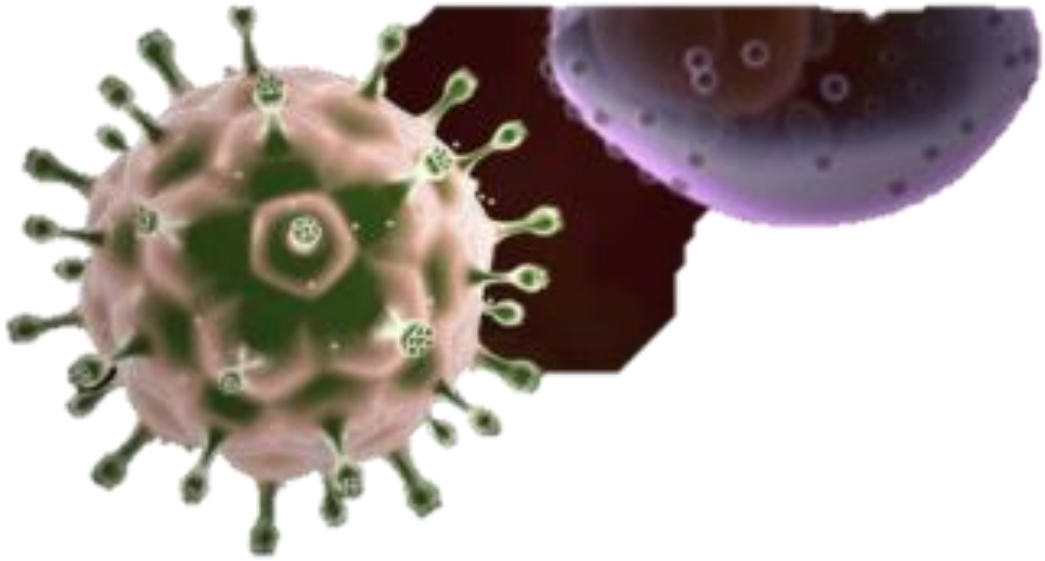


HIV Virology

Anna Maria Geretti

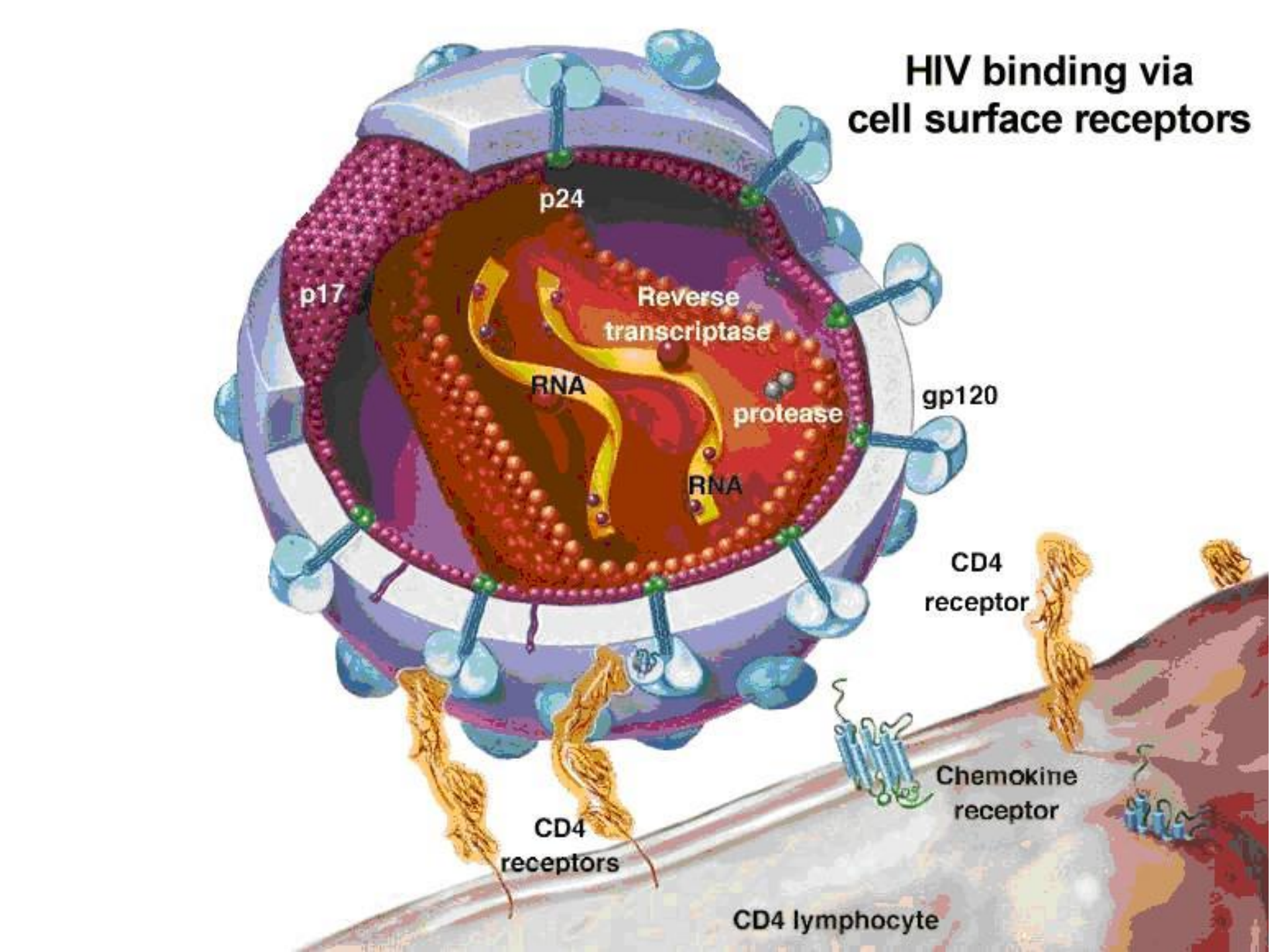
Institute of Infection & Global Health

UNIVERSITY OF LIVERPOOL

Your turn ☺

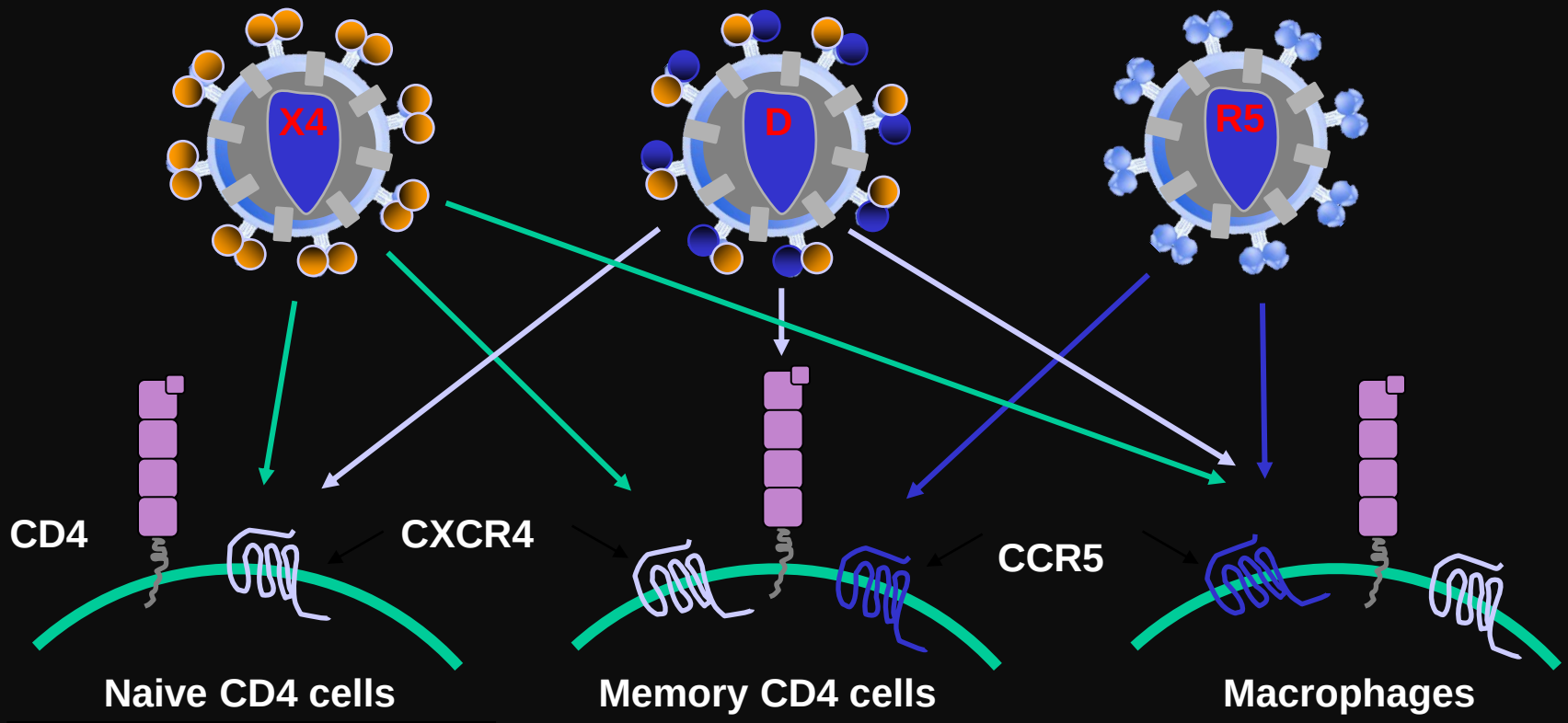
Which of the following applies to your setting?

1. **I use viral load monitoring routinely**
2. **I use resistance testing routinely**
3. **I have access to virology tests but only in selected cases**
4. **I do not have access to virology tests**

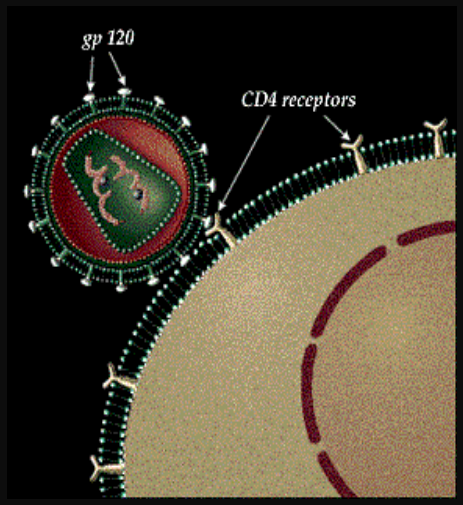

HIV binding via
cell surface receptors
p24
p17
Reverse
transcriptase
RNA
protease
RNA
gp120
CD4
receptor
Chemokine
receptor
CD4
receptors
CD4 lymphocyte

HIV Tropism

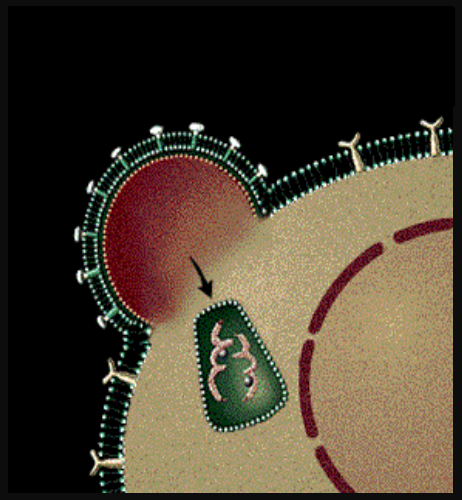
- Defined by the use of co-receptors and their cellular distribution



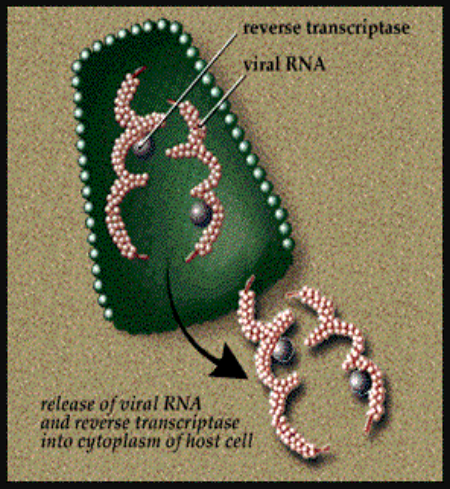
Esté et al. Lancet 2007



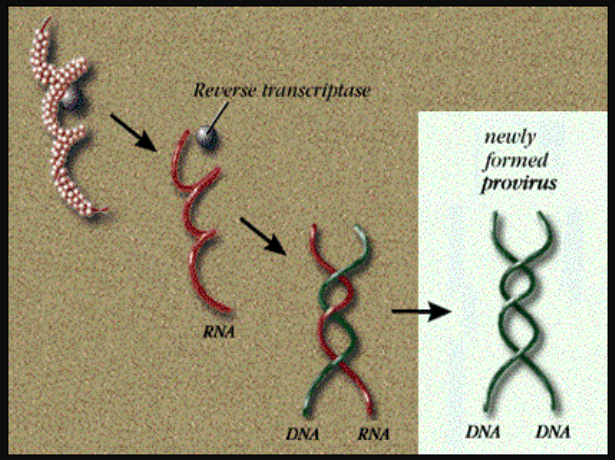
Attachment



Fusion

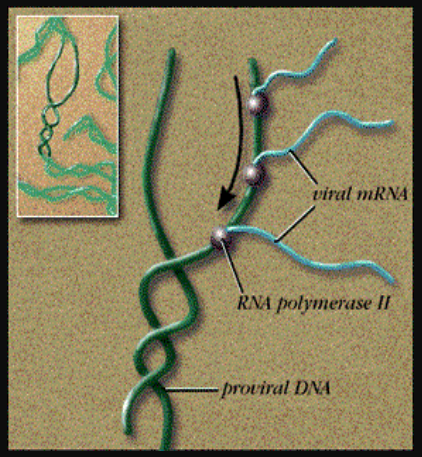


Release of RNA

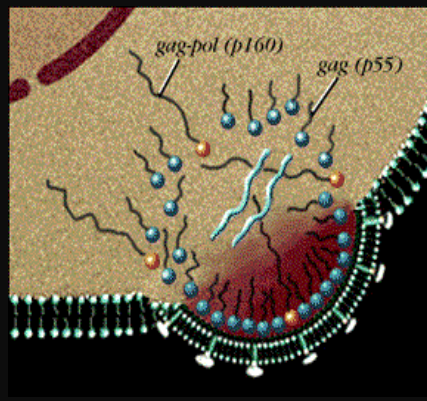


Reverse transcription

Integration

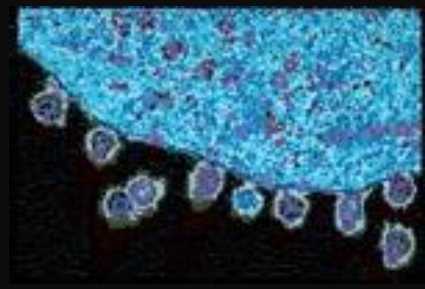


Transcription



Assembly

Maturation & budding



Key virological characteristics of HIV infection

- **High replication rate**
  10^9-10^{10} virus particles produced each day
- **Rapid virus clearance**
  $T_{½}$ virus producing cells: <1 day
  $T_{½}$ plasma free: a few hours
- **Genetic evolution**
  All possible point mutations in the viral genome are generated daily
- **Virus latency – integration into host DNA**
  ~ 1:10^6 resting CD4 T cells

The HIV Virology Timeline

HIV-1 isolated — 1982

HIV-1 genome sequenced — 1985

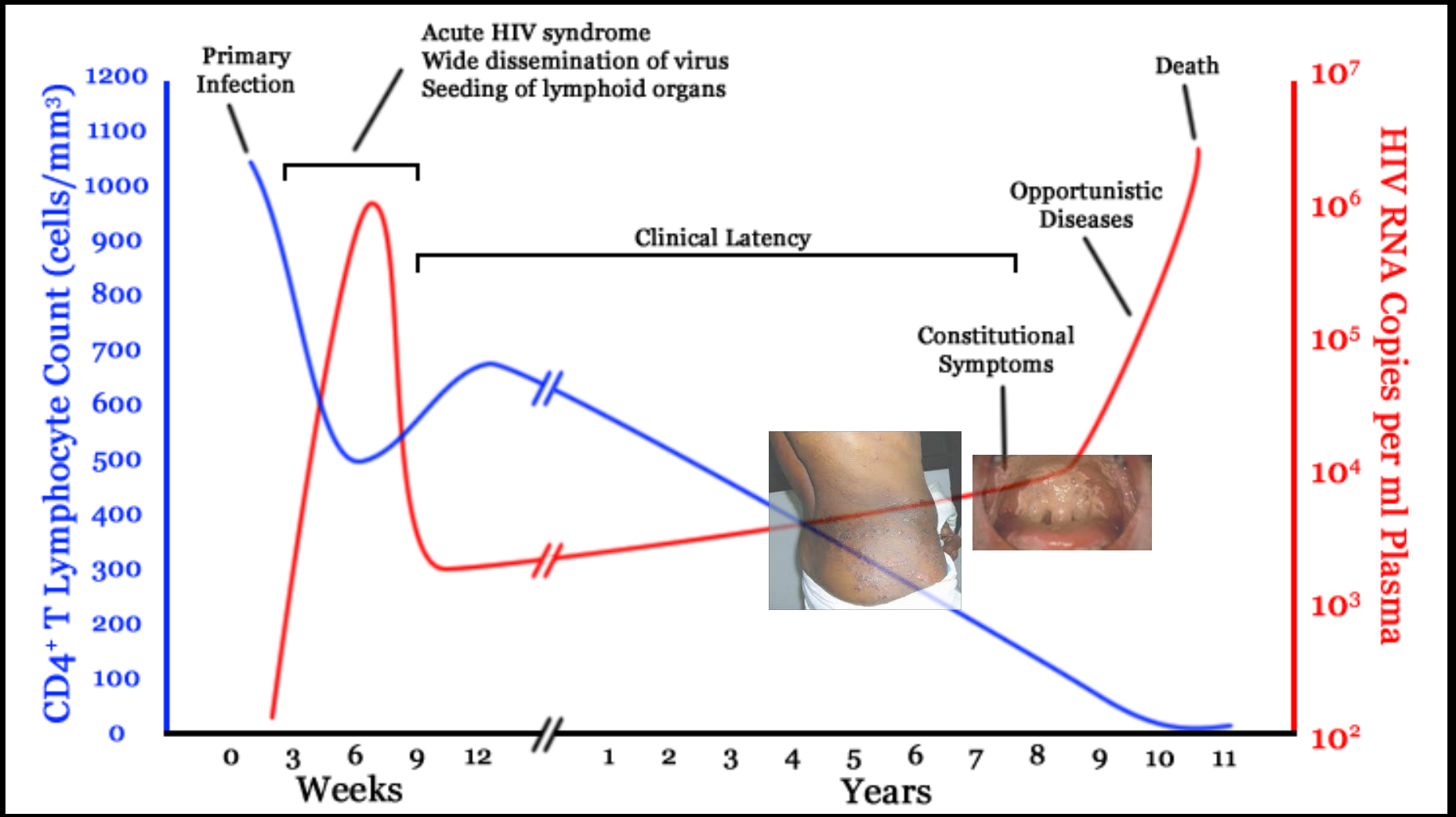
HIV replicates at high levels throughout the infection — 1991

HIV replication drives immune compromise — 1995

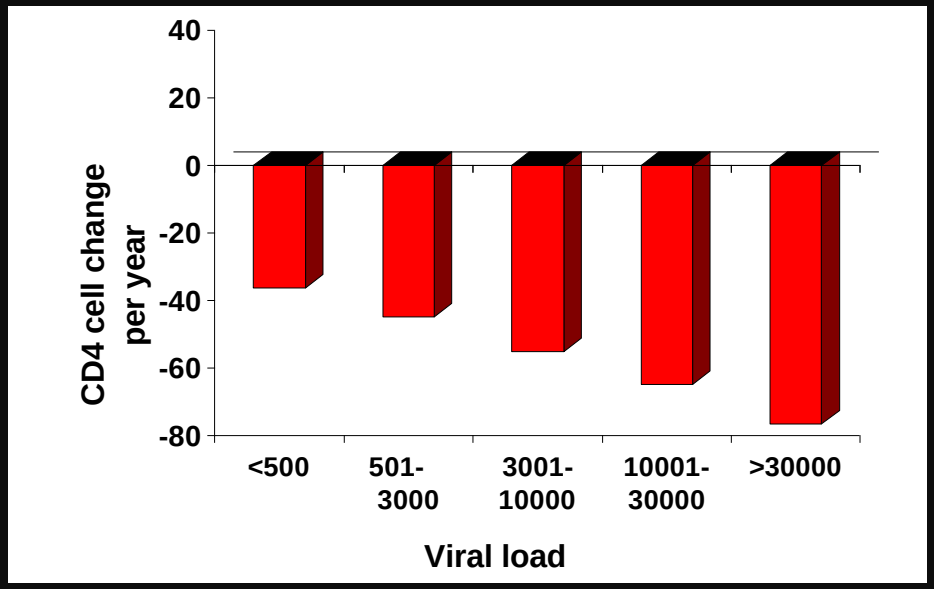
Highly active antiretroviral therapy — 1996

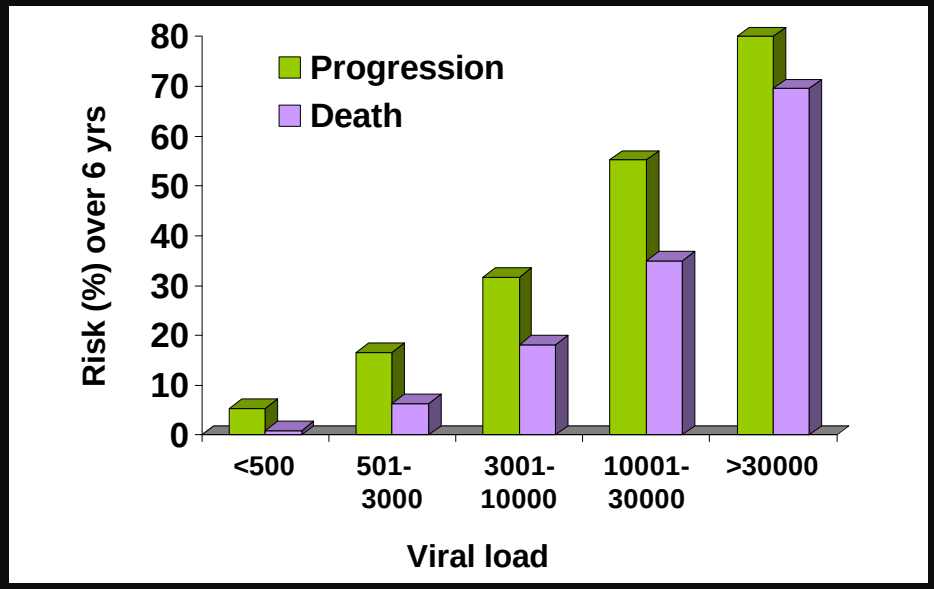
Plasma HIV RNA ("viral load") suppression as goal of therapy — 1996

Natural course of HIV infection



HIV viral load predicts the rate of CD4 cell loss and disease progression





Mellors et al. Science 1996

The HIV Virology Timeline

HIV-1 isolated

HIV-1 genome sequenced

HIV replicates at high levels throughout the infection

HIV replication drives immune compromise

Highly active antiretroviral therapy

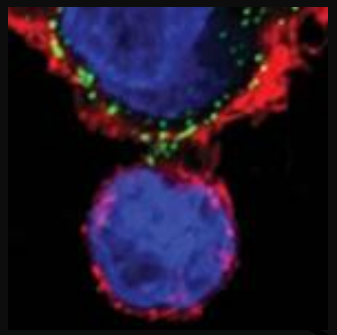
Plasma HIV RNA ("viral load") suppression as goal of therapy

HIV replication causes disease through immune activation & inflammation

HIV eradication attempts

1982 1985 1991 1995 1996 2009 2010 →

Pathogenesis of HIV infection



Immune activation
Inflammation

Immune compromise

Cardiovascular, Renal, Bone, Neurocognitive disease; Cancer; etc

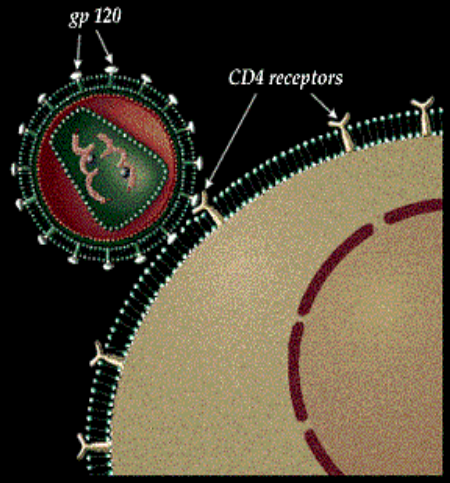
Liver disease due to hep B or hep C

Accelerated aging

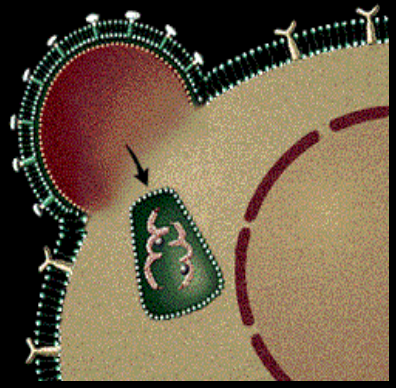
AIDS, Cancer

The goal of antiretroviral therapy

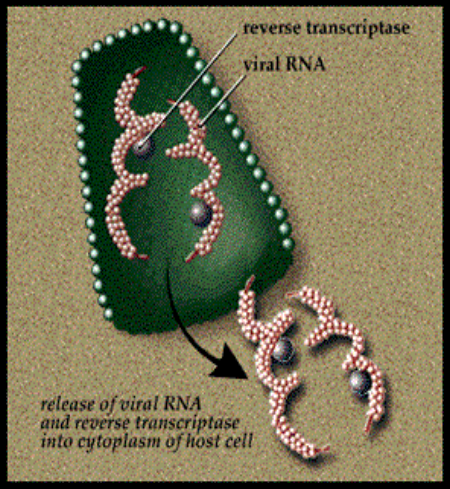
- *To induce and maintain plasma viral load suppression as the key surrogate marker of clinical efficacy*



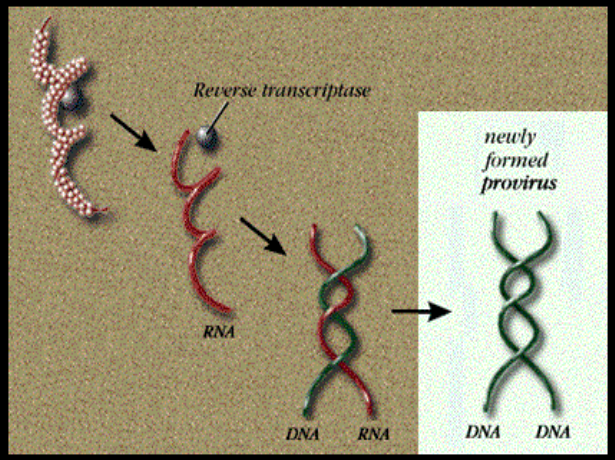
Attachment



Fusion

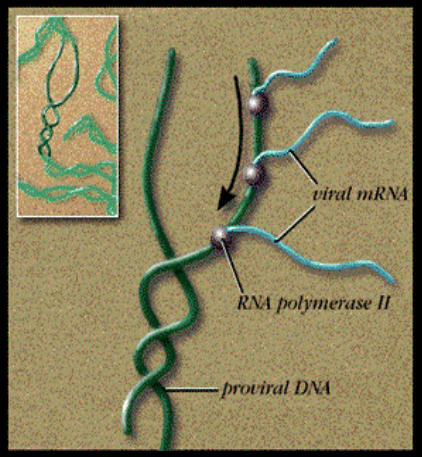


Release of RNA

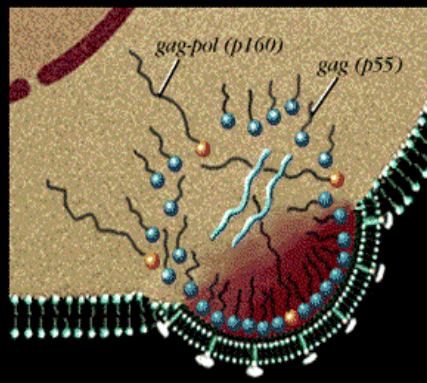


Reverse transcription

Integration

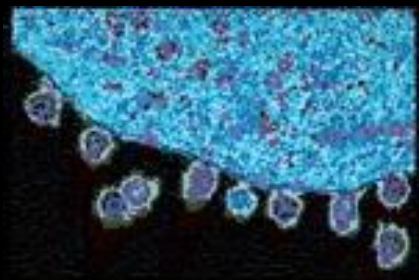


Transcription

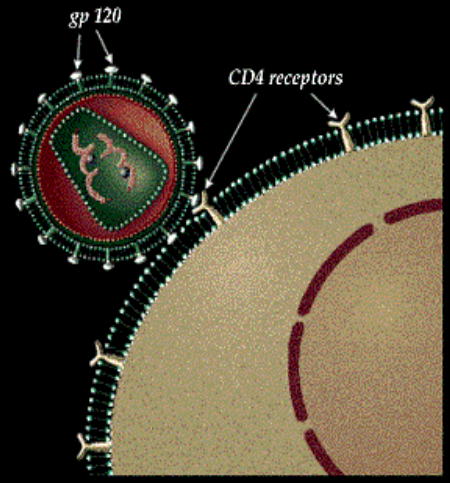


Assembly

Maturation & budding

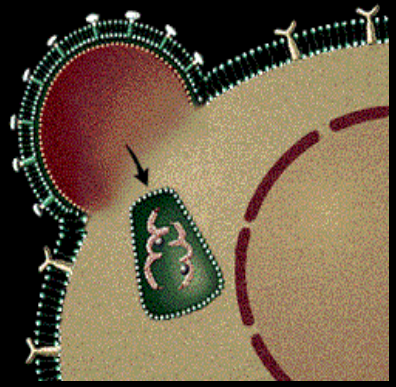


CCR5 antagonists

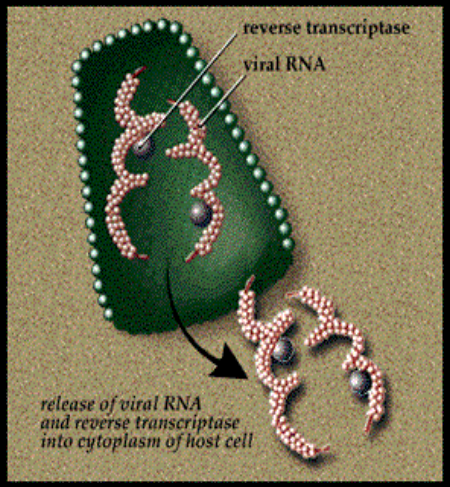


Attachment

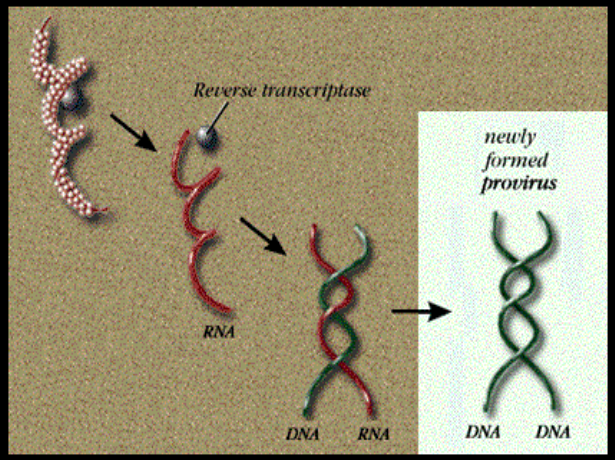
Fusion inhibitors



Fusion



Release of RNA

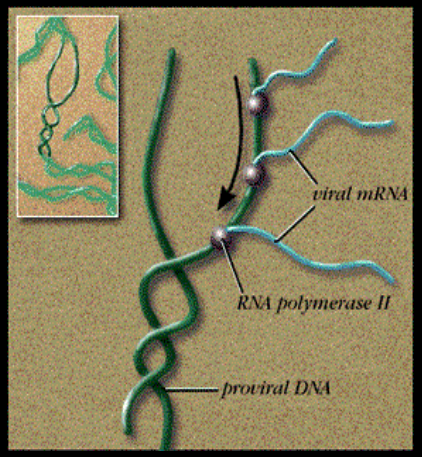


Reverse transcription

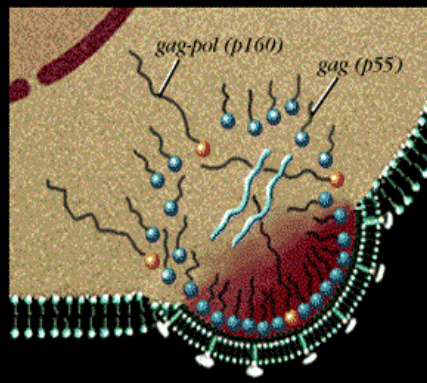
Nucleos(t)ide and Non-nucleoside RT inhibitors

Integration

Integrase inhibitors



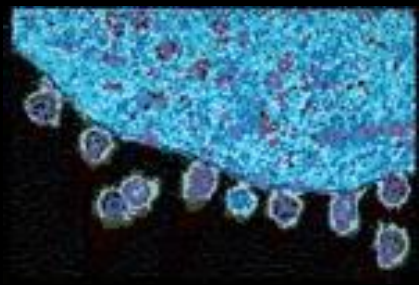
Transcription



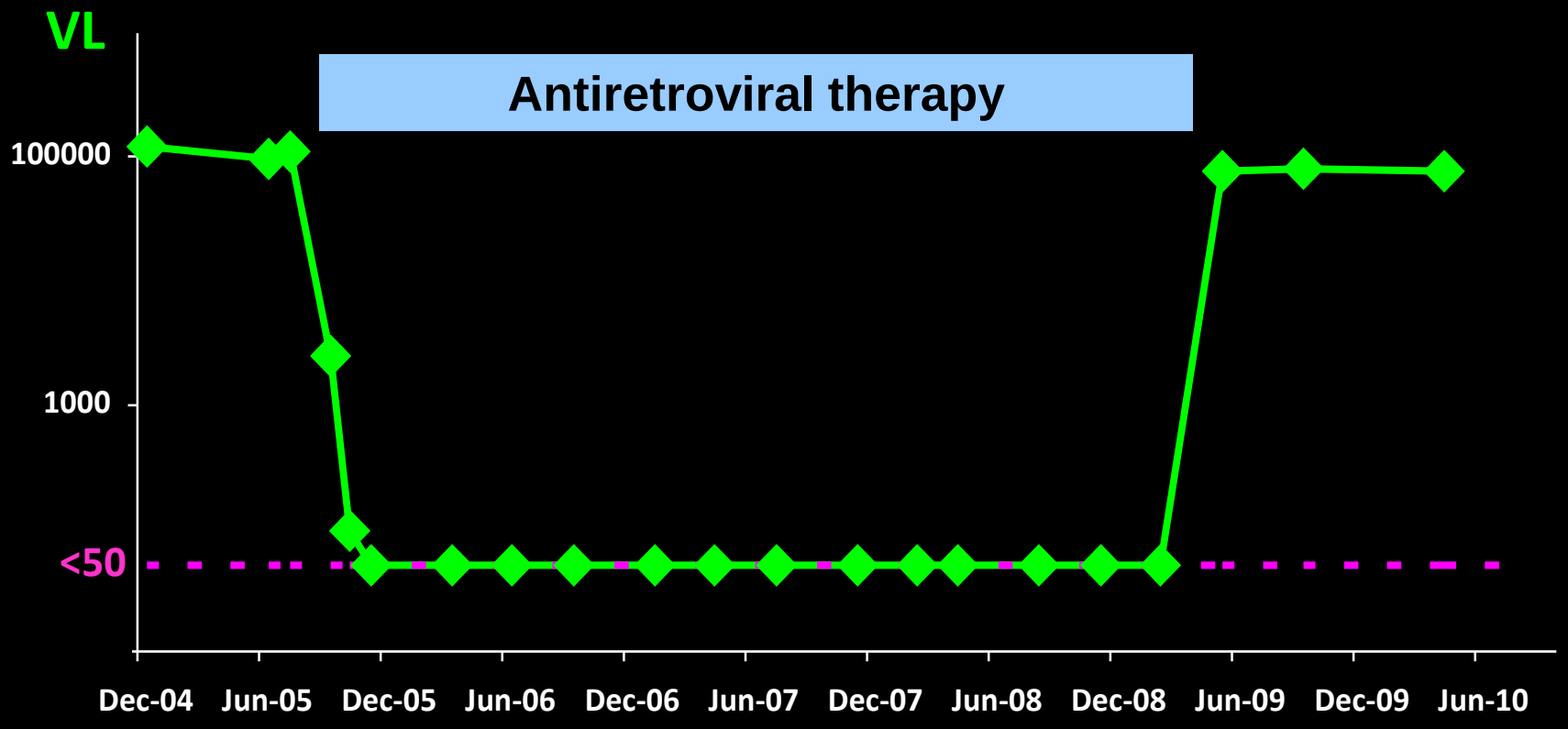
Assembly

Maturation & budding

Protease inhibitors



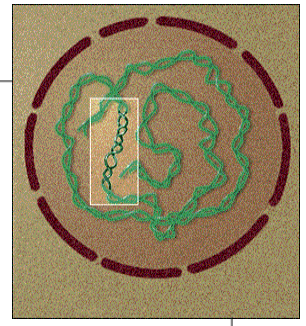
The viral load rebounds after stopping therapy



- Antiretroviral therapy cannot achieve virus eradication
- After therapy discontinuation the viral load rebounds to pre-treatment levels

Persistence of cell-associated HIV-1 DNA during long-term therapy

- HIV-1 DNA quantified in PBMC from 104 patients receiving suppressive ART for 1 to 15 years



PBMC = Peripheral Blood Mononuclear Cells

Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

Case study: Mr B

- 45 yrs old man
- HIV-positive in 2000
- HBV and HCV negative
- Baseline CD4 349 cells
- Baseline VL 71,000 cps
- Started ART in 2001

Date	VL cps	*ARVs*
Nov 01 - Apr 04	<50	*ABC ddI NFV*
May 04 - Mar 08	<50	*ZDV 3TC NVP*
Apr 08	<50	*TDF FTC NVP*
Jul - Feb 09	<50	
Jul 09	53	
Dec 09 - Mar 10	<50	
Jul - Dec 10	97-77	

HBV = Hepatitis B virus; HCV = Hepatitis C virus
VL = Viral load; ART = Antiretroviral therapy
ARVs = Antiretrovirals
ABC = Abacavir; ddI = Didanosine; NFV = Nelfinavir; ZDV = Zidovudine;
3TC = Lamivudine NVP = Nevirapine; TDF = Tenofovir; FTC = Emtricitabine

Your turn ☺

Which of the following correctly defines virological failure?

1. **Any confirmed HIV RNA detection**
2. **Confirmed viral load >50 cps**
3. **Confirmed viral load >200 cps**
4. **Confirmed viral load >400 cps**
5. **Confirmed viral load >1000 cps**

Variable definitions of virological failure

EACS 2014: Confirmed >50 cps ≥6 months after ART initiation or modification

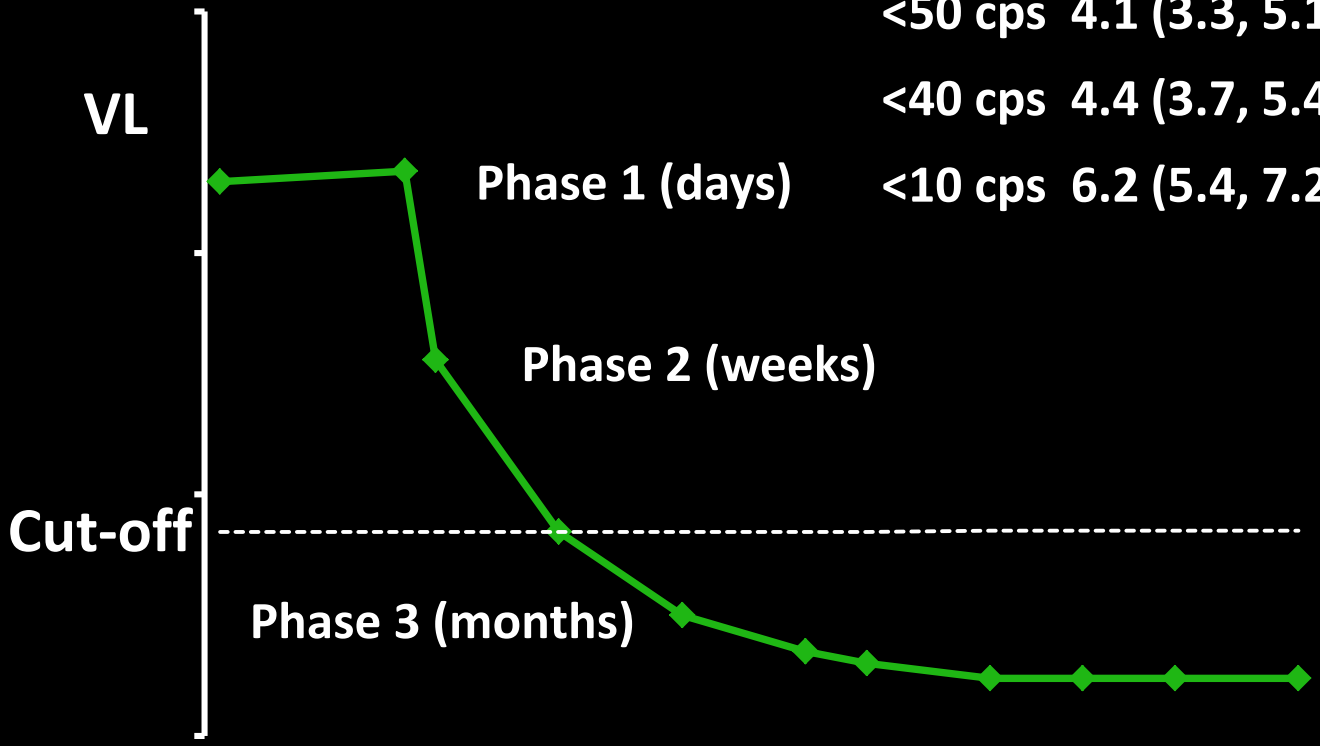
DHHS 2014: Inability to achieve or maintain <200 cps

IAS-USA 2014: HIV-1 RNA level >200 cps should prompt evaluation of factors leading to failure and consideration of switching ART

BHIVA 2013: Failure to achieve <50 cps 6 months after starting ART, or confirmed rebound >400 cps after suppression <50 cps

WHO 2014: Confirmed >1000 cps after ≥6 months of ART

HIV-1 RNA kinetics after starting ART



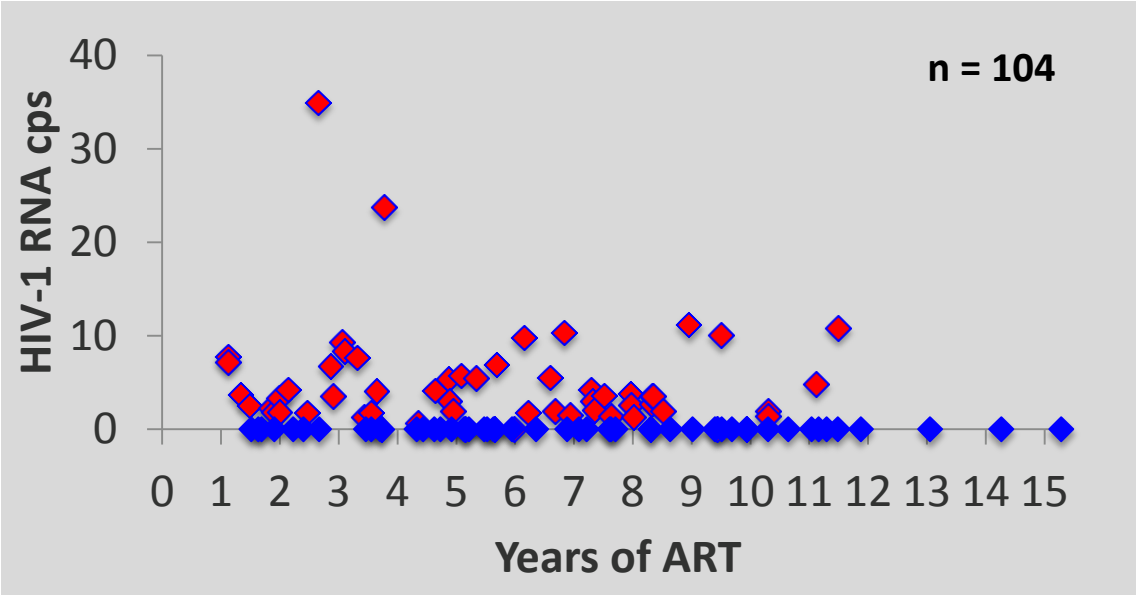
Median months to cut-off (95%CI)[1]

<50 cps	4.1 (3.3, 5.1)
<40 cps	4.4 (3.7, 5.4)
<10 cps	6.2 (5.4, 7.2)

1. Doyle et al. Clin Inf Dis 2012

"Residual" HIV-1 RNA detection during ART

- 1st-line NNRTI-based ART with VL consistently <50 cps
- Single copy HIV-1 RNA assay

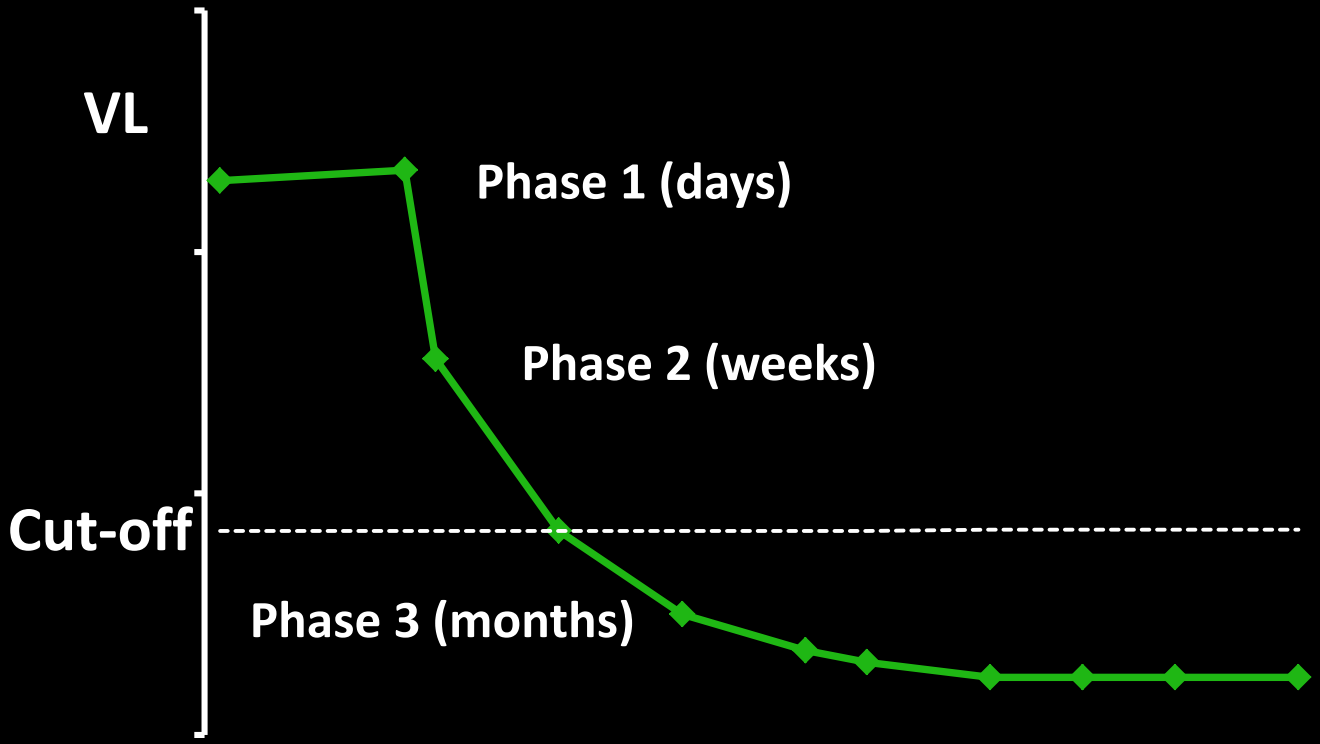


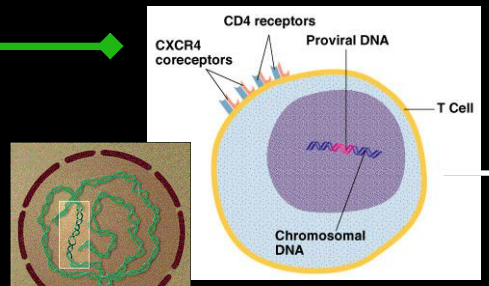
- HIV-1 RNA detection not associated with age, sex, race, risk group, duration of HIV diagnosis, nadir & current CD4 count, pre-ART VL, NNRTI used, NNRTI concentration

HIV-1 RNA cps/ml	Years of ART			Total (n=104)	*P*
	0-4 (n=31)	5-7 (n=33)	8-15 (n=40)		
Median (range)	3 (1, 35)	3 (1, 10)	3 (1, 11)	3 (1, 35)	*0.451*

Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

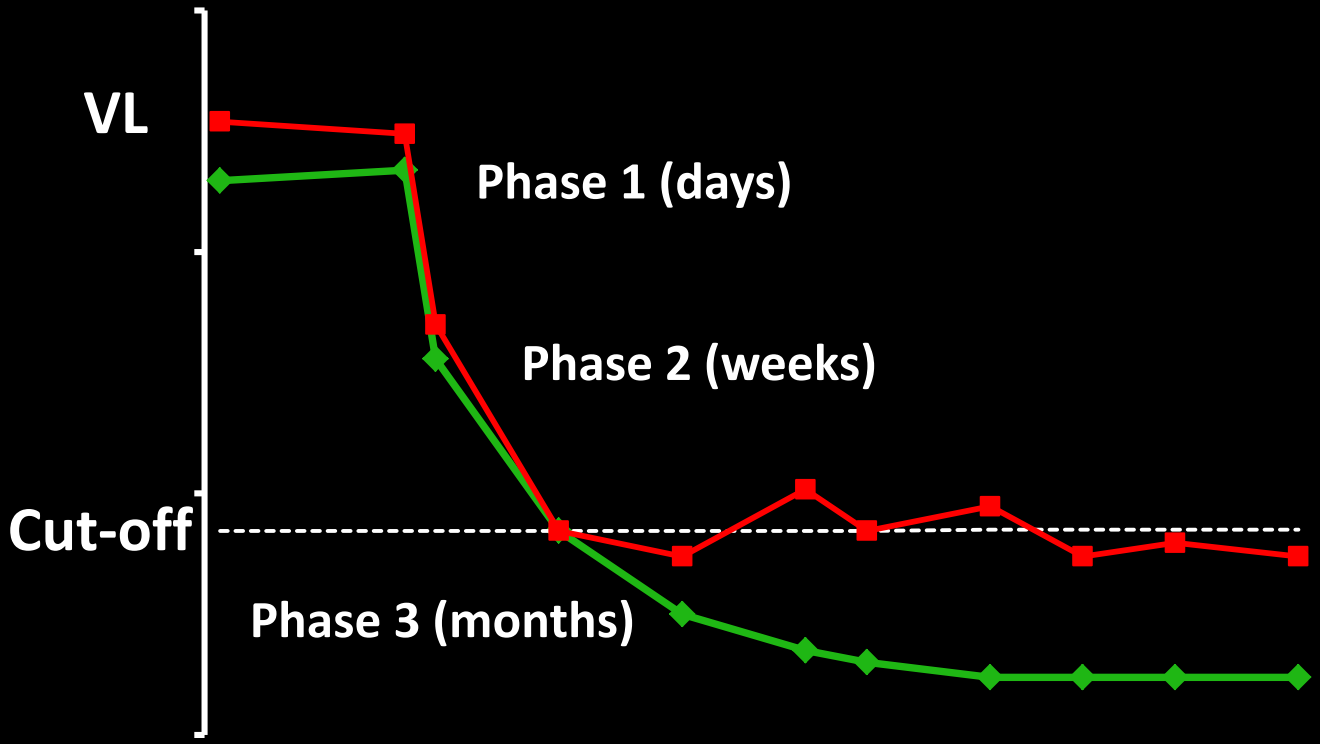
HIV-1 RNA kinetics after starting ART

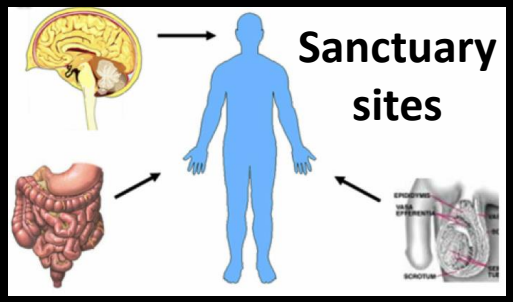


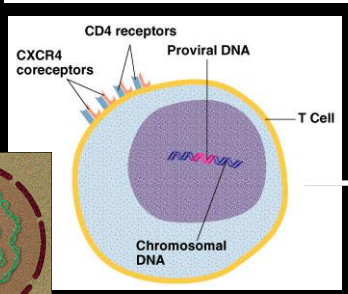


Latently infected cell

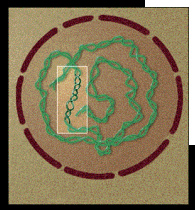
HIV-1 RNA kinetics after starting ART





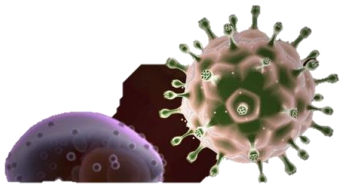


Latently infected cell →



Take away points: Viral load

- Prognosis, ART initiation, ART efficacy, Risk of transmission
- HIV-1 RNA declines in 3 phases after starting ART
- HIV-1 RNA >10 cps after >7 months predicts rebound
  - *Dose-dependent effect*
  - *Management uncertain, discrepancies in guidelines*
- During long-term ART with viral load persistently <50 cps HIV-1 RNA remains detectable at ~3 cps
  - *Not detectable by current commercial assays*
  - *Different population from that with HIV-1 RNA >10 cps*
  - *Source unclear, not associated with risk of rebound*



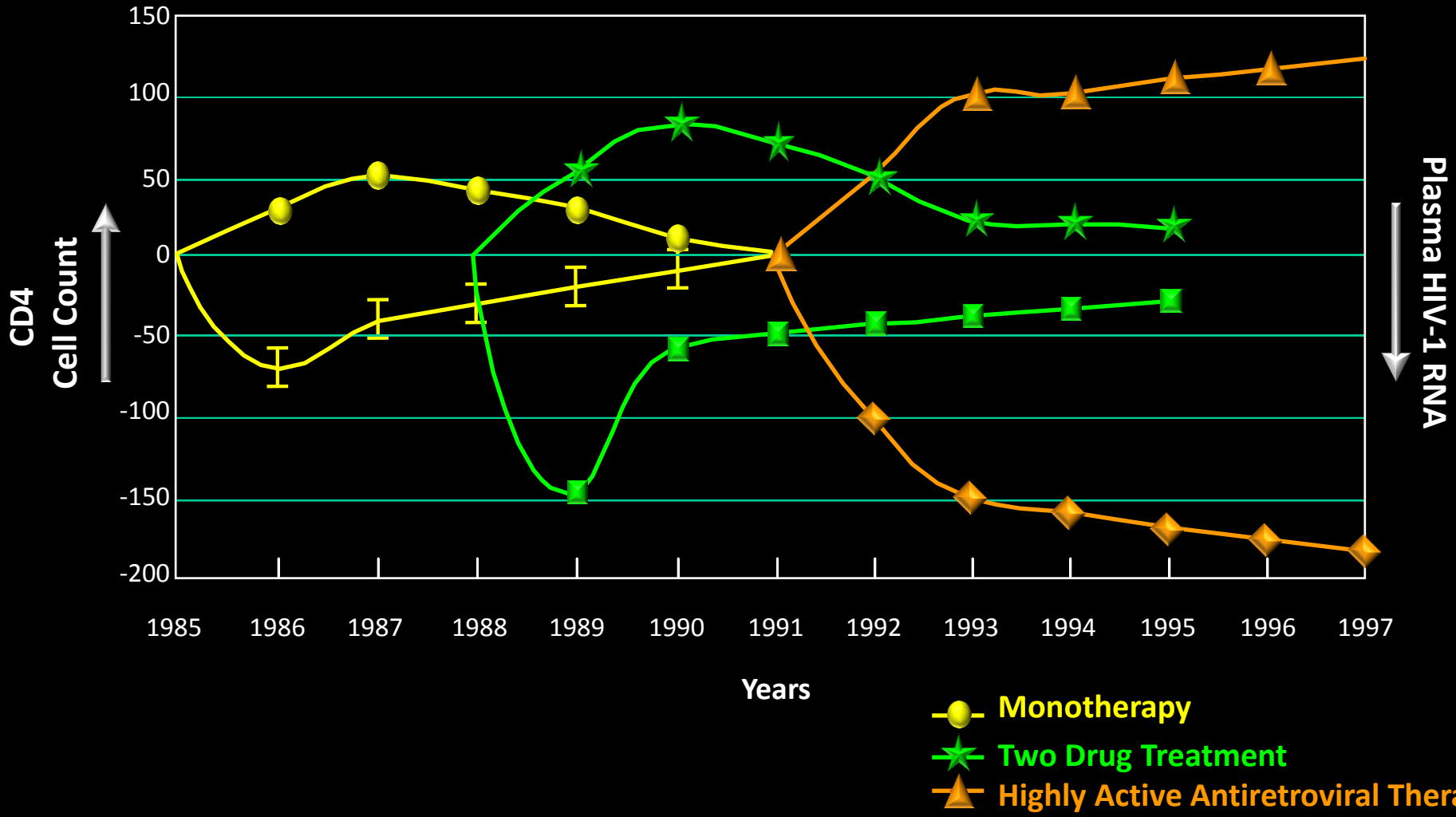
Case study: Mr B

- VL rebound >400 cps
- Intermittent problems with adherence
- Persistent low-level viraemia
- Drug resistance

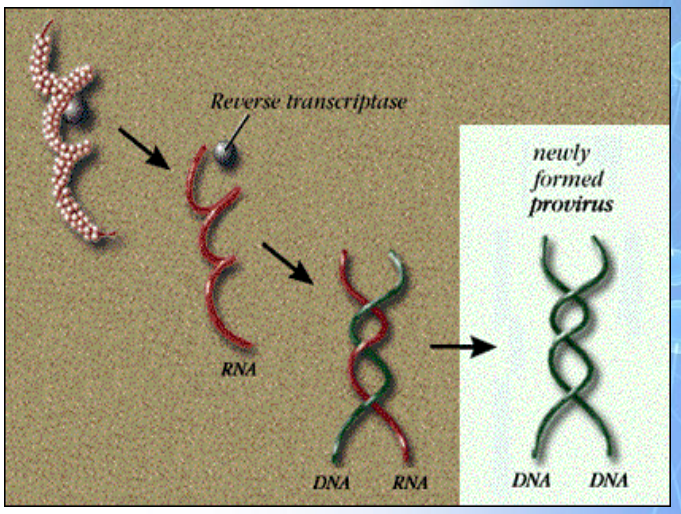
Date	VL cps	*ARVs*
Nov 01 - Apr 04	<50	*ABC ddI NFV*
May 04 - Mar 08	<50	*ZDV 3TC NVP*
Apr 08	<50	*TDF FTC NVP*
Jul - Feb 09	<50	
Jul 09	53	
Dec 09 - Mar 10	<50	
Jul - Dec 10	97-77	
Jan 11	451	***RT: K65R***
Feb 11	81	*ZDV TDF ATV/r*
May - Sept 11	<40	
Jan - May 12	47-49	
Aug - Dec 12	<40	
Apr – Nov 13	99-201	
Mar-Jun 14	102-475	***PR: I50L; R5***

RT = Reverse transcriptase
PR = Protease
R5 = CCR5-tropic HIV-1

Treatment strategies through the years



Mechanisms of HIV genetic evolution



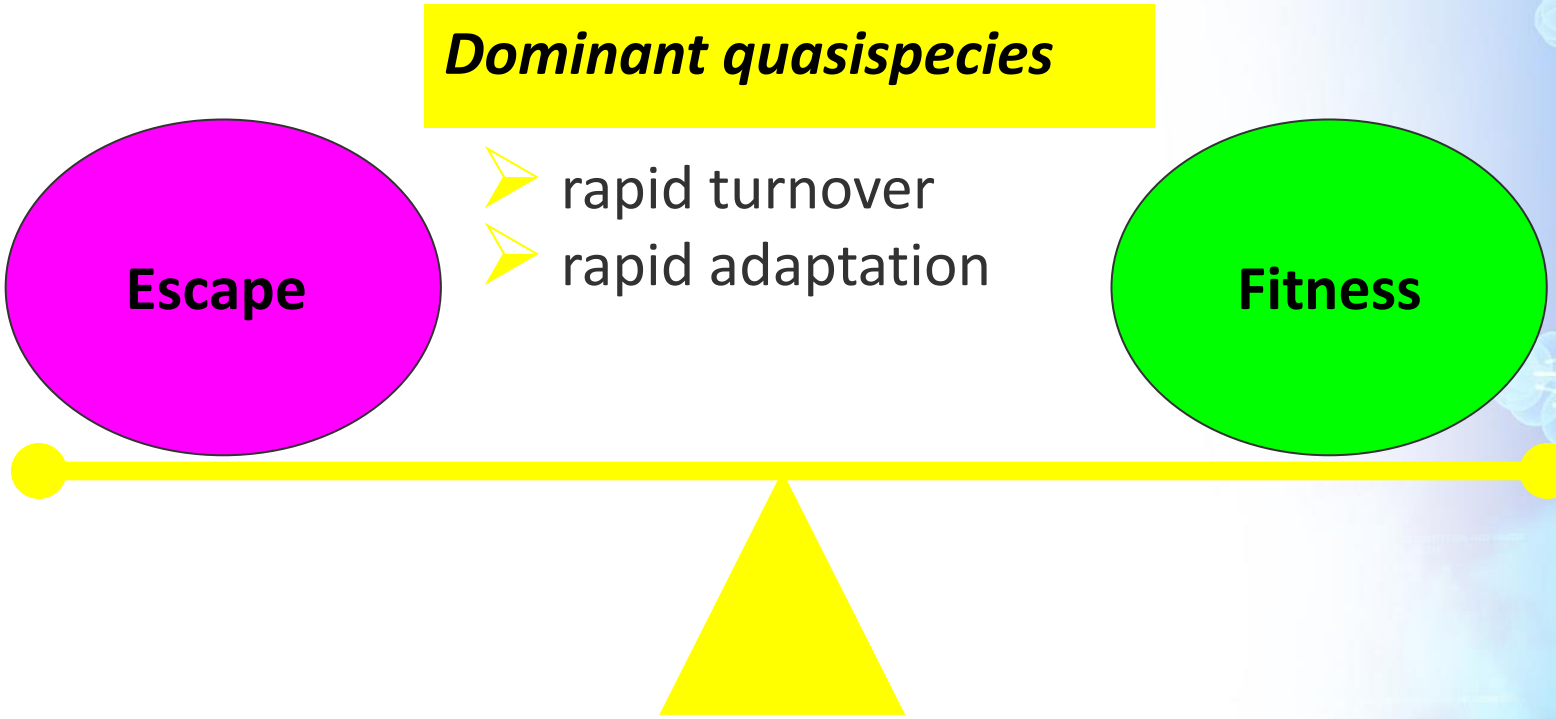
1. **Errors by viral reverse transcriptase**

   ~1 mis-incorporation per genome round

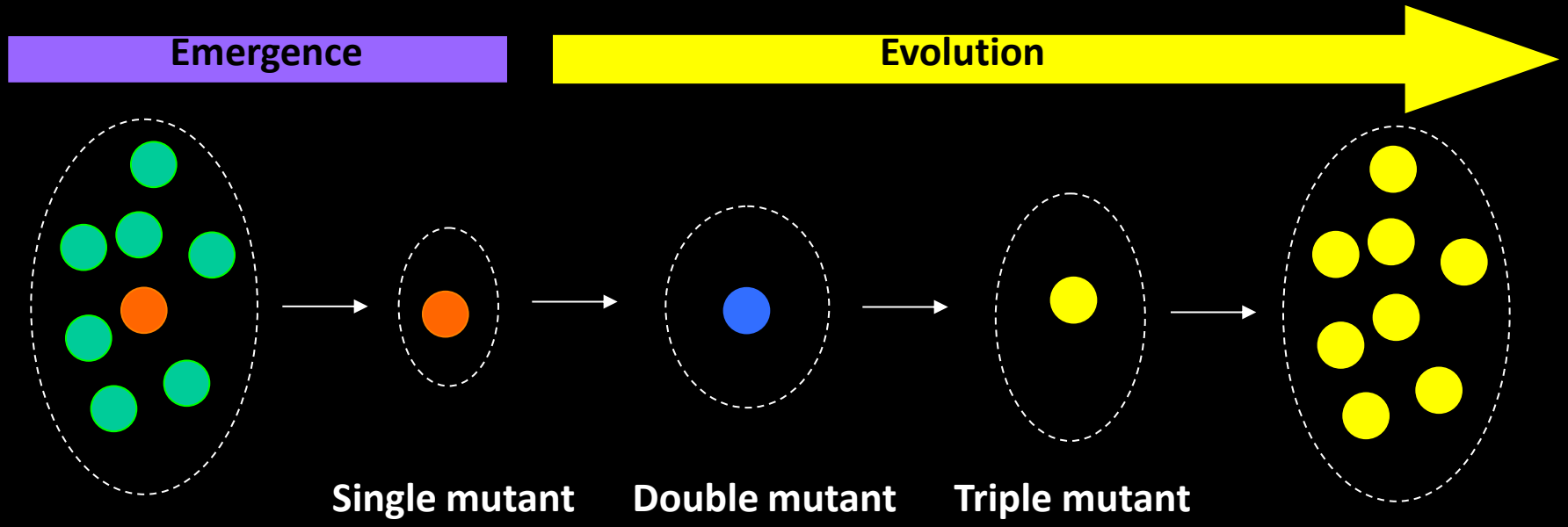
2. Errors by cellular RNA polymerase II
3. APOBEC-driven G➔A hypermutation

   Deamination of cytosine residues in nascent viral DNA

4. Recombination between HIV strains

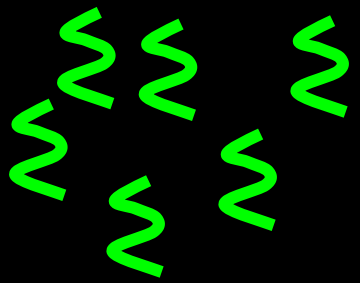

Dominant quasispecies
rapid turnover
rapid adaptation
Escape
Fitness

Emergence and evolution of resistance

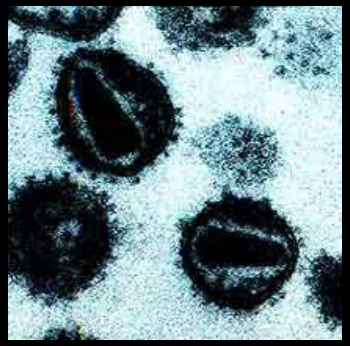


Genetic barrier and cross-resistance

Class	ARVs	Genetic barrier	Cross Resistance
NRTIs	ZDV/3TC, d4T/3TC	+/++	+++
	ABC/3TC, TDF/3TC	+	+++
	TDF/FTC	+/++	+++
NNRTIs	EFV, NVP, RPV	+	+++
	ETR	+/++	+++
PIs	Unboosted	+/++	++/+++
	Boosted	+++/++++	+/++
Fusion inhibitors	T20	+	NA
CCR5 antagonists	MVC	+/++	NA
Integrase inhibitors	RAL, EVG	+	+++
	DTG	++/+++	++



PCR



Viral gene (e.g., RT)

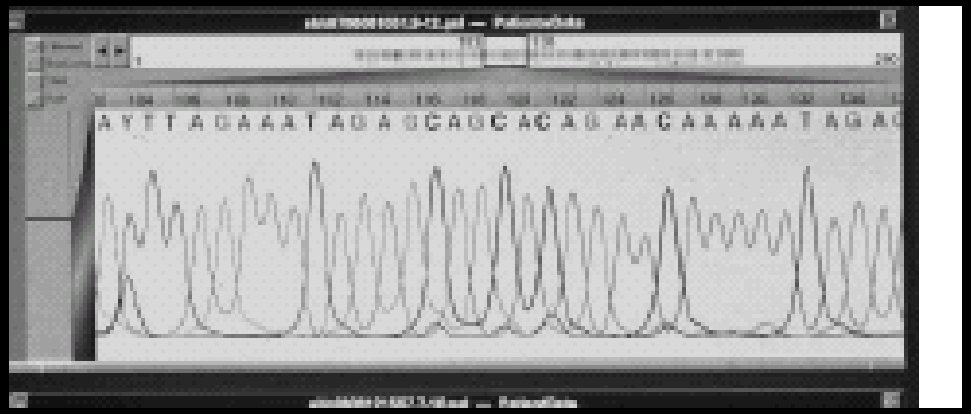
HIV RNA

Plasma

How we detect resistance in routine practice

Sequencing

Mutations

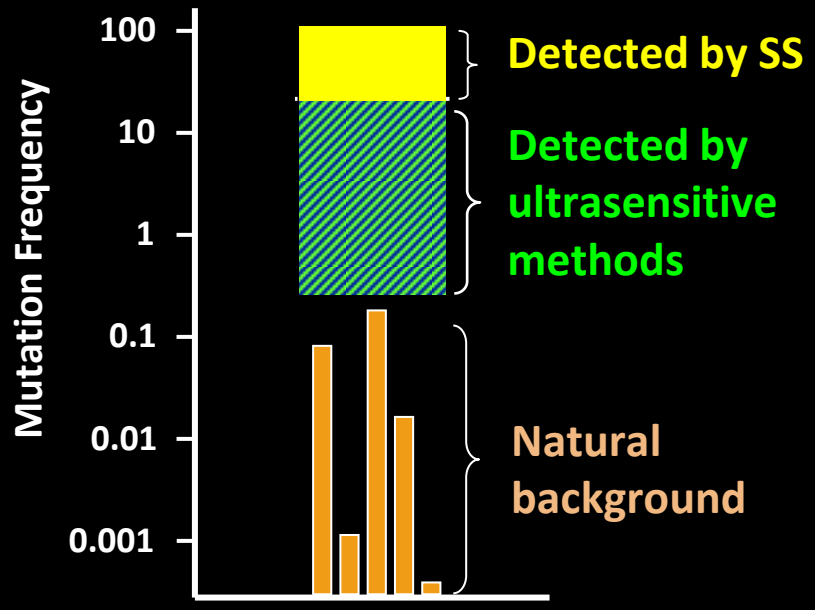


RT M184V

Methionine Valine

@ codon 184 of RT

ATG / AUG GTG / GUG

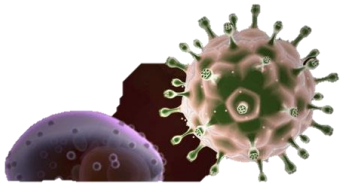


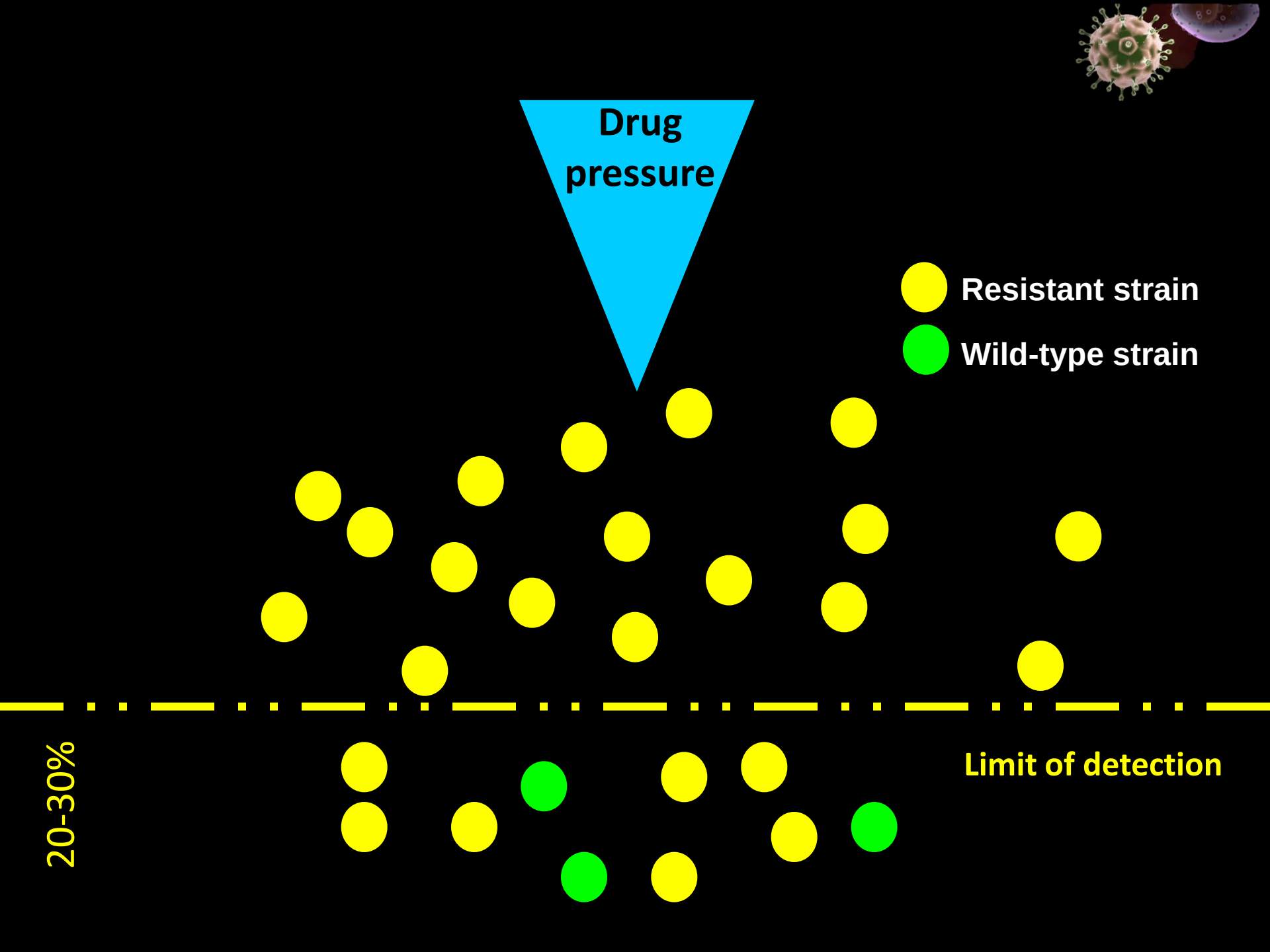
15-30%

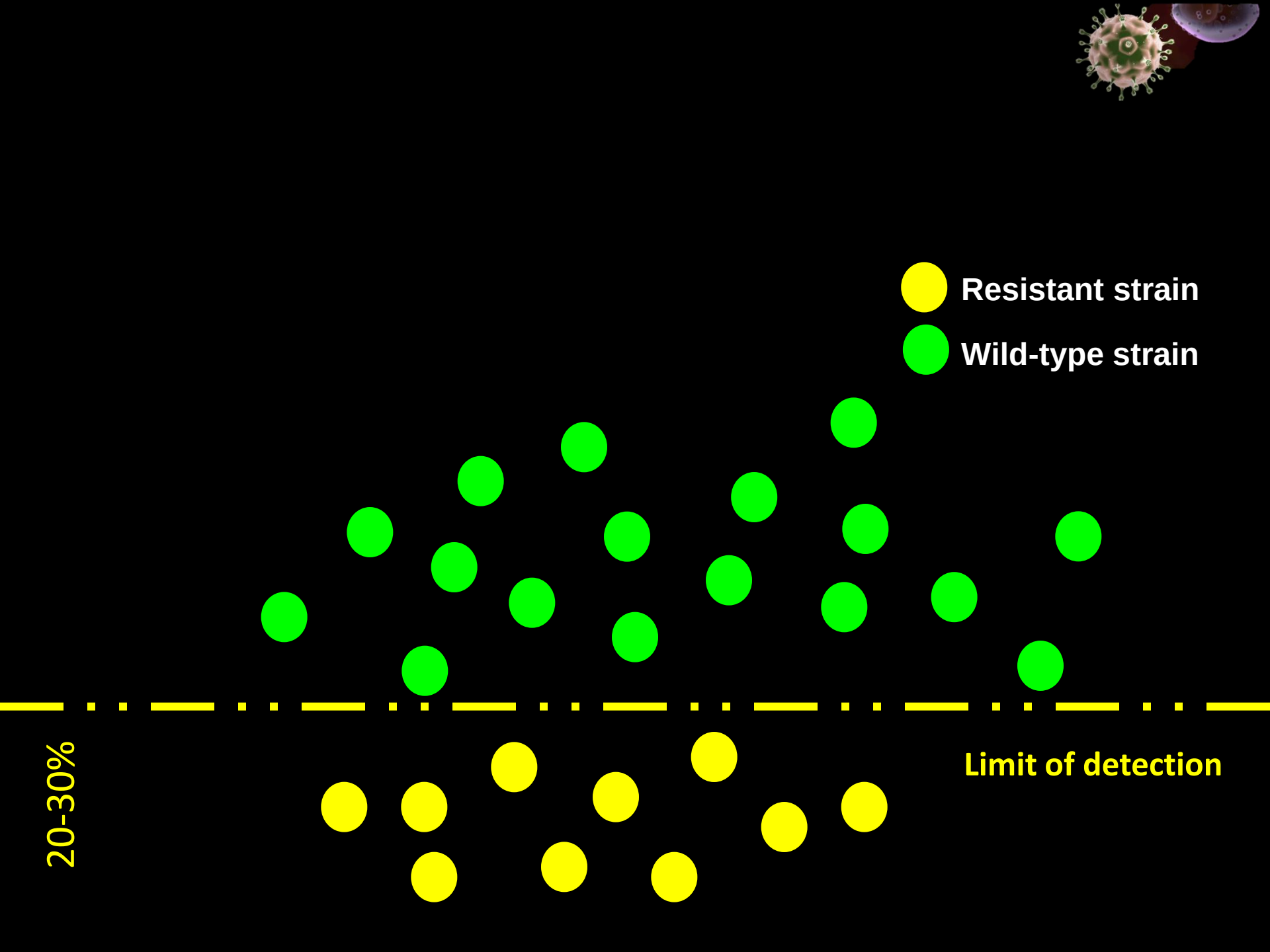
Limit of detection of Sanger sequencing (SS)

Take away points: Drug resistance

- Drug-resistant mutants emerge ”spontaneously “ during HIV replication
- Due to impaired fitness, the spontaneous mutants exists only at very low level and cannot be detected
- Once therapy is introduced, if virus replication continues, the mutants expand becoming dominant and detectable
- Natural evolution ➔ *increasing resistance and fitness*



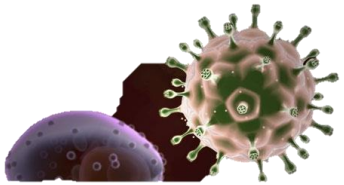

Drug pressure
Resistant strain
Wild-type strain
Limit of detection
20-30%


Resistant strain
Wild-type strain
Limit of detection
20-30%

Take away points: Drug resistance

- Routine (Sanger) sequencing only detects dominant species
- Once drug pressure is removed, resistant mutants are outgrown by fitter wild-type virus and become undetectable by routine sequencing
- Resistant mutants persist at low frequency in plasma and are “archived” in latently infected cells

➢ *The memory of HIV drug resistance is long-lived*



Your turn ☺

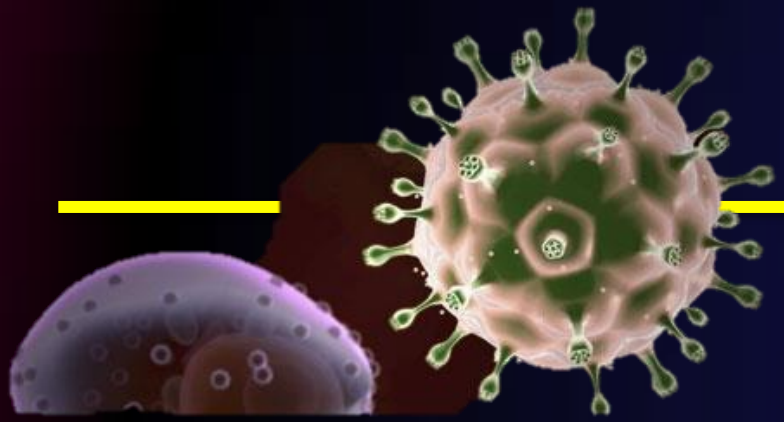
Which of the following correctly describes HIV?

1. **DNA virus, high replication**
2. **RNA virus, high replication during AIDS phase only**
3. **RNA virus, high replication, stable genetic make-up**
4. **RNA virus, high replication, rapid genetic evolution**

You turn ☺

Which of the following correctly describes HIV?

1. **DNA virus, high replication**
2. **RNA virus, high replication during AIDS phase only**
3. **RNA virus, high replication, stable genetic make-up**
4. **RNA virus, high replication, rapid genetic evolution**



Thank you