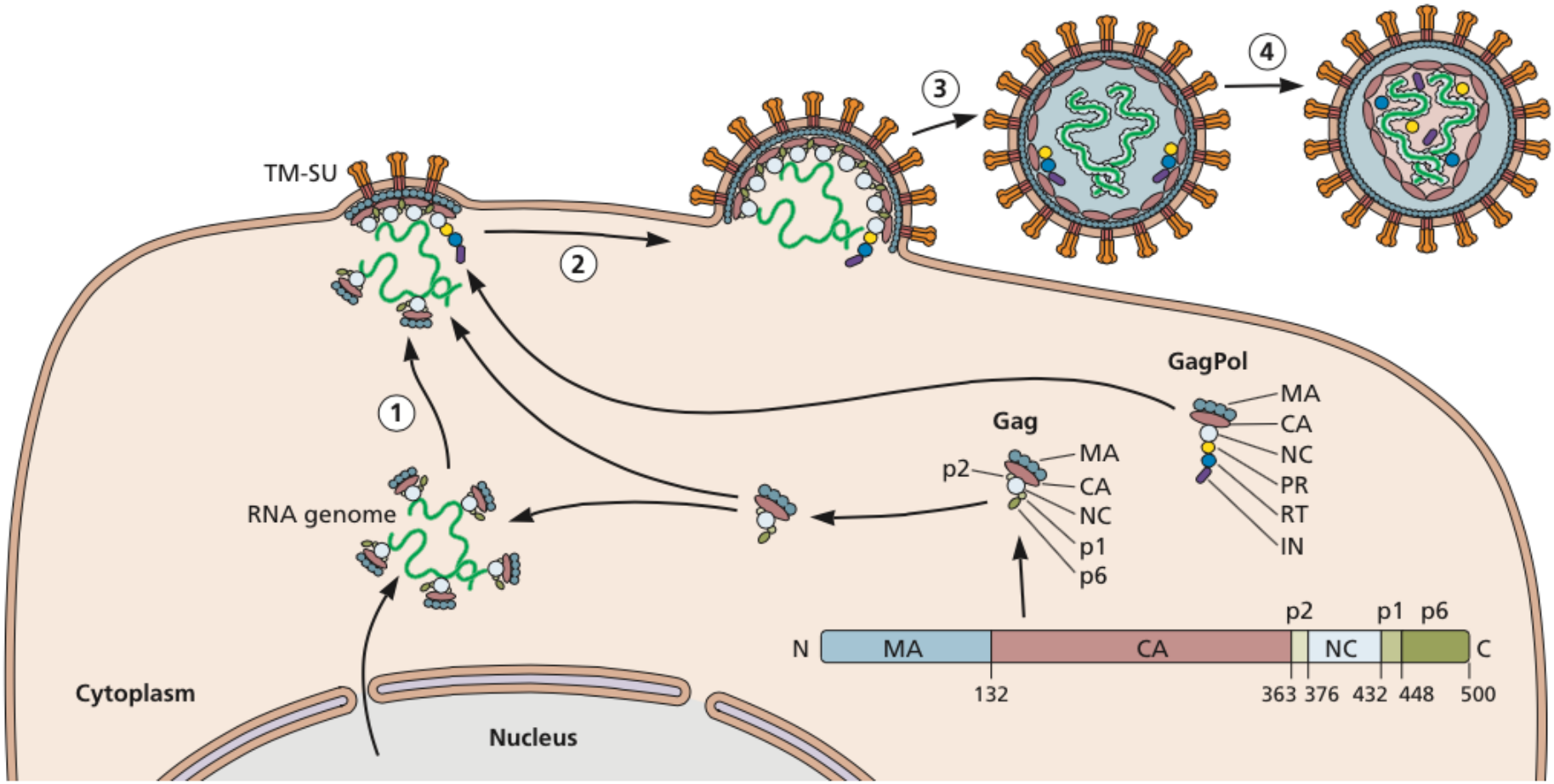
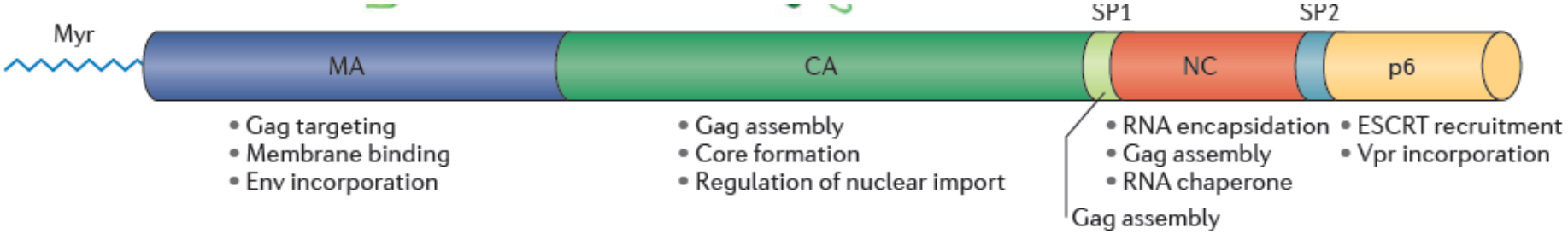
Assembly and release



Assembly and release

Endosomal sorting complex required for transport (ESCRT). A multi-complex machinery that comprises ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, and that promotes membrane scission reactions (for example, during vesicle budding, cytokinesis and enveloped-virus budding).



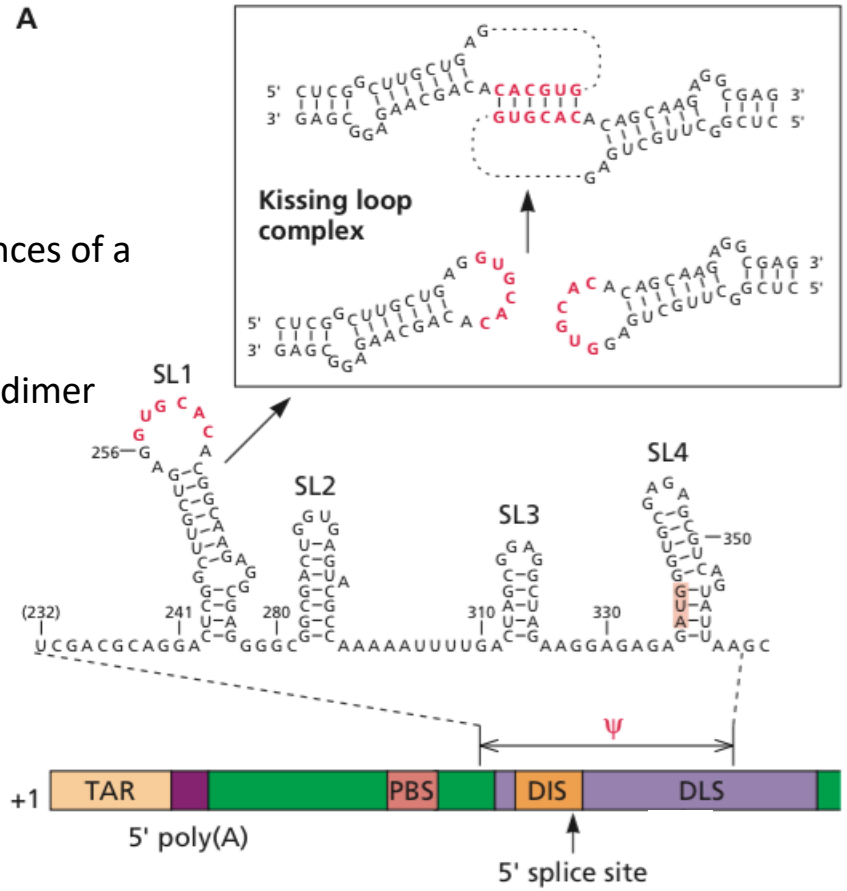
The NC domain of Gag is the primary viral determinant that drives RNA packaging.
Gag NC domains direct the packaging of viral genomic RNA by binding to the packaging signal (ψ site).
An RNA dimer is the recognition unit for packaging into assembling virions.

Assembly and release

In virions, the dimeric genome is held together by many noncovalent interactions between the RNA molecules.

In vitro – Evidence for base pairing between loop sequences of a specific herpin (SL1) within the DIS.

Mutations in this sequence – inhibition of genomic RNA dimer formation in vivo

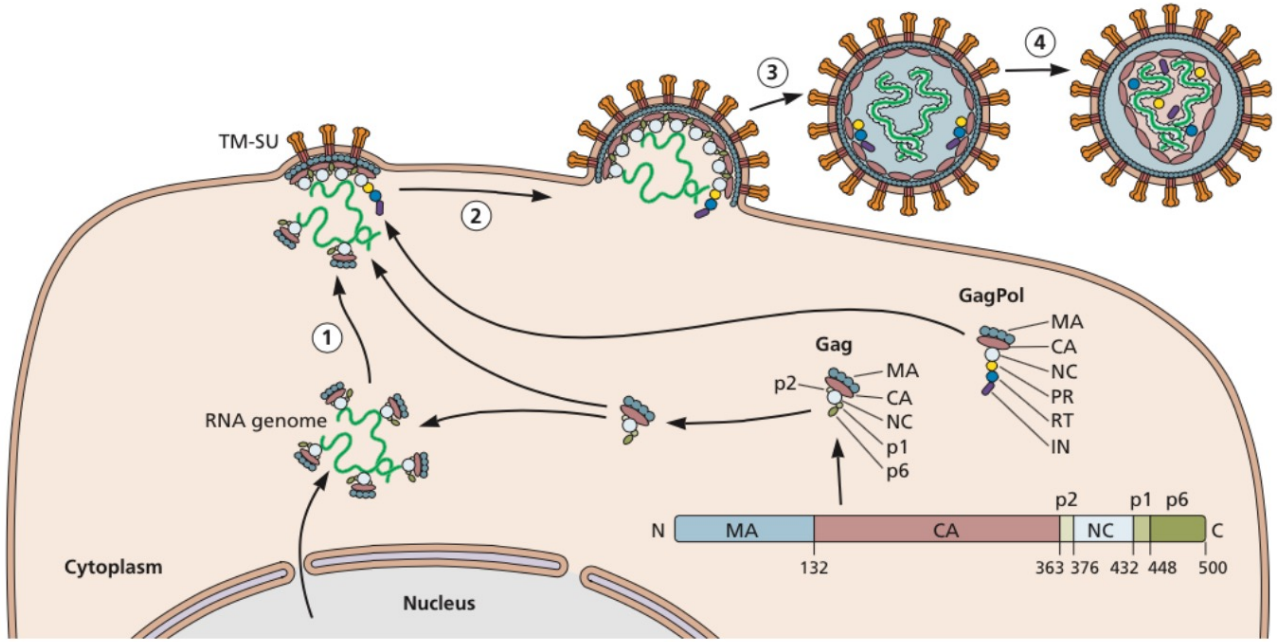


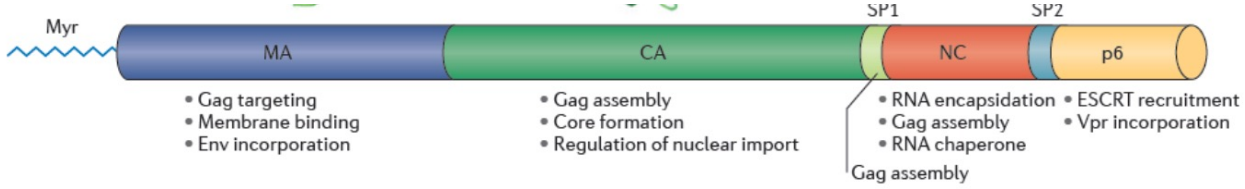
DIS - dimer initiation site
DLS – dimer linkage structure

Assembly and release

MA drives GAG targeting to the plasma membrane (1)

CA promotes GAG multimerization (2)

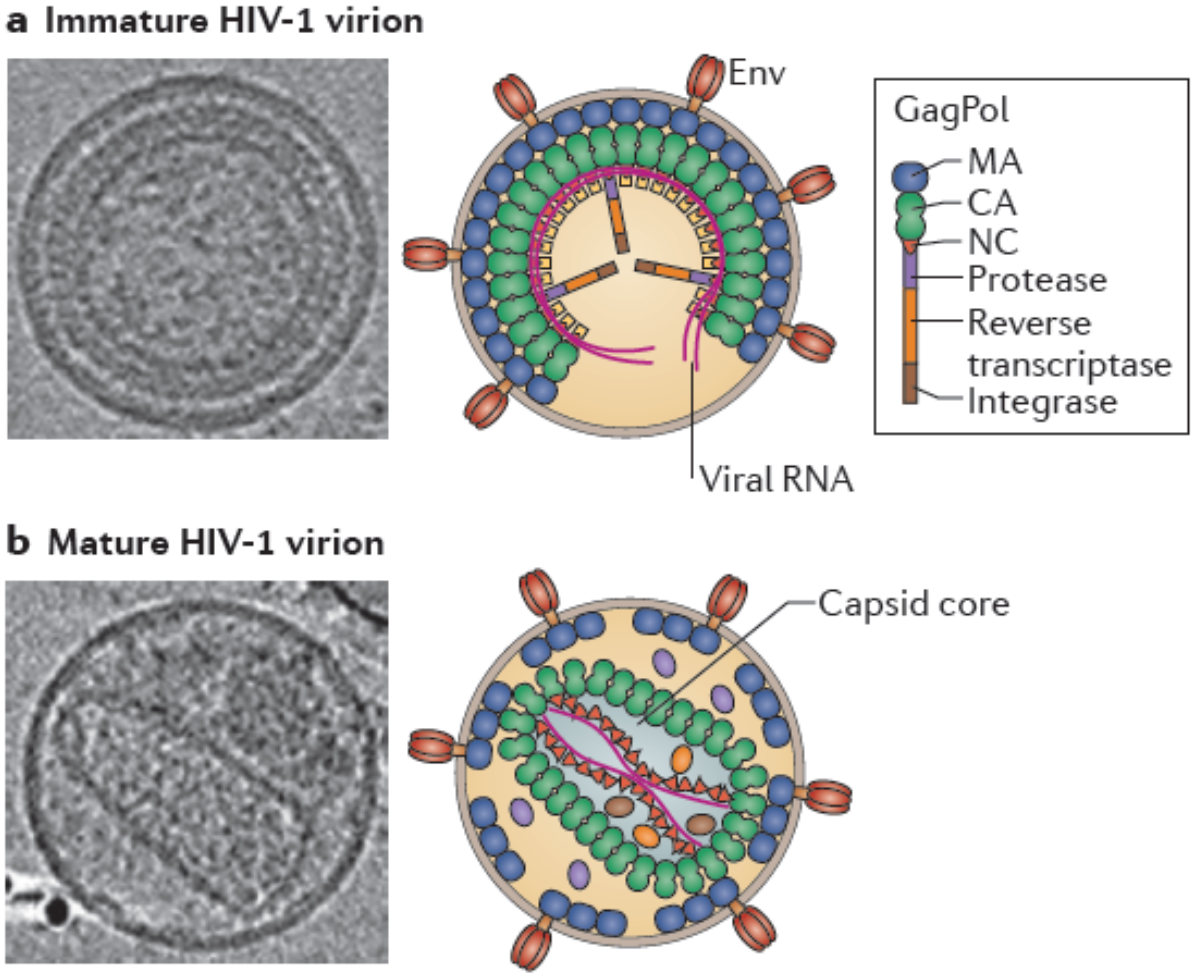




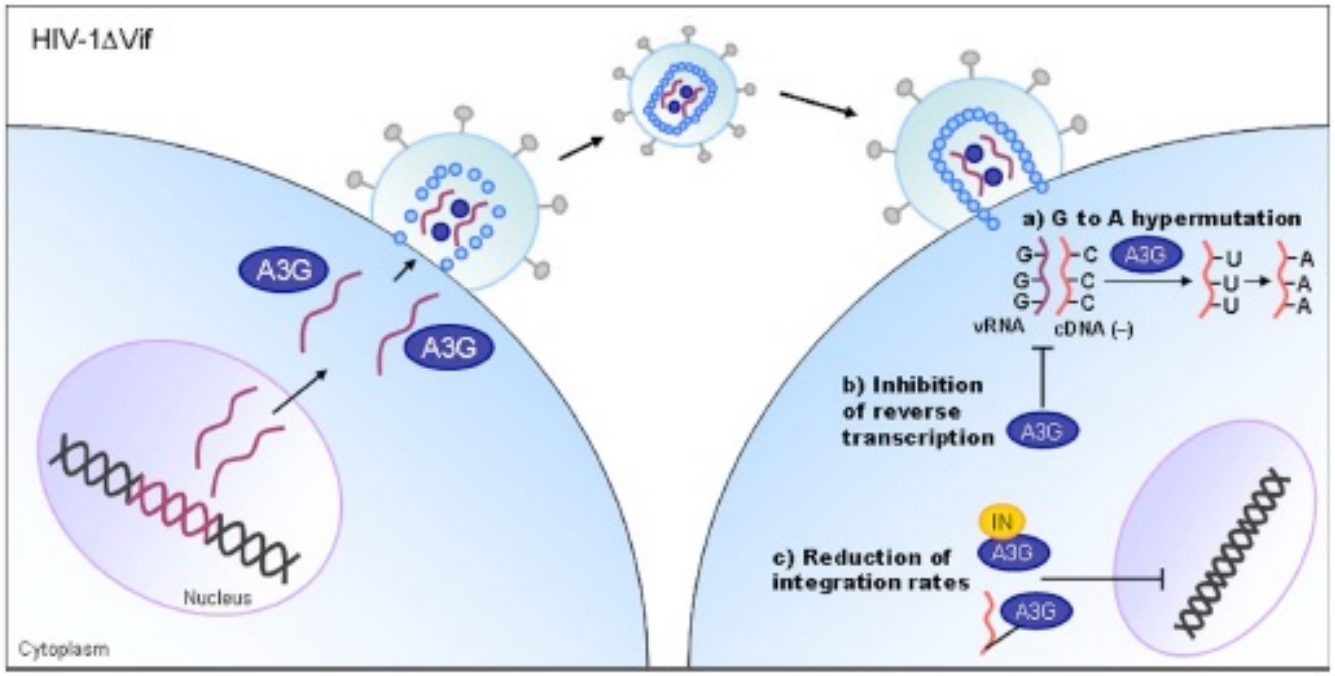
Maturation

The virus buds and the protease cuts itself free of the GAG-POL polyprotein

Further proteolytic cleavage occurs and the virion matures



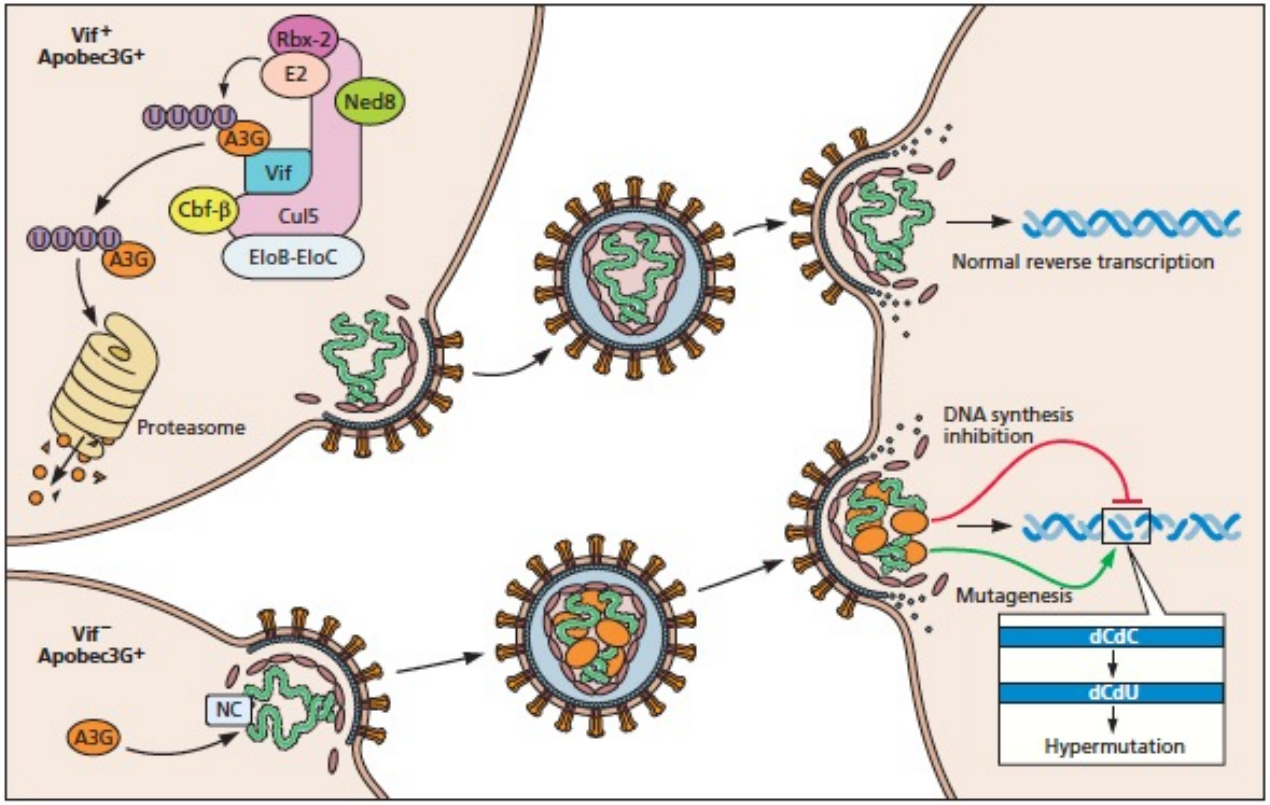
VIF: *Virion Infectivity Factor (23 kDa)*



Apobec3G (member of an RNA-binding family of cellular cytidine deaminases) is incorporated into progeny virus particles via interactions with the viral RNA and possibly NC protein. Apobec3G appears to inhibit virus reproduction in a number of ways. It has been proposed that binding to viral RNA may account, in part, for the deaminase-independent inhibition of reverse transcription in newly infected cells. The deaminase activity of Apobec3G leads to formation of deoxyuridine (dU), most frequently at preferred deoxycytidine (dC) sites in the first (-) strand of viral DNA to be synthesized by reverse transcriptase. Consequently, the (+) strand complement of the deaminated (-) strand will contain deoxyadenosine in place of the normal deoxyguanosine at such sites. Indeed, the frequency of G→A transitions is abnormally high in the genomes of vif -defective particles produced in nonpermissive cells, and incomplete protection from Apobec3 proteins by Vif may explain why such transitions are the most frequent point mutations in HIV genomes. It has been suggested that the Apobec3 proteins represent an ancient intrinsic cellular defense against retroviruses.

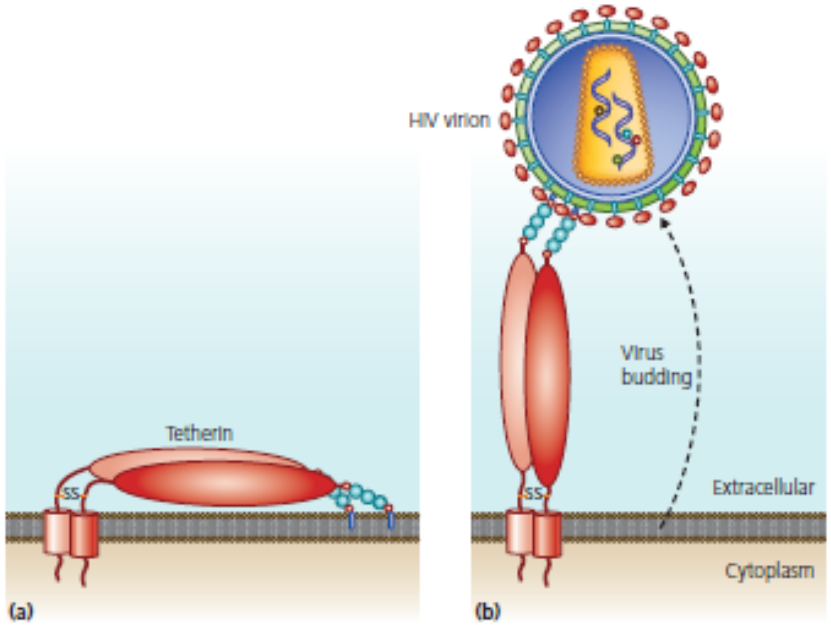
Front. Microbiol., 26 July 2012

VIF: *Virion Infectivity Factor (23 kDa)*



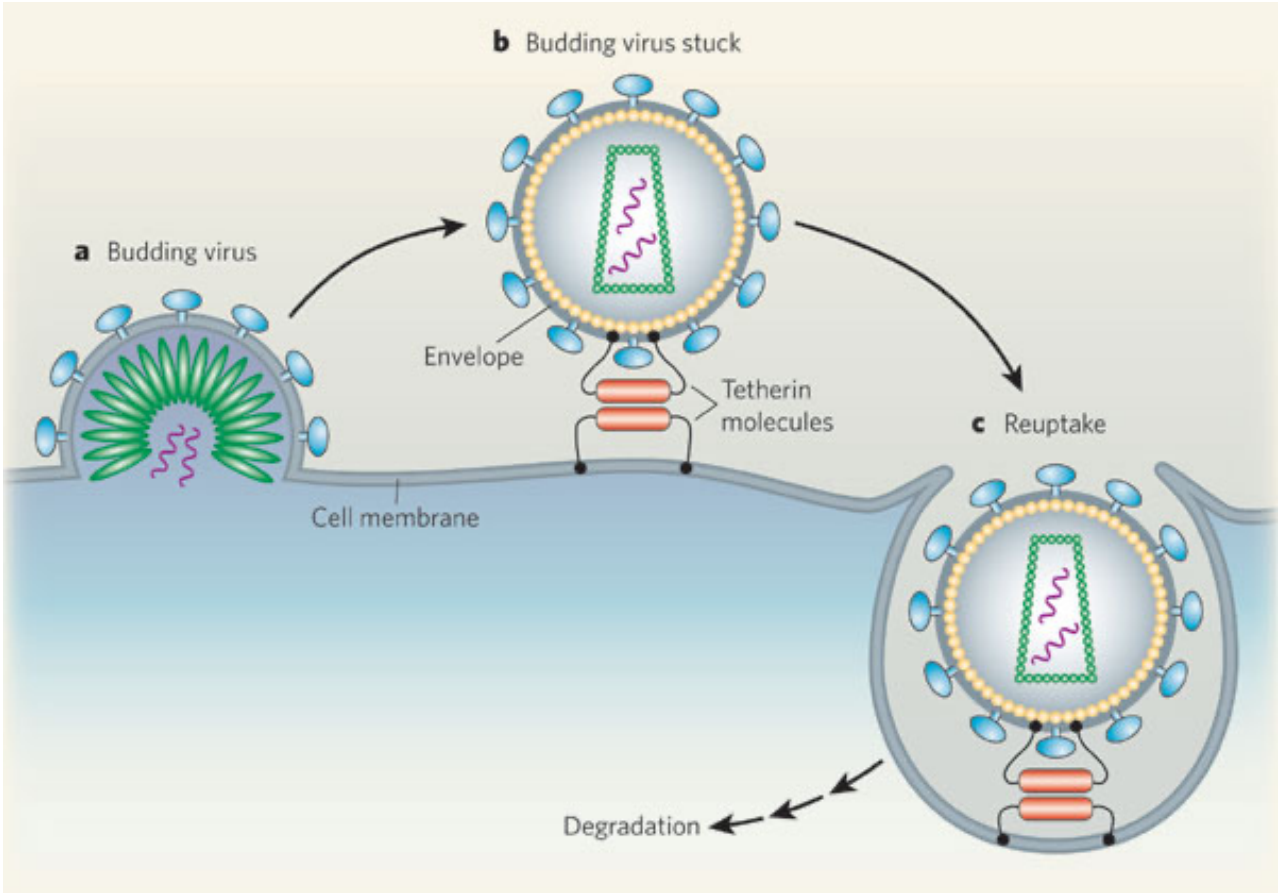
(Top) Vif counteracts the antiviral effects of Apobec3G (A3G) by mediating its polyubiquitination, which leads to proteosomal degradation. (Bottom) In the absence of Vif, A3G is incorporated into newly formed virus particles through interaction between viral RNA and NC protein. In the newly infected cell, reverse transcription is inhibited by A3G, and cytosines in the newly synthesized DNA are converted to uracil, causing hypermutation through eventual C to A transversions.

Tetherin has been recognized as a potent repressive factor for HIV-1 replication**. It is a cell-surface protein that, in addition to being anchored in the plasma membrane by a hydrophobic membrane-spanning region, also has a second membrane anchor in the form of a GPI (glycosyl phosphatidyl inositol) modification at the C terminus of its extracellular domain**; it also dimerizes via its extracellular domains which become linked by disulphide bonds. Possession of a GPI anchor causes the protein to locate in lipid rafts within the plasma membrane, which are known to be sites for budding of HIV and several other enveloped viruses. Thus, when HIV particles exit the cell, they carry with them the tetherin GPI anchor and so remain attached to the cell by a tetherin bridge.



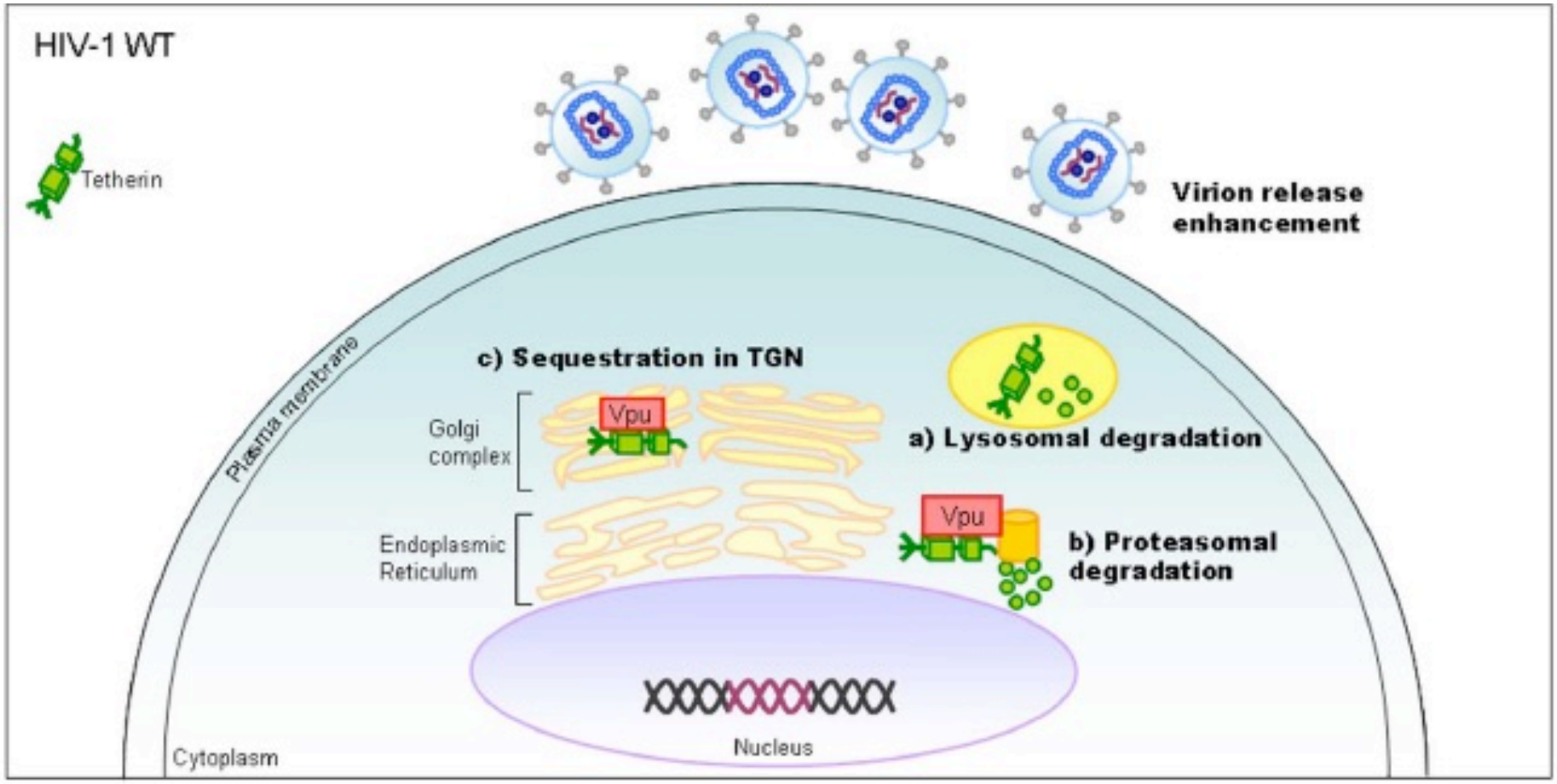
Schematic representation of the topology of tetherin protein dimers in (a) an uninfected cell and (b) an HIV-infected cell, in which it prevents virus release by anchoring exiting virions to the producer cell. In HIV-1 infection, this effect is opposed by the action of the viral **Vpu** protein to allow more efficient spread of virus between cells.

VPU: *Viral Protein Unknown (9,2 kDa)*



Nature **451**, 406-408(24 January 2008)

VPU: *Viral Protein Unknown (9,2 kDa)*



Front. Microbiol., 26 July 2012

Accessory genes

VPU: ***Viral Protein Unknown (9,2 kDa)***

- Promotes the degradation of the CD4/gp160 complex in the endoplasmic reticulum, facilitating the gp160 transport to the plasma membrane for assembling of new viral particles.
- Reduces CD4 and MHC-I expression promoting the virion budding and the escape of the virus from IS

VPR : ***Viral Protein, Regolatory (15 kDa)***

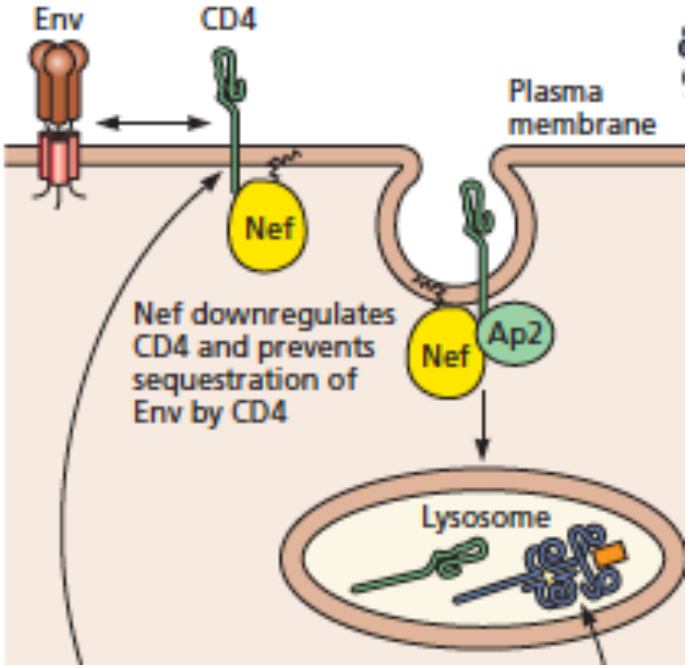
- Is a late HIV product packaged into the virion nucleocapsid
- Contains a nuclear localization signal (NLS) and is involved in the pre-integration complex (PIC) formation and nuclear transport
- Induces cells to arrest in the G2 phase of the cell cycle (LTR promoter is more active in G2-arrested cells)
- Negatively modulates the expression of CD4 on the cell surface
- Acts as a weak transactivator of viral transcription. It is important for proviral DNA expression occurring before integration

NEF: *Negative Factor (27 kDa)*

- Nef protein is synthesized early in infection.
- Nef is a myristoylated protein localized at the cell membrane of infected cells.
- NEF is important for HIV replication *in vivo* but there seems to be much less effect of NEF in an in vitro cell culture situation.

Attenuated HIV-1 strains exist that lack nef

NEF: *Negative Factor (27 kDa)*

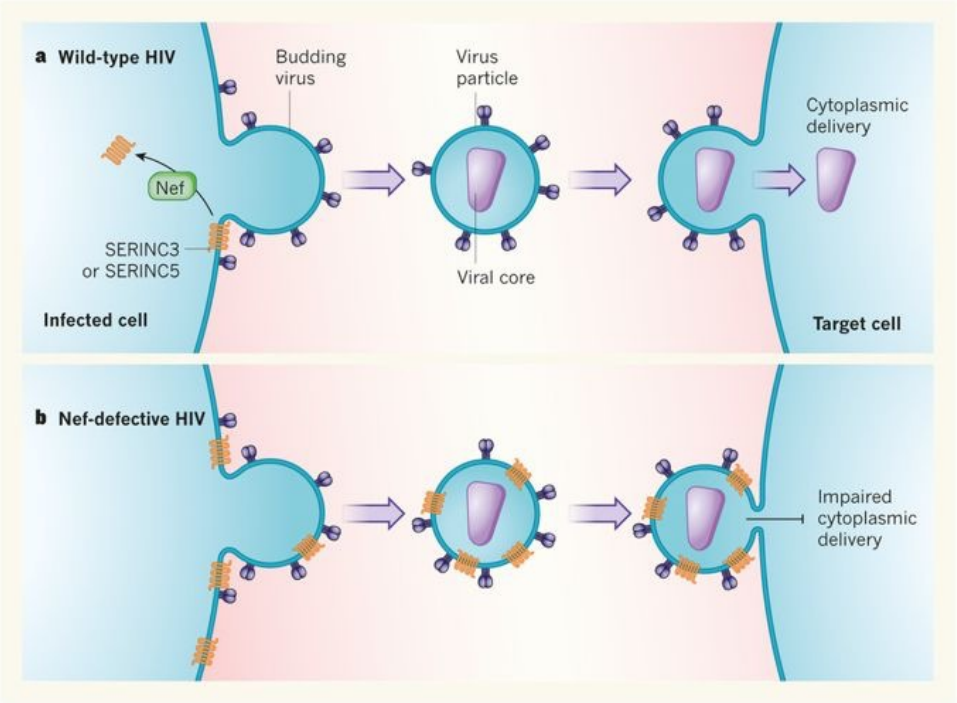


NEF causes the internalization of CD4 antigen from the cell surface and its destruction in lysosomes, enhancing Env incorporation into budding virus particles.

NEF reduces surface expression of MHC class I molecules. This alters antigen presentation by the infected cell and is proposed to protect the infected cell from attack by cytotoxic T cells.

Antiviral action countered by Nef

Both SERINC3 and SERINC5 are members of a family of proteins named for a putative activity on membranes ('serine incorporator'). The SERINC proteins are integral membrane proteins that form scaffolds for enzymes involved in the synthesis of specific membrane phospholipid molecules. How do SERINC3 and SERINC5 lower HIV infectivity? It has been showed that, at high levels of expression, these proteins reduce the efficiency of virus fusion with target cells. Nef induces SERINC5 to move from the cell surface to an intracellular compartment, preventing it from being incorporated into the budding virus.



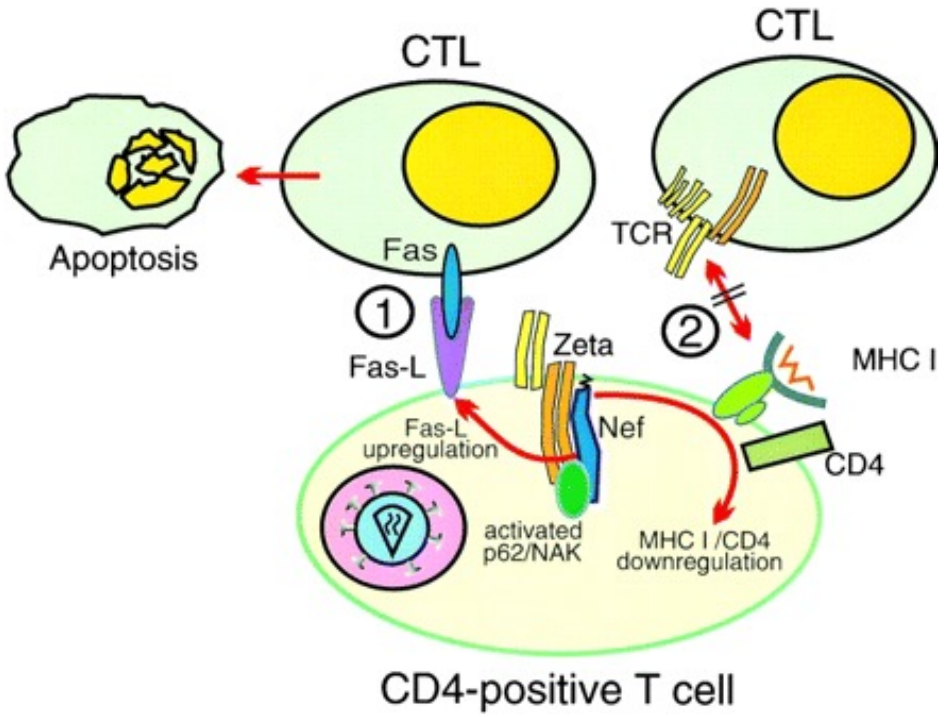
SERINC3 and SERINC5 are membrane proteins found to have antiviral activity against HIV. **a**, HIV protein Nef prevents the SERINC proteins from being incorporated into a growing virus particle as it buds from the membrane of an infected cell. The resulting virus particle is able to correctly fuse with another target cell and deliver its viral core to the host-cell cytoplasm. **b**, It has been proposed that, in the absence of Nef, SERINC3 and SERINC5 are successfully incorporated into viral particles, and prevent delivery of the viral core by inhibiting the expansion of the fusion pore.

Nature **volume 526**, pages 202–203 (08 October 2015)

Nef and apoptosis

Pro-apoptotic Role

Nef stimulates expression of FasL on the surface of infected cells (the mechanism involves the signaling pathway of the TCR). This results in the protection of infected cells from CTL attack by killing Fas+ viral-specific CTLs in the process (1)

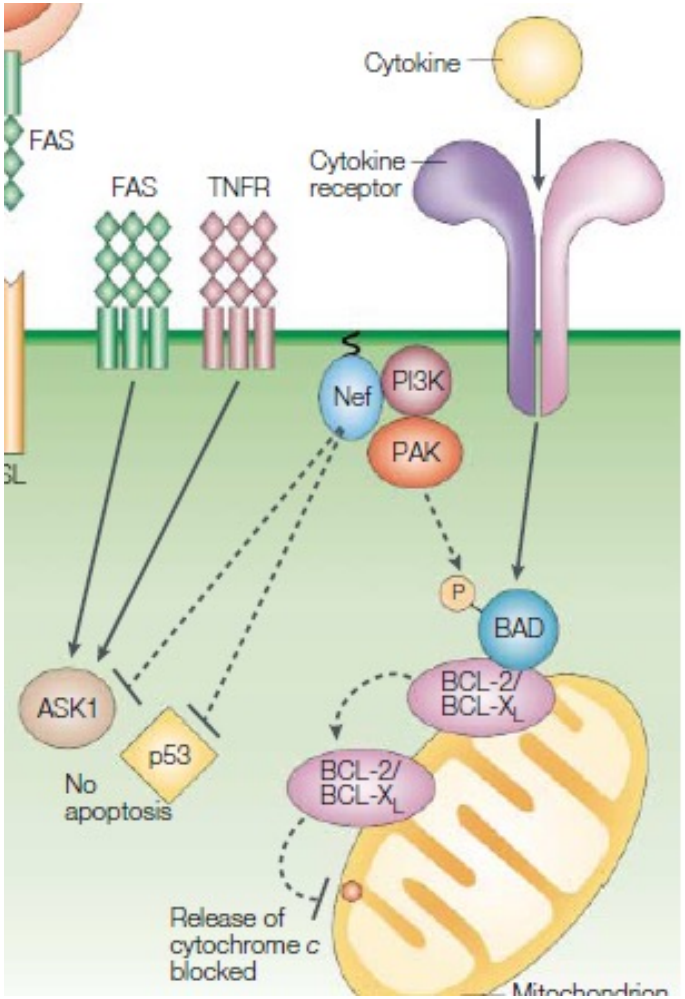


Xu X et al. J Exp Med 1999;189:1489-1496

Nef and apoptosis

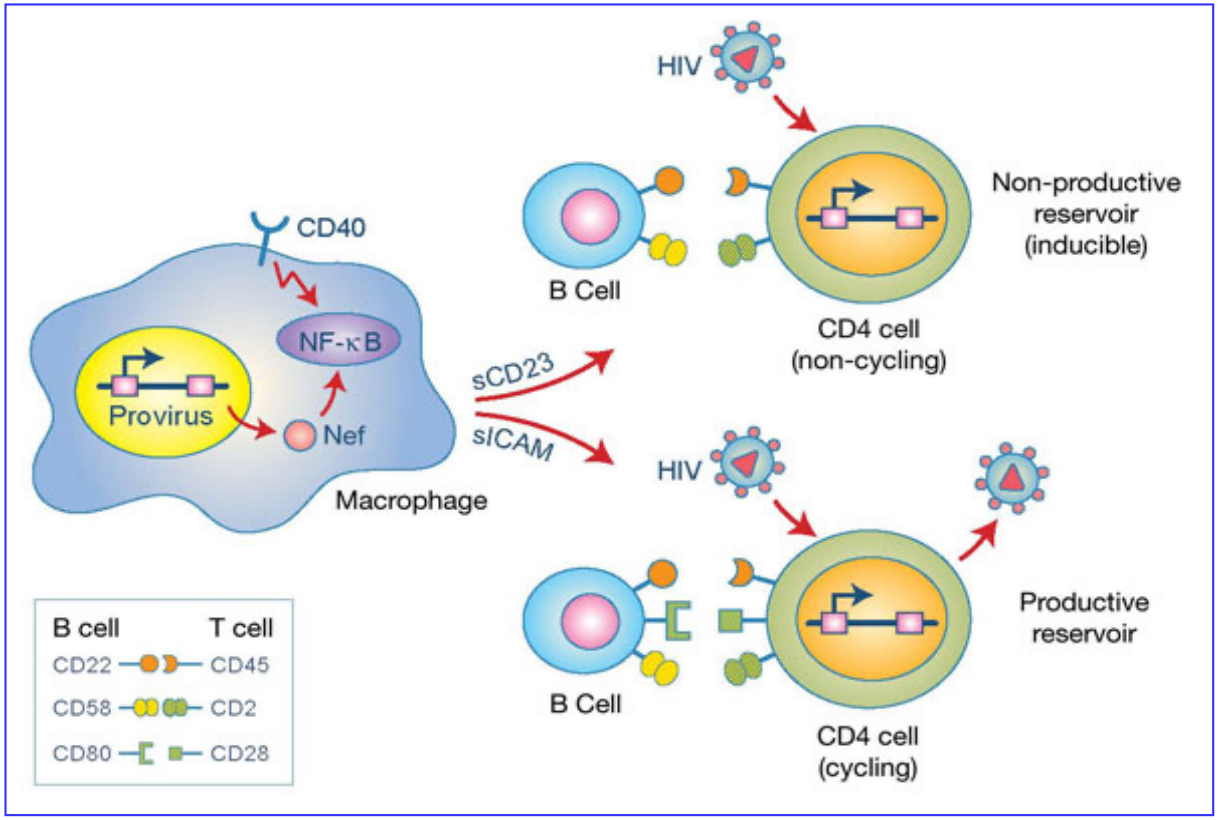
Anti-apoptotic role

In infected cells, Nef blocks apoptotic pathways mediated by FAS and tumour-necrosis factor receptor (TNFR) (through inhibition of apoptosis signal-regulating kinase 1, ASK1) and by p53 (through direct binding), and unleashes the anti-apoptotic effects of BCL-2 and BCL-XL (by inducing the PAK-mediated phosphorylation of BAD, releasing the anti-apoptotic effectors, and thereby mimicking cytokine-induced signals).



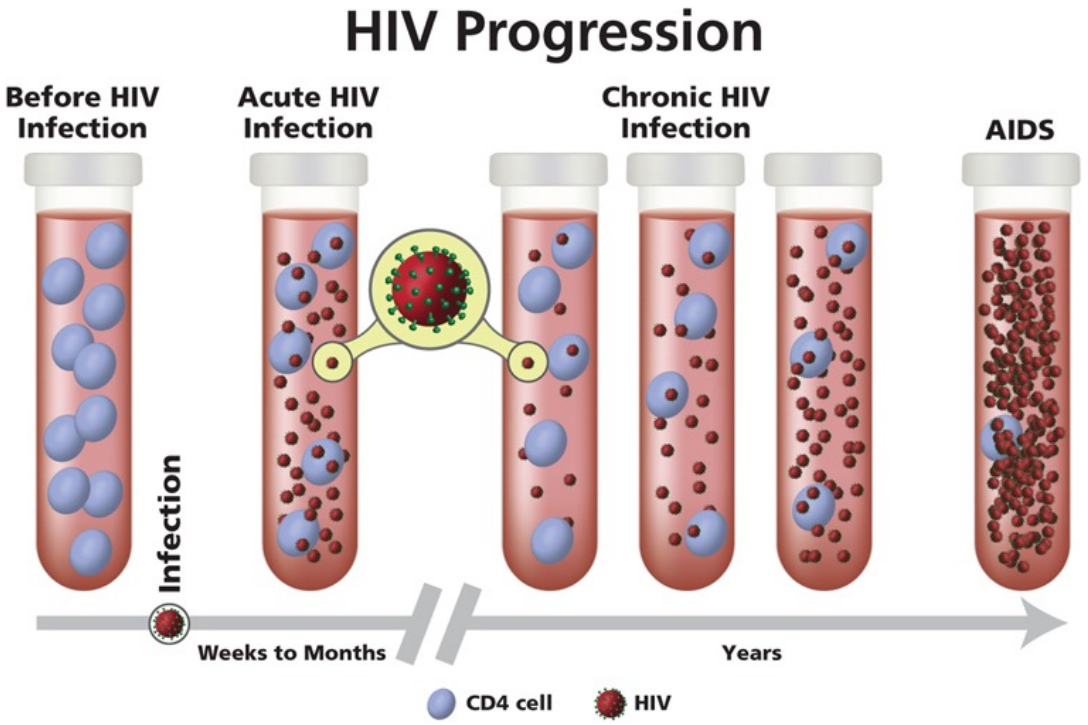
NATURE REVIEWS | IMMUNOLOGY

Nef induces T resting lymphocytes permissivity to HIV-1 infection



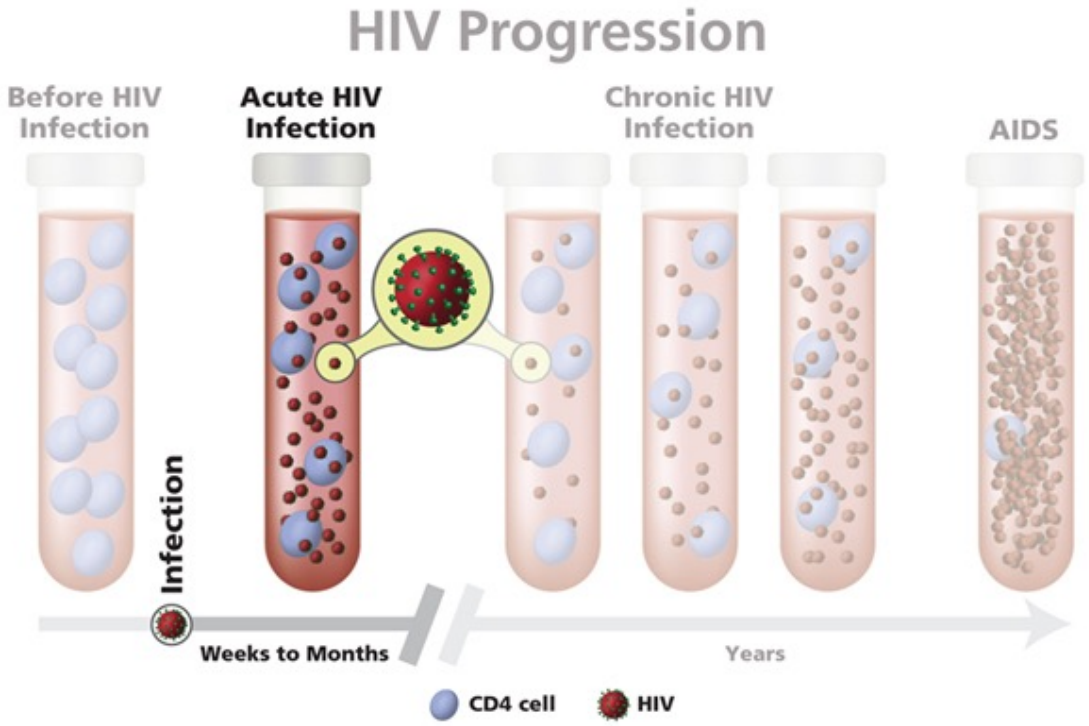
Physiological stimulation of CD40 in macrophages leads to NF-kappaB activation. This signalling pathway is intersected by HIV-1 Nef. In macrophages, activation of CD40 or expression of Nef induces the release of sCD23 and sICAM, which upregulate the expression of co-stimulatory receptors on B lymphocytes. These, in turn, interact with their corresponding ligands on T lymphocytes, rendering them permissive to HIV-1 infection. The types of receptor that are upregulated on B cells dictate the outcome of the infection. The induction of CD22 and CD58 on B cells is mediated primarily by sCD23. The action of these receptors on T cells does not lead to T-cell proliferation, but is sufficient to permit virus entry, but not virion release. Induction of CD80 on B cells, as mediated primarily by sICAM, provides signals that promote entry of T cells into the cell cycle, thereby allowing the productive infection of these cells.

Swingler S, *et al.* Nature 2003



The three stages of HIV infection are (1) acute HIV infection, (2) chronic HIV infection, and (3) acquired immunodeficiency syndrome (AIDS).
There is no cure for HIV, but treatment with HIV medicines (called antiretroviral therapy or ART) can slow or prevent HIV from advancing from one stage to the next. HIV medicines help people with HIV live longer, healthier lives.

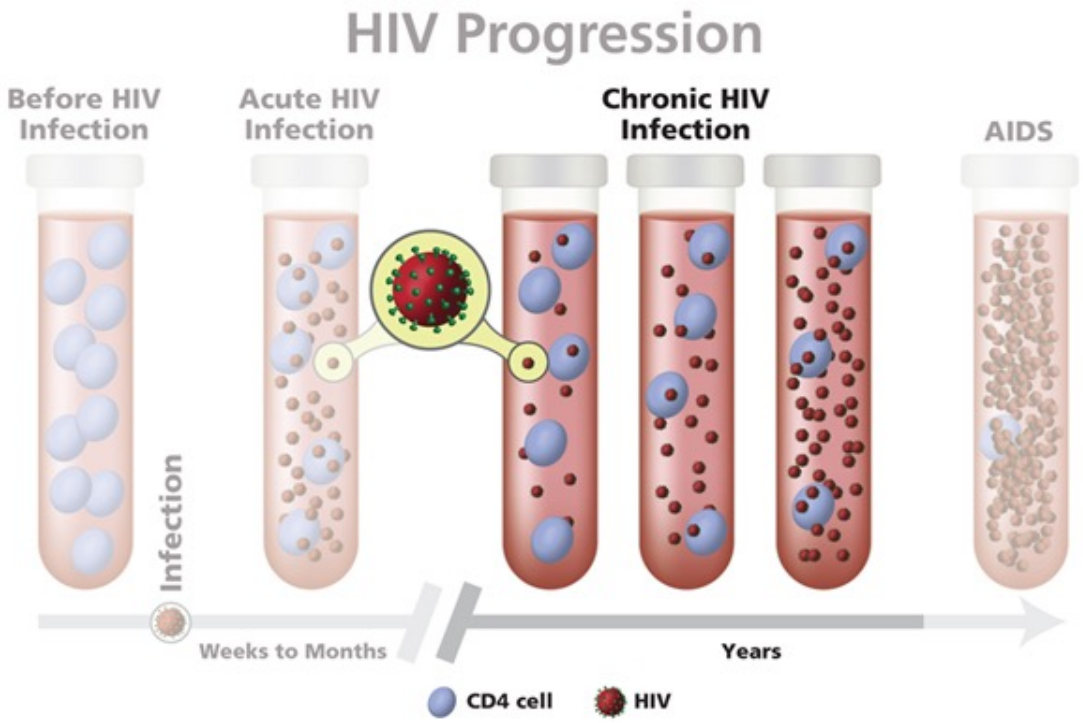
https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection



Acute HIV infection

Early stage of HIV infection that extends approximately 2 to 4 weeks from initial infection until the body produces enough HIV antibodies to be detected by an HIV antibody test. During acute HIV infection, HIV is highly infectious because the virus is multiplying rapidly. The rapid increase in HIV viral load can be detected before HIV antibodies are present. During this time, some people have flu-like symptoms, such as fever, headache, and rash. The virus attacks and destroys the infection-fighting CD4 cells of the immune system. A person may experience significant health benefits if they start ART during this stage.

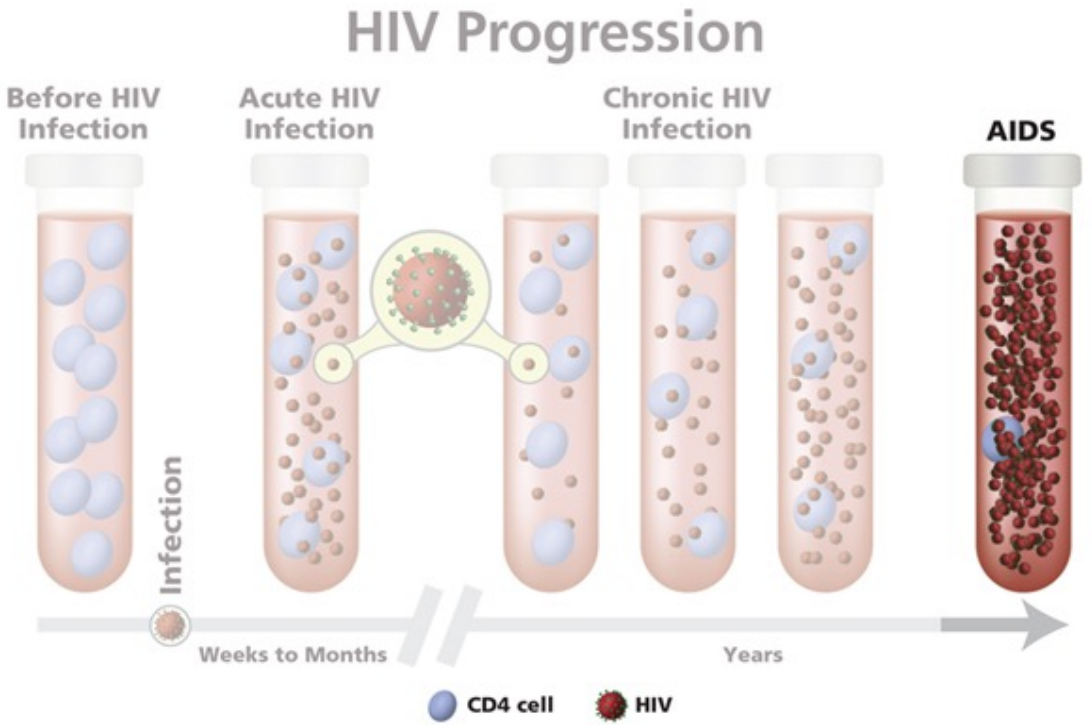
https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection



Chronic HIV Infection

The second stage of HIV infection is chronic HIV infection (also called asymptomatic HIV infection or clinical latency). During this stage, HIV continues to multiply in the body but at very low levels. People with chronic HIV infection may not have any HIV-related symptoms. Without ART, chronic HIV infection usually advances to AIDS in 10 years or longer, though in some people it may advance faster. People who are taking ART may be in this stage for several decades. While it is still possible to transmit HIV to others during this stage, people who take ART exactly as prescribed and maintain an undetectable viral load have effectively no risk of transmitting HIV to an HIV-negative partner through sex.

https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection

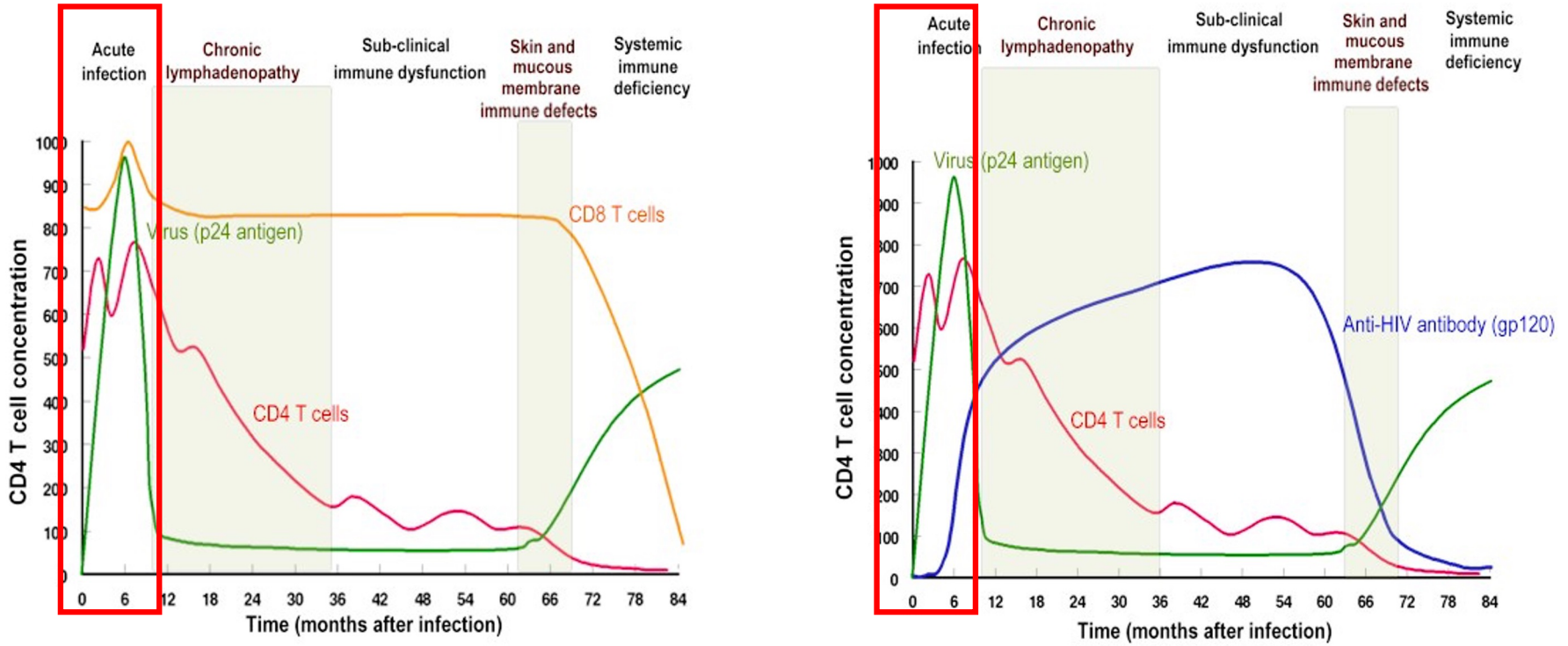


AIDS

AIDS is the final, most severe stage of HIV infection. Because HIV has severely damaged the immune system, the body can't fight off opportunistic infections. (Opportunistic infections are infections and infection-related cancers that occur more frequently or are more severe in people with weakened immune systems than in people with healthy immune systems.) People with HIV are diagnosed with AIDS if they have a CD4 count of less than 200 cells/mm3 or if they have certain opportunistic infections. Once a person is diagnosed with AIDS, they can have a high viral load and are able to transmit HIV to others very easily. Without treatment, people with AIDS typically survive about 3 years.

https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection

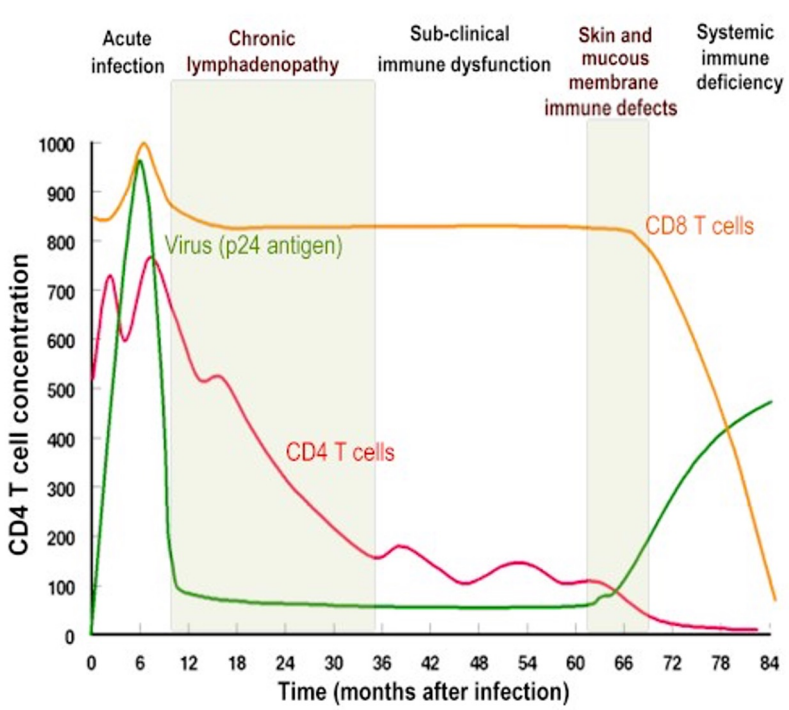
Course of the disease

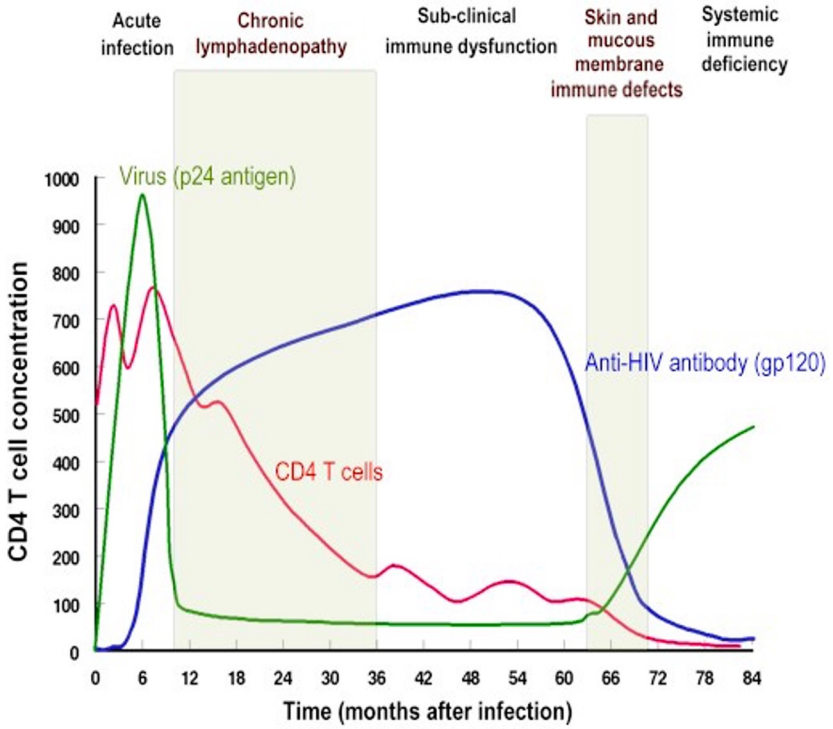


Cytotoxic B and T lymphocytes mount a strong defense and virus largely disappears from the circulation. After the increased cell-mediated immune response, there is a rise in humoral anti-HIV antibodies. During this period of strong immune response to the virus, more than 10 billion new HIV particles are produced each day, but they are rapidly cleared by the immune system and have a half-life of only 5 to 6 hours

The infected cells that are producing the virus are destroyed either by the immune system or by the virus and have a half-life about 1 day. However, the rate of production of $CD4^+$ cells can compensate for the loss of cells and a steady state is set up in which most CD4+ cells are uninfected.

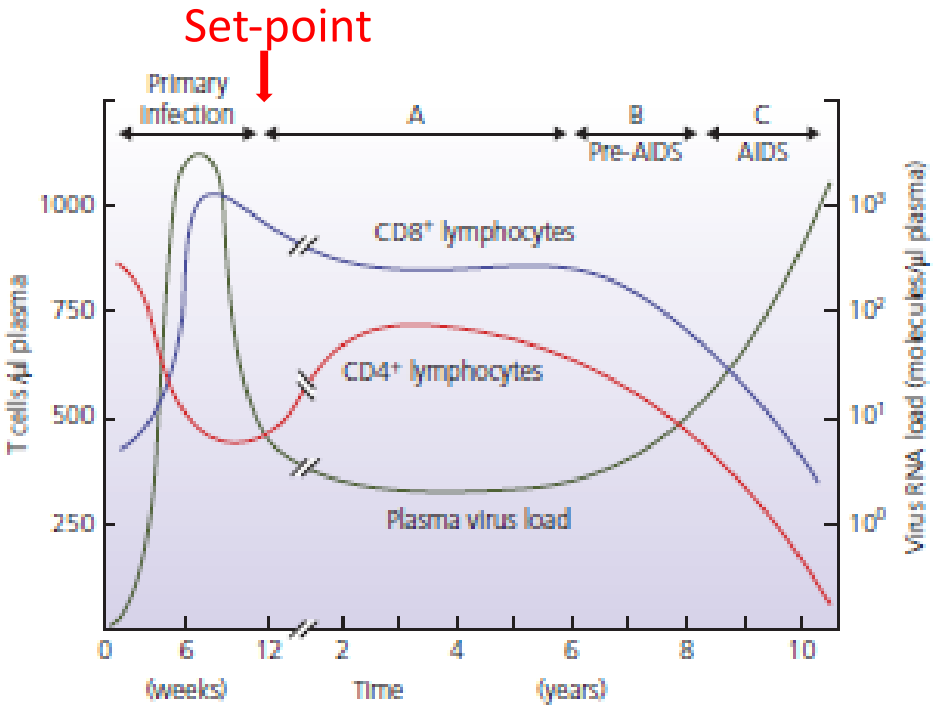
Course of the disease





As a result of the strong immune defense, the number of viral particles in the blood stream declines and the patient enters *clinical latency* . Little virus can now be found in the bloodstream or in peripheral blood lymphocytes and, initially, the number of blood CD4+ cells is only slightly decreased. Nevertheless, the virus persists elsewhere, particularly in lymph nodes and here viral replication continues as follicular dendritic cells interact with more CD4+ cells that become infected. The virus is also replicated by macrophages. The killer cells needed to control HIV also damage the helper T cells that they need to function efficiently. With the lack of CD4+ cells, new cytotoxic T cell responses cannot occur as helper cells are lacking and such new responses are required as the virus mutates. As the CD4+ cells fall below 200 per cu mm, virus titers rise rapidly and immune activity drops precipitously.

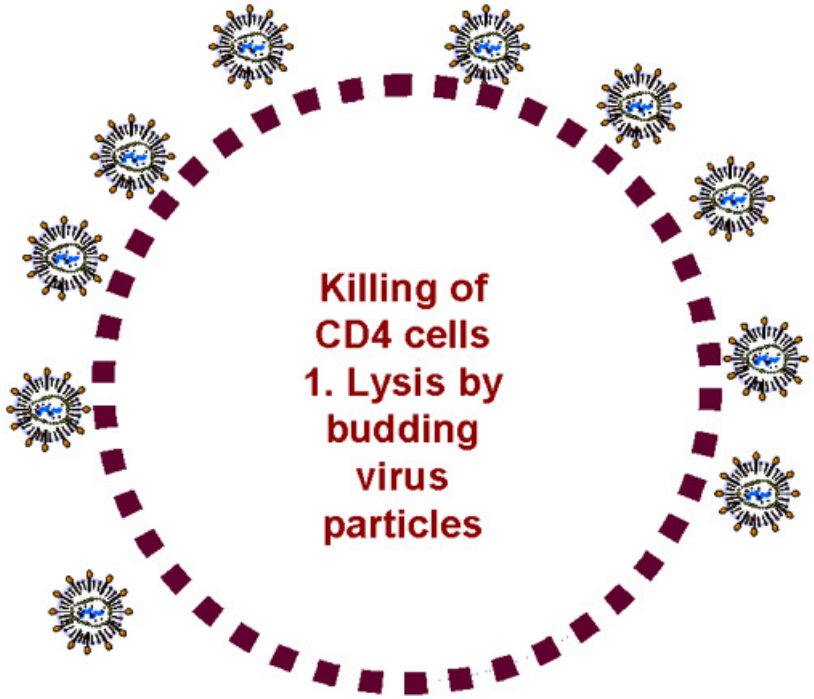
Course of the disease



During the initial/primary infection by HIV-1, virus-specific CD4+ T cells are stimulated to proliferate by viral antigens. As virus infects and replicates in activated CD4+ cells, these same cells are preferentially destroyed. At the same time, there is a dramatic expansion of virus-specific CD8+ T cells which coincides with the suppression of viraemia and a recovery in CD4+ T cell numbers. However, CD8+ T cell proliferation is dependent on CD4+ T cell help. Thus there is a fine balance between virus destroying CD4+ T cells and leaving enough CD4+ T cells to help produce virus-specific activated CD8+ cells. It is suggested that this balance determines the plasma virus load (concentration) at the end of the acute phase, also known as the 'set point'. The set point load appears to be a critical determinant of the rate of progression to AIDS; a low set point load means a longer subclinical period, and a high load means rapid progression to AIDS. However, virus loads ultimately rise again as immune function collapses

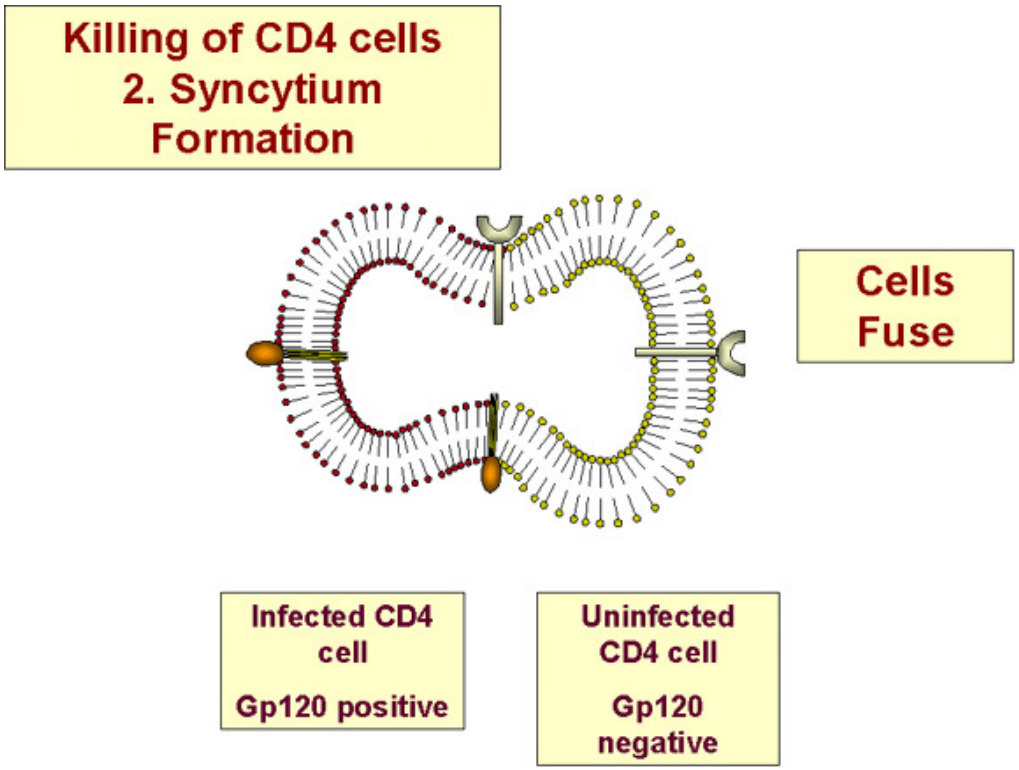
With less than 1000 copies/ml of blood, disease will probably occur with a latency period of more than 10 years. With less than 200 copies/ml, disease does not appear to occur at all. Most patients with more than 100,000 copies per ml, lose their CD4+ cells more rapidly and progress to AIDS before 10 years. Most untreated patients have between 10,000 and 100,000 copies per ml in the clinical latency phase.

LOSS OF CD4 CELLS



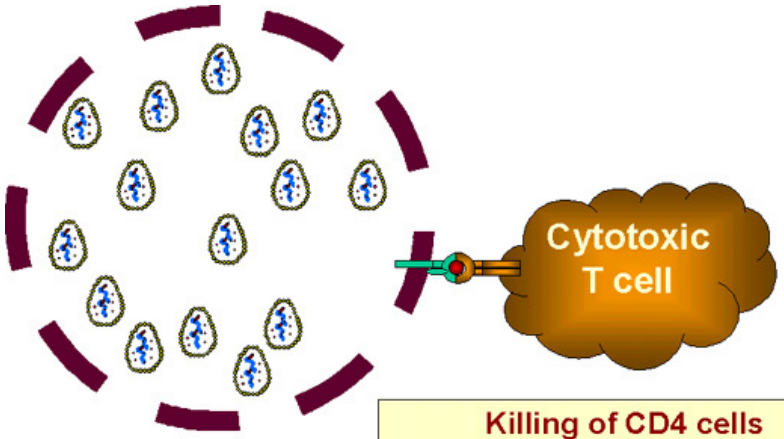
In an activated, infected CD4 cell, huge numbers of virions are synthesized. These bud from the cell and result in punctured membranes.

LOSS OF CD4 CELLS



Since the membrane of HIV fuses with the membrane of the cell to be infected by a pH-independent mechanism, syncytia formation can occur leading syncytium formation

LOSS OF CD4 CELLS



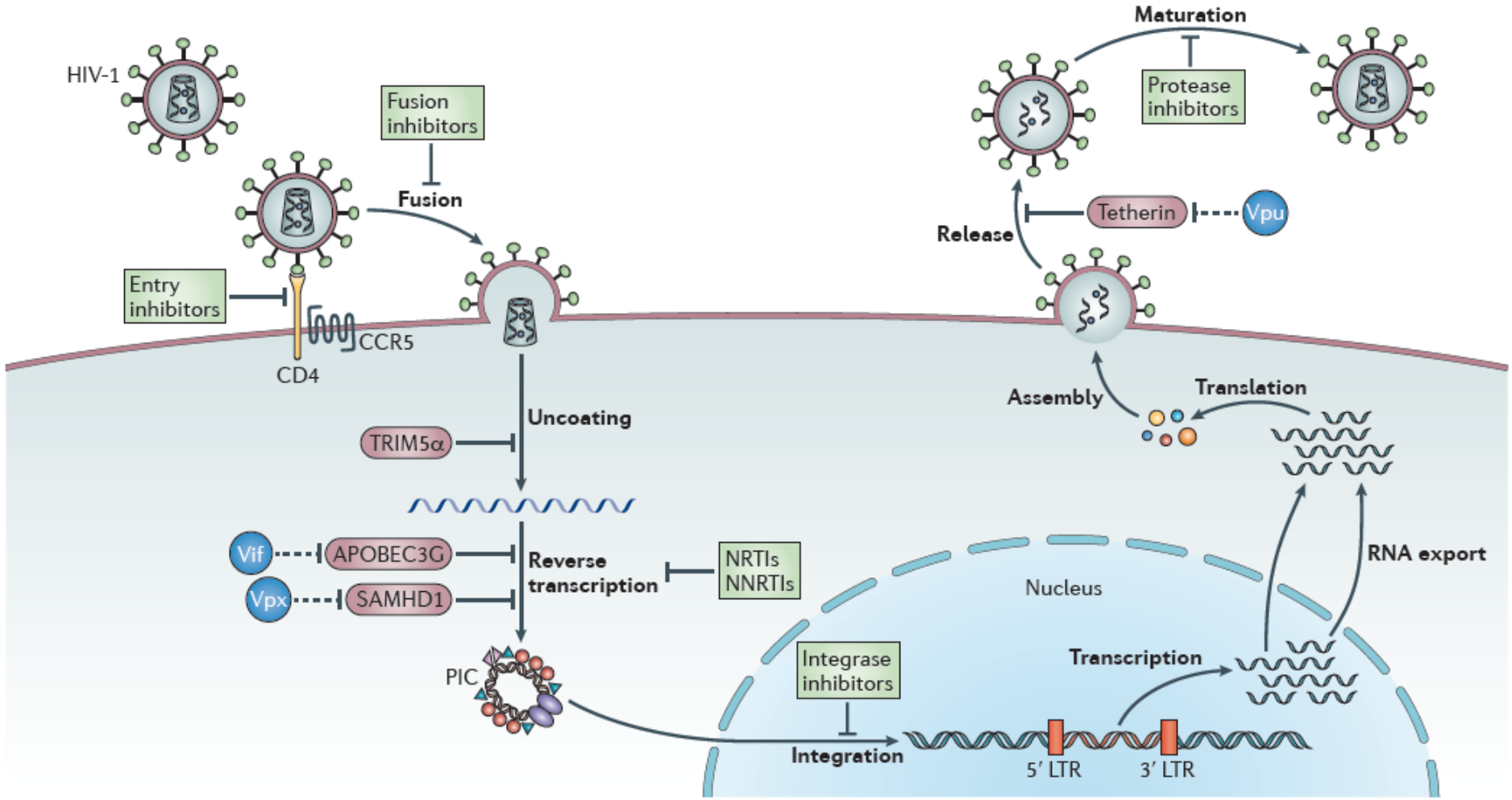
Killing of CD4 cells
3. Cytotoxic T cell-mediated lysis

Cell undergoes apoptosis via receptor on infected cell or Induction of leakage via perforin

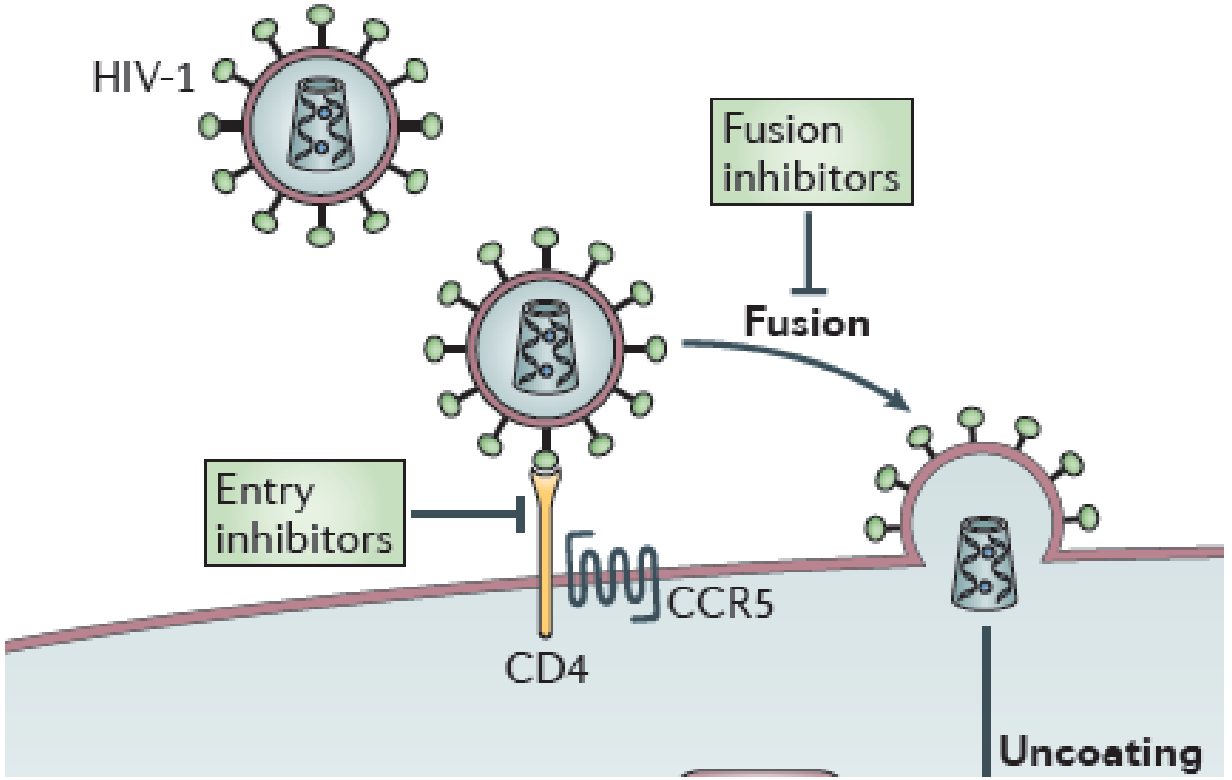
Infected cells that are producing viral proteins (but not those in the latent state) will present those proteins on the cell surface in association with class I MHC histocompatibility antigens. The infected cell, like other virally-infected cells, will be destroyed by cytotoxic T cells. Again this only happens in cells that are infected by HIV.

Gp120 is linked to the Gp41 on the virus surface by non-covalent interactions and is frequently shed from infected cells or from virus particles. This binds to uninfected cells via CD4 antigen. As a result, they appear to be infected and are destroyed by the immune system.

ANTI-HIV CHEMOTHERAPY



ANTI-HIV CHEMOTHERAPY



Agents that block interaction of gp120 with co-receptors

Agents that block fusion by interacting with gp41

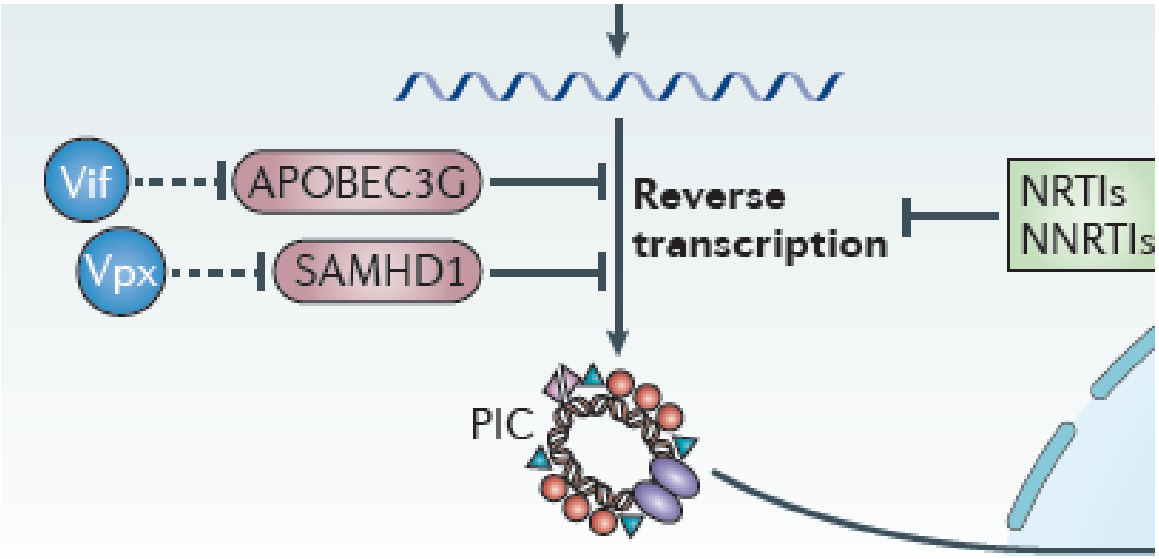
ANTI-HIV CHEMOTHERAPY

Nucleoside-Analog Reverse Transcriptase Inhibitors (NRTI).
They are incorporated into viral DNA, act as chain terminators.

AZT e d4T ⟶ timidina

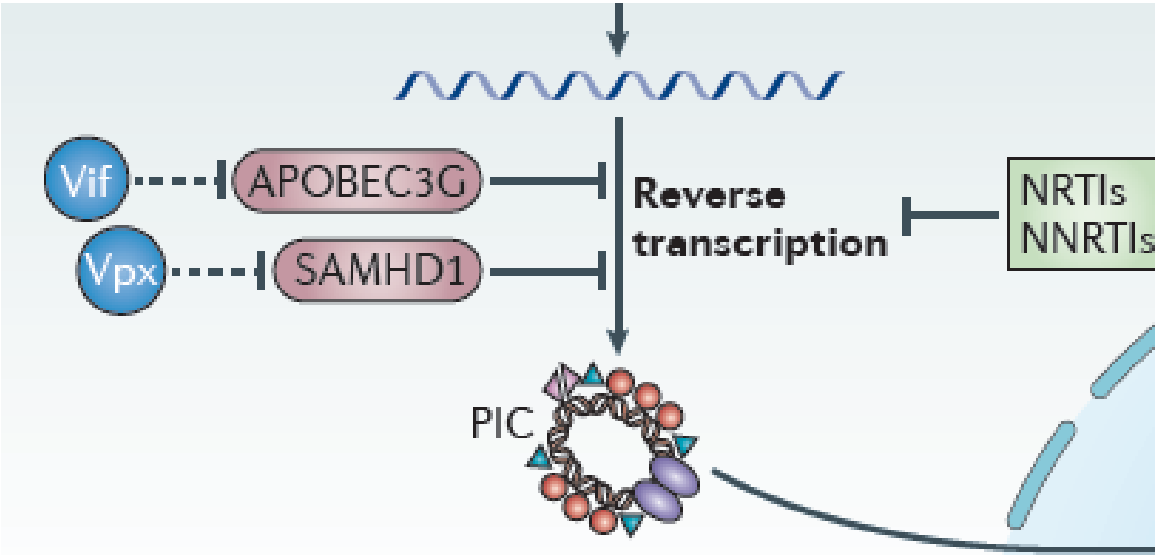
FTC e 3TC ⟶ citidina

Abacavir ⟶ guanosina



They were among the first anti-HIV-1 drugs. However, after long term administration, several side effects have been observed (mainly MITOCHONDRIAL DAMAGE)

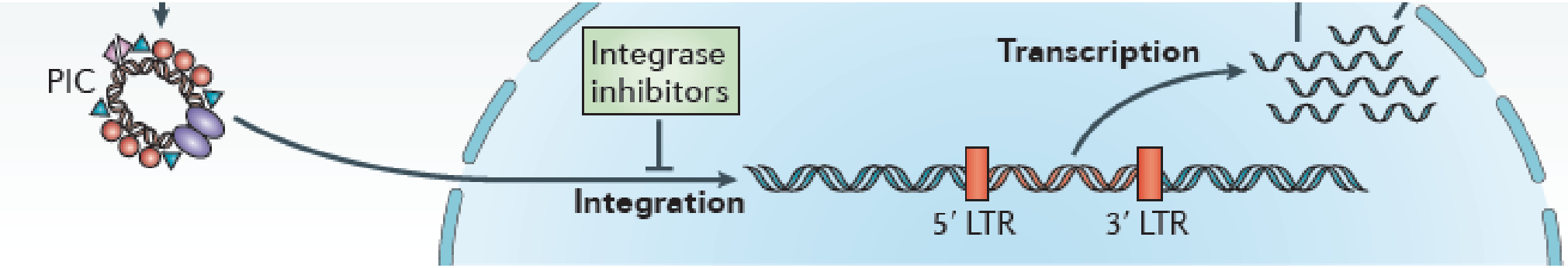
ANTI-HIV CHEMOTHERAPY



Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are small molecule drugs that bind directly to the active site of HIV-1 reverse transcriptase, disrupting its RNA-dependent and DNA-dependent DNA polymerase activities. Unlike nucleoside analogs, they have minimal toxicity in tests with cultured cells. There is some toxicity when used in humans, mainly gastrointestinal problems or elevated liver enzymes

Nevirapine – Delaviridine - Efavirenz

Drugs that inhibit integrase (IN) activity



Viral DNA integration requires several steps. These can be targeted by different IN inhibitors:

IN associates with the viral DNA in the cytoplasm to form the pre-integration complex (PIC).

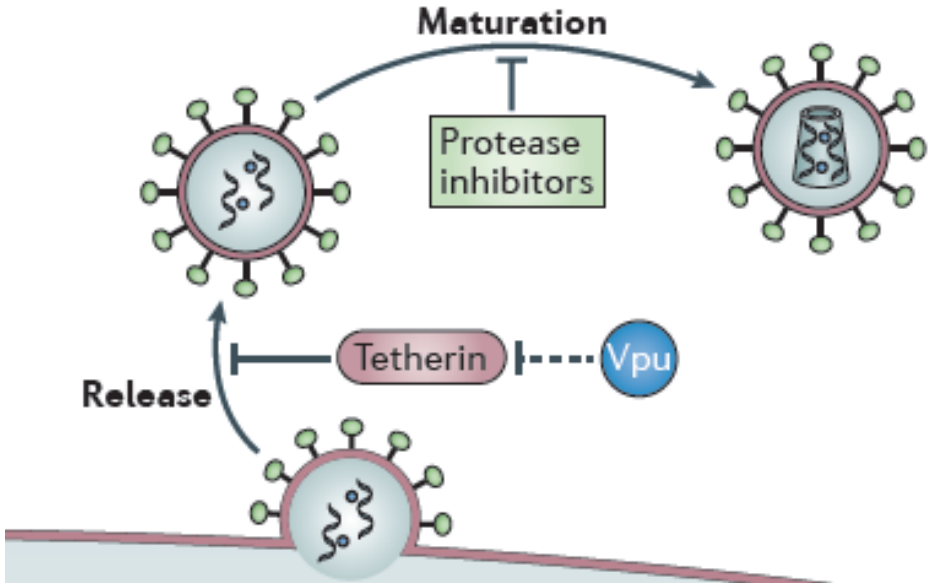
In a first catalytic step IN cuts a dinucleotide from both ends of the viral DNA to produce hydroxylated 3’ ends in the PIC

In the nucleus IN binds the host DNA to allow annealing of hydroxylated ends of the proviral DNA to the host DNA

ANTI-HIV CHEMOTHERAPY

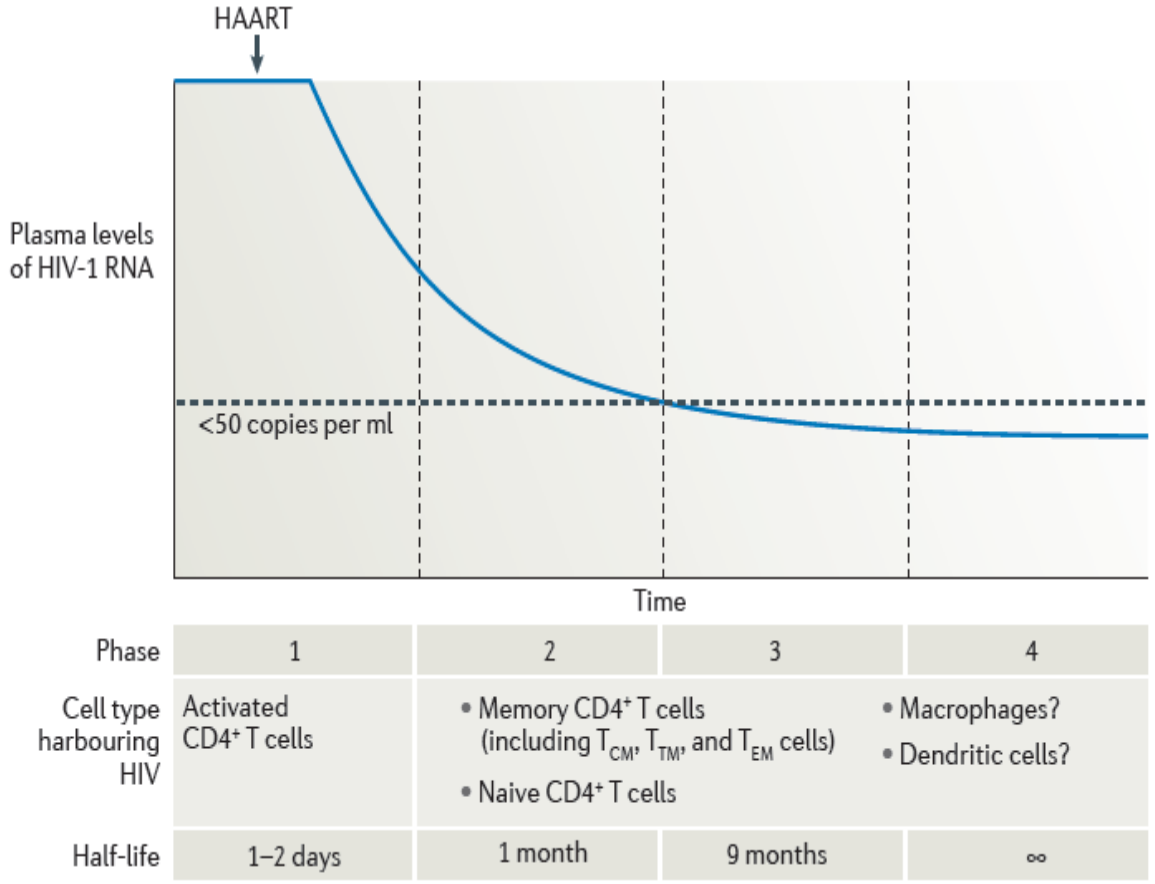
Protease Inhibitors. They are all substrate analogs, that is they mimic a peptide that can bind to the active site of the viral protease.

Indinavir – Ritonavir - Saquinavir



There have a few side effects in patients. These are mild: nausea, diarrhea, upset stomach, and heartburn.

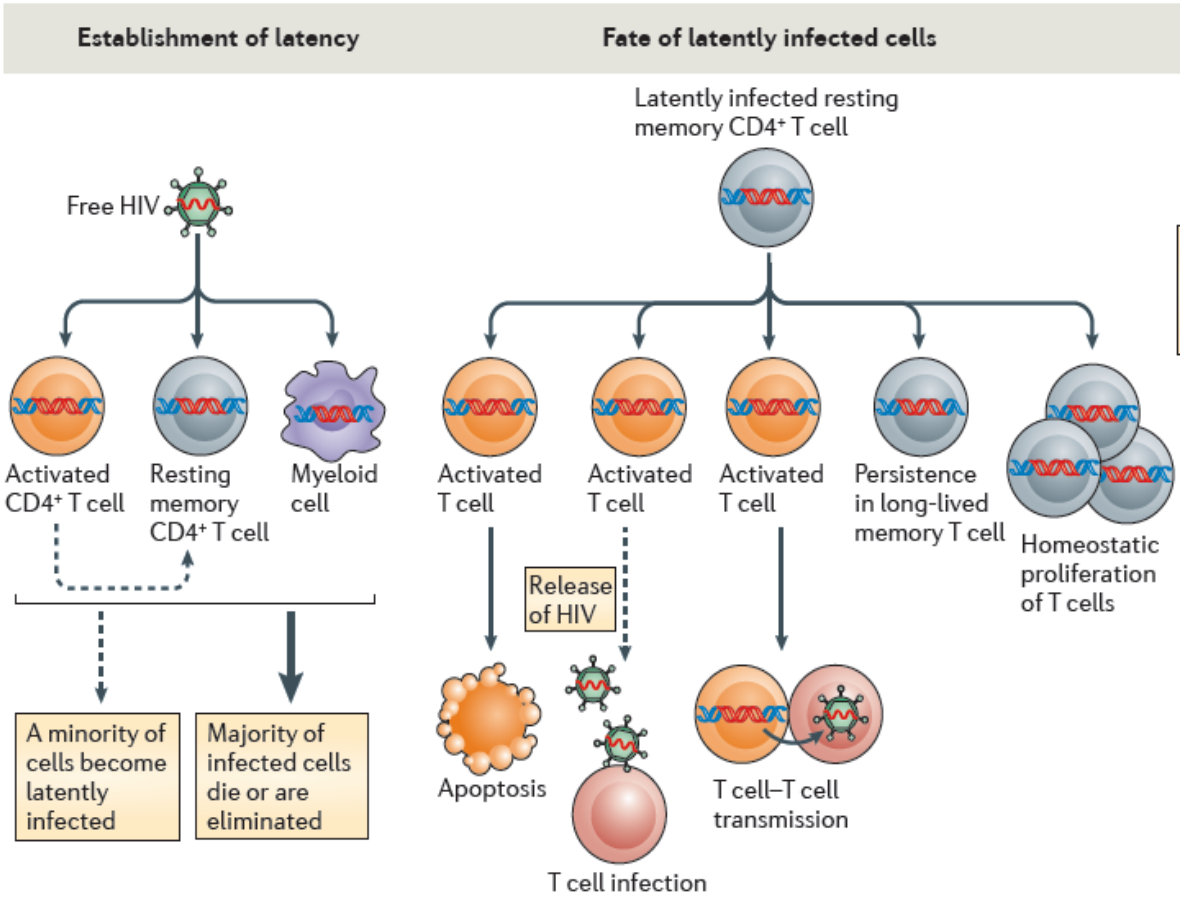
The impact of antiretroviral therapy on HIV reservoirs.



Most patients who adhere to antiretroviral therapy have dramatic and rapid decreases in plasma levels of HIV RNA. Persistent viraemia largely reflects the release of the virus from stable cellular reservoirs. The source of the virus during effective antiretroviral therapy is primarily defined by the half-life of the cells that were infected before therapy was initiated. After several years of therapy, long-lived populations of resting CD4+ central memory T (T_{CM}) cells become the dominant source of HIV persistence. HAART, highly active antiretroviral therapy; T_{EM}, effector memory T; T_{TM}, transitional memory T.

NATURE REVIEWS | IMMUNOLOGY

Mechanisms of HIV persistence during antiretroviral therapy



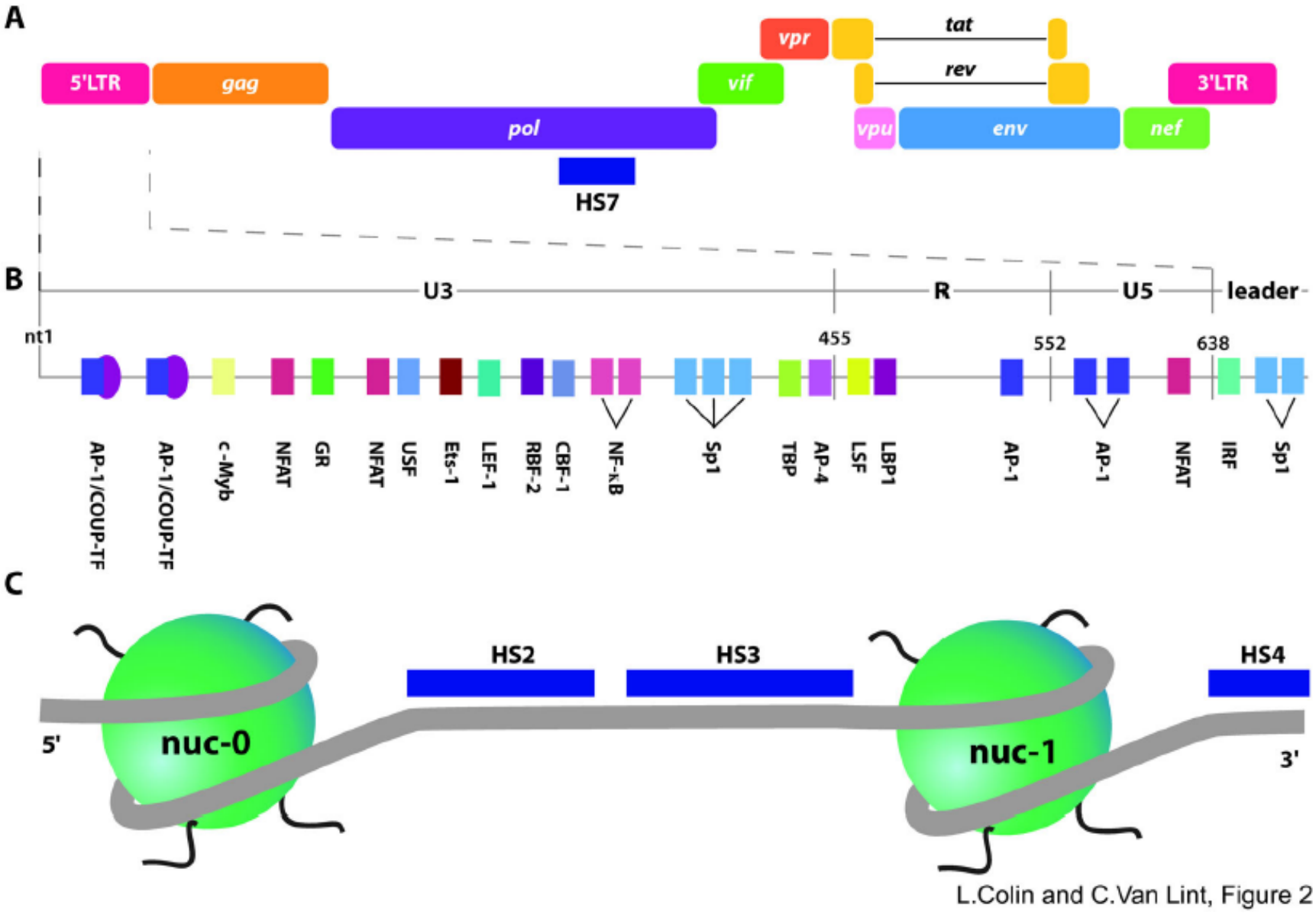
The left panel illustrates how latent HIV infection can be established in T cell and myeloid cell reservoirs. The primary mechanism is probably infection of activated memory CD4+ T cells. Most of these cells die, but a minority revert to a resting state. The right panel illustrates the fate of these now resting 'latently infected' memory CD4+ T cells. These cells either die slowly, become a source of new infections, persist as long-lived cells or expand through homeostatic mechanisms.

NATURE REVIEWS | IMMUNOLOGY

… post-integration latency :

- The integrated provirus may not be accessible to the transcription machinery in resting cells.
- There could be a lack of necessary host cell-provided transcription factors in resting T4 cells (eg NFAT and NF-kB are sequestered in the cytoplasm of resting cells)

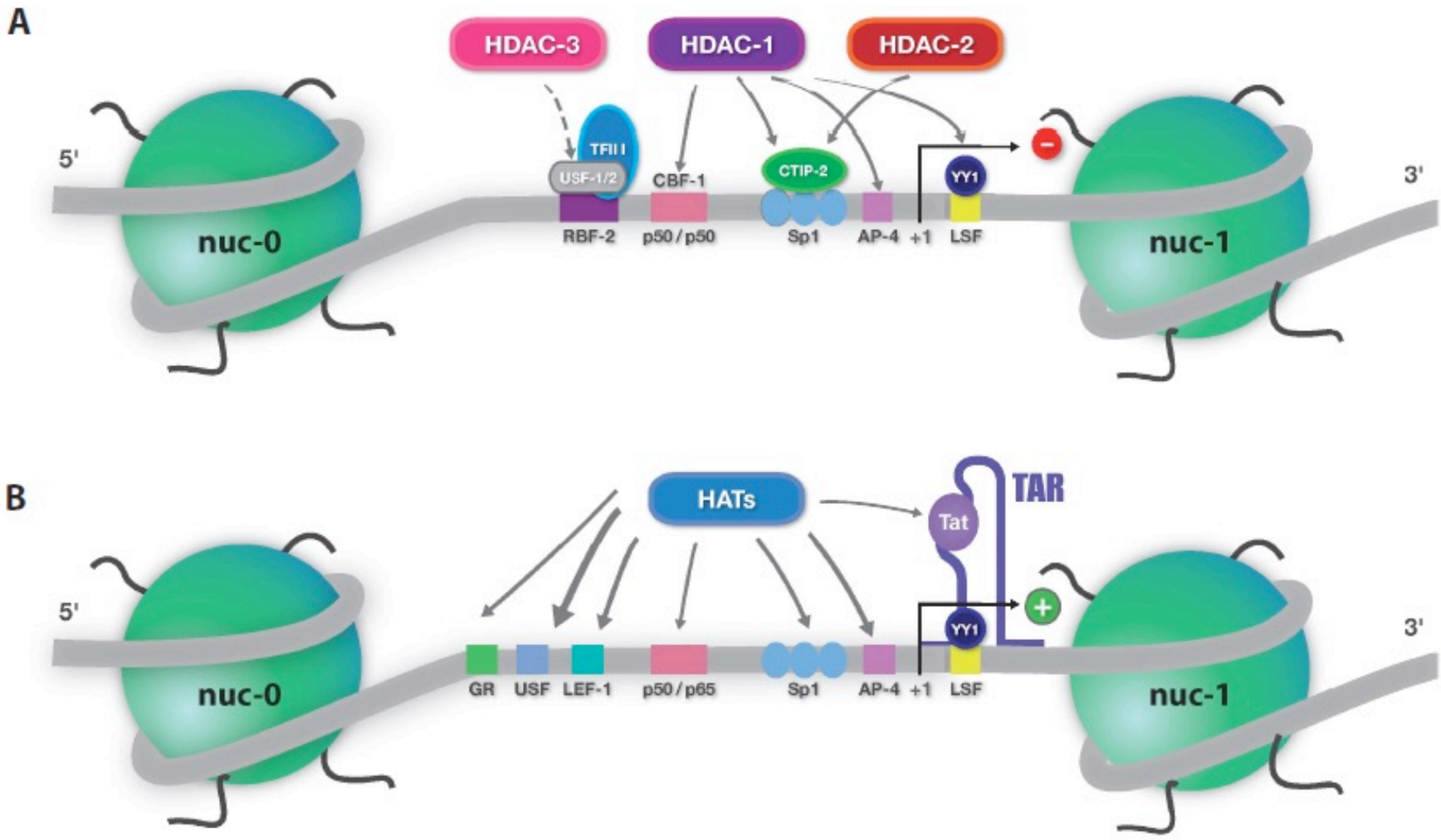
Molecular mechanisms that allow or restrict HIV proviral expression in quiescent T cells



L.Colin and C.Van Lint, Figure 2

Transcription factor binding sites and chromatin organization in the 5'LTR and leader region of HIV-1. (A) Representation of the HIV-1 genome. The intragenic hypersensitive site HS7 located in the *pol* gene is indicated. (B) Schematic representation of the main transcription factor binding sites located in the 5'LTR and in the beginning of the leader region of HIV-1. The U3, R, U5 and leader regions are indicated. Nucleotide 1 (nt1) is the start of U3 in the 5'LTR. The transcription start site corresponds to the junction of U3 and R. (C) Schematic representation of the nucleosomal organization of the HIV-1 genome 5' region. Hypersensitive sites HS2, HS3 and HS4 are indicated. The assignment of nucleosome position in this region is based on DNase I, micrococcal nuclease and restriction enzyme digestion profiles. During transcriptional activation, a single nucleosome, named nuc-1 and located immediately downstream of the transcription start site, is specifically and rapidly remodeled.

Molecular mechanisms that allow or restrict HIV proviral expression in quiescent T cells



*Retrovirology*2009

HDAC and HAT recruitment to the HIV-1 5'LTR. (A) During latency, nuc-1 blocks transcriptional initiation and/or elongation because it is maintained hypoacetylated by nearby recruited HDACs. The targeting of nuc-1 by these HDACs is mediated by their recruitment to the 5'LTR via several transcription factor binding sites. Thin arrows indicate that the implicated transcription factors were demonstrated to recruit HDACs to the 5'LTR (by ChIP experiments or following knockdown of the corresponding transcription factor). The dotted arrow indicates that the USF transcription factor could potentially recruit HDAC-3 to the nuc-1 region based on its interactome partners in the literature, but this recruitment has not been demonstrated so far in the specific context of the HIV-1 promoter. (B) Nuc-1 is a major obstacle to transcription and has to be remodelled to activate transcription. This disruption could happen following local recruitment of HATs by DNA-binding factors, and/or by the viral protein Tat, which binds to the neo-synthesized TAR element. This would result in nuc-1 hyperacetylation and remodelling, thereby eliminating the block to transcription at least for certain forms of viral latency. This acetylation-based activation model has been validated notably regarding the involvement of the transcription factors NF-kB p65 and Tat.

HIV - Life History

Latency – Cellular – The problem of memory T4 cells

Only activated T4 cells can replicate virus

Most infected T4 cells are rapidly lyzed but are replaced

Some T4 cells revert to resting state as memory cells which are long-lived

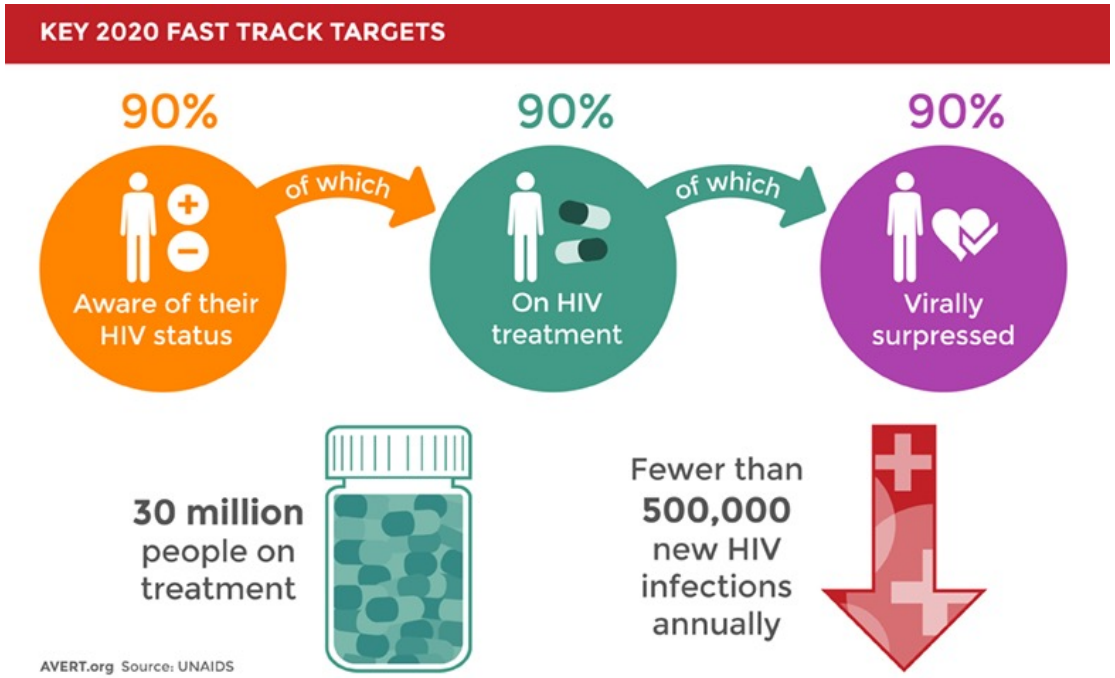
Memory T4 cells cannot replicate the virus unless they become activated

Clinical Latency

HIV infection is not manifested as disease for years

During apparent clinical latency, virus is being replicated and cleared

The UNAIDS Fast-Track strategy, launched in 2014, aims to greatly step up the HIV response in low- and middle-income countries to end the epidemic by 2030
The Fast-Track treatment targets are known as the 90-90-90 targets:



In September 2016 Sweden became the first country to achieve these targets.

Taking this action would mean that in 2020, there would be:
•fewer than 500,000 people newly infected with HIV
•fewer than 500,000 people dying from AIDS-related illnesses
•elimination of HIV-related discrimination.

The 90-90-90 targets refer to the pathway by which a person is tested, linked and retained in HIV care, and initiates and adheres to antiretroviral drugs (ARVs).
See more at: http://www.avert.org/professionals/hiv-around-world/global-response/

Global HIV epidemic – incidence and mortality since 2010

2022

Globally

39 million

People living with HIV

+20%

Relative to 2010

1.3 million

People newly infected

- 38%

New infections/year relative to 2010

0.63 million

HIV-related deaths

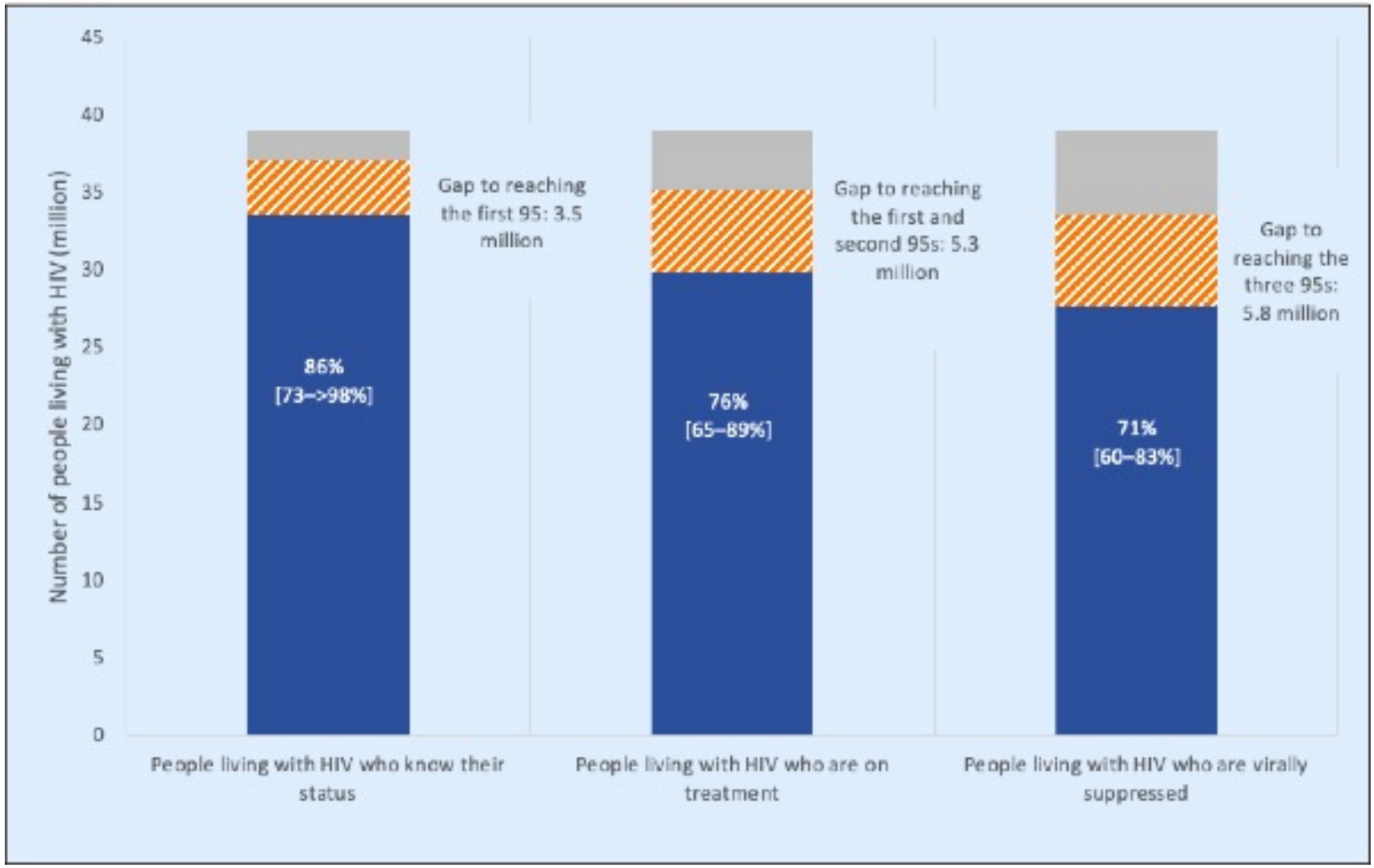
- 51%

Deaths/year relati

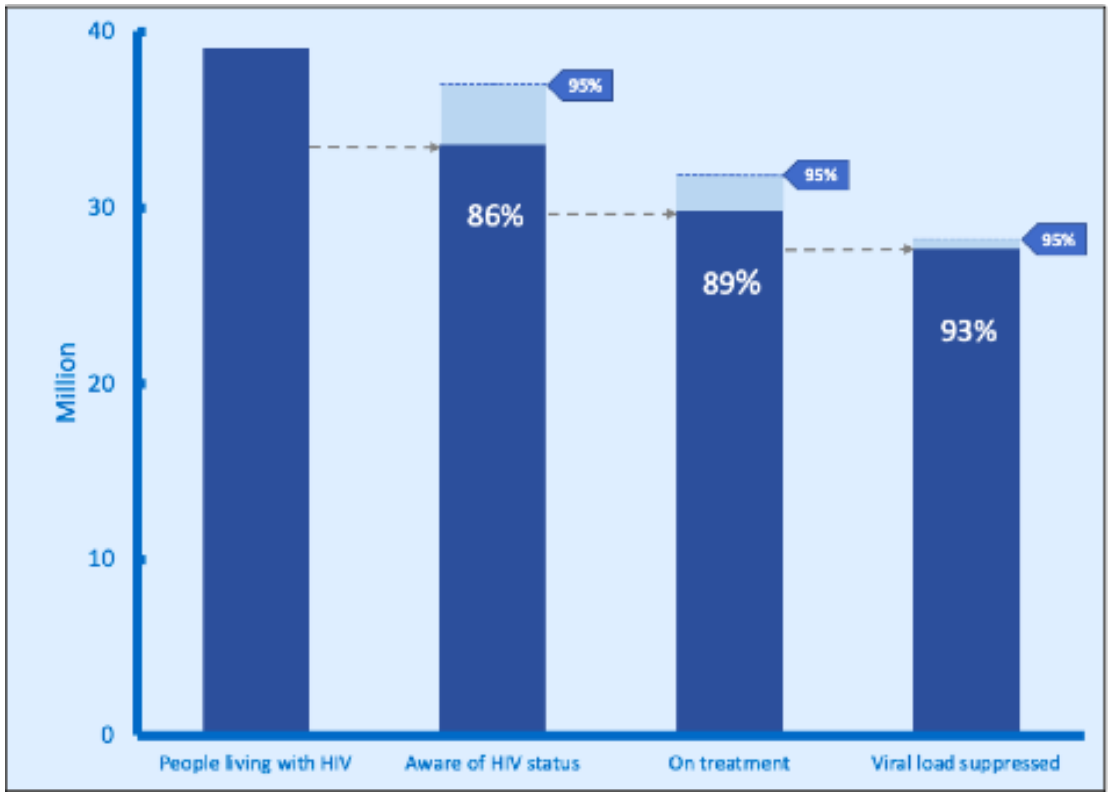
Source: UNAIDS/WHO and HIV.gov

W
Organization

World Health Organization



Progress towards 95-95-95 targets, global, 2022



95-95-95 targets by 2030 (displayed here):
-95 percent of people living with HIV know their status
-95 percent of people living with HIV who know their status are receiving treatment -95 percent of people on treatment have suppressed viral loads

Increase in people receiving antiretroviral therapy, global, 2010–2022