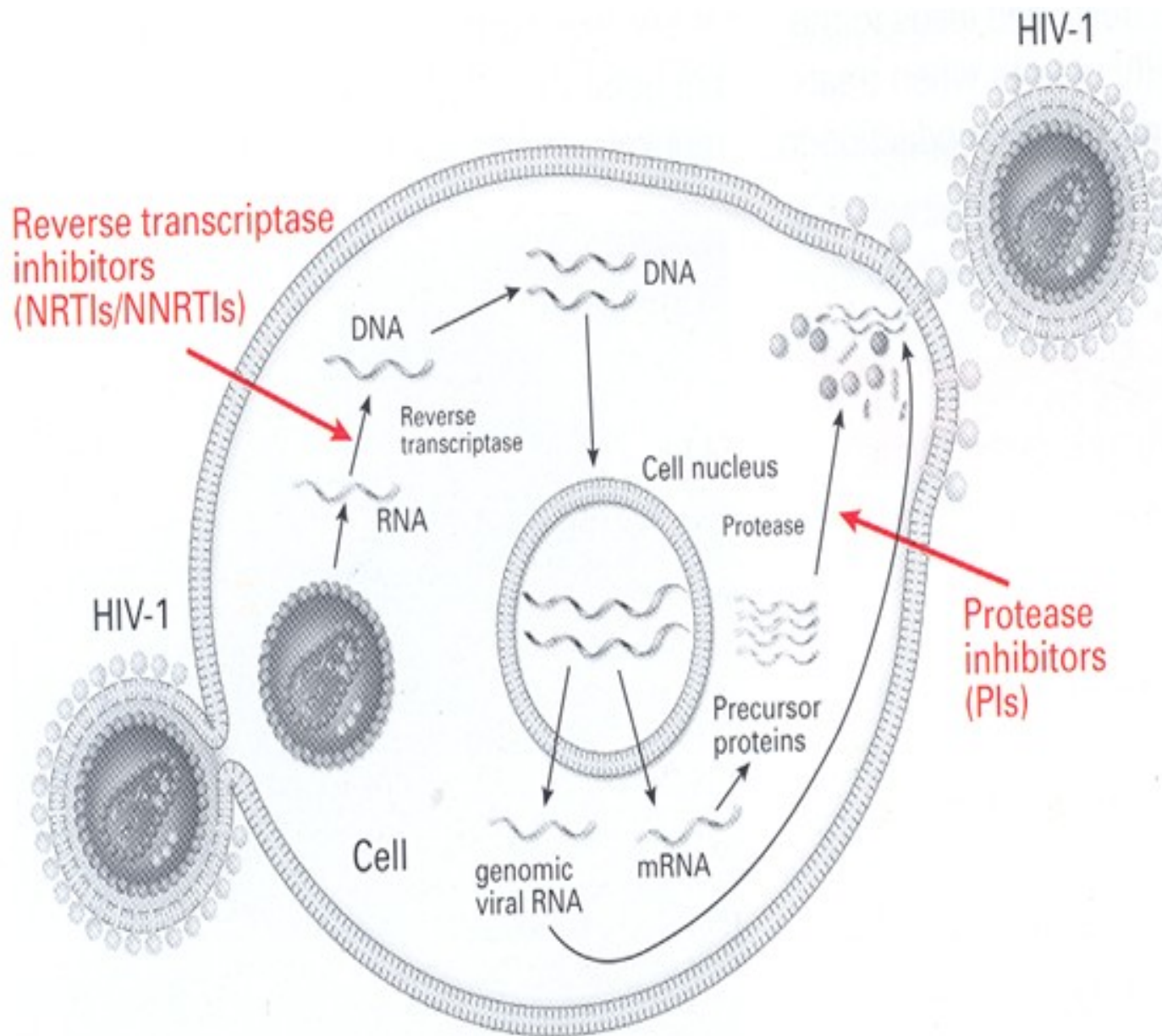
3. Specific anti -viral agents

RT inhibitors

- **Abacavir**
- **Zalcitabine**
- **Lamivudine**
- **Zidovudine (PMTCT)**
- **Didanosine**

Protease inhibitors

- **Ritonavir**
- **Indinavir**
- **Amprenavir**
- **Saquinavir**



Major side effects

- **Hypersensitivity reactions**
- **Nausea**
- **Neuropathy**
- **Rise in transaminases**
- **Diarrhea**
- **Disorder of fat metabolism , etc**

Vaccine prospects

- Purified viral envelope gps: **gp¹²⁰ /gp¹⁶⁰**
- Whole inactivated virus
- Live attenuated HIV strains (lacking certain genes)
- Live recombinant virus vector expressing HIV proteins

Prevention & control

- **Follow universal precautions**
- **Screen all blood & blood products**
- **Identify & treat STIs/other infections**
- **Provision of ART**
- **PMTCT**
- **Supportive care**
- **Avoiding stigma & discrimination**
- **Education (behavioral change)**