Human Immunodeficiency Virus
An Evolutionary Masterpiece

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University of California, Davis

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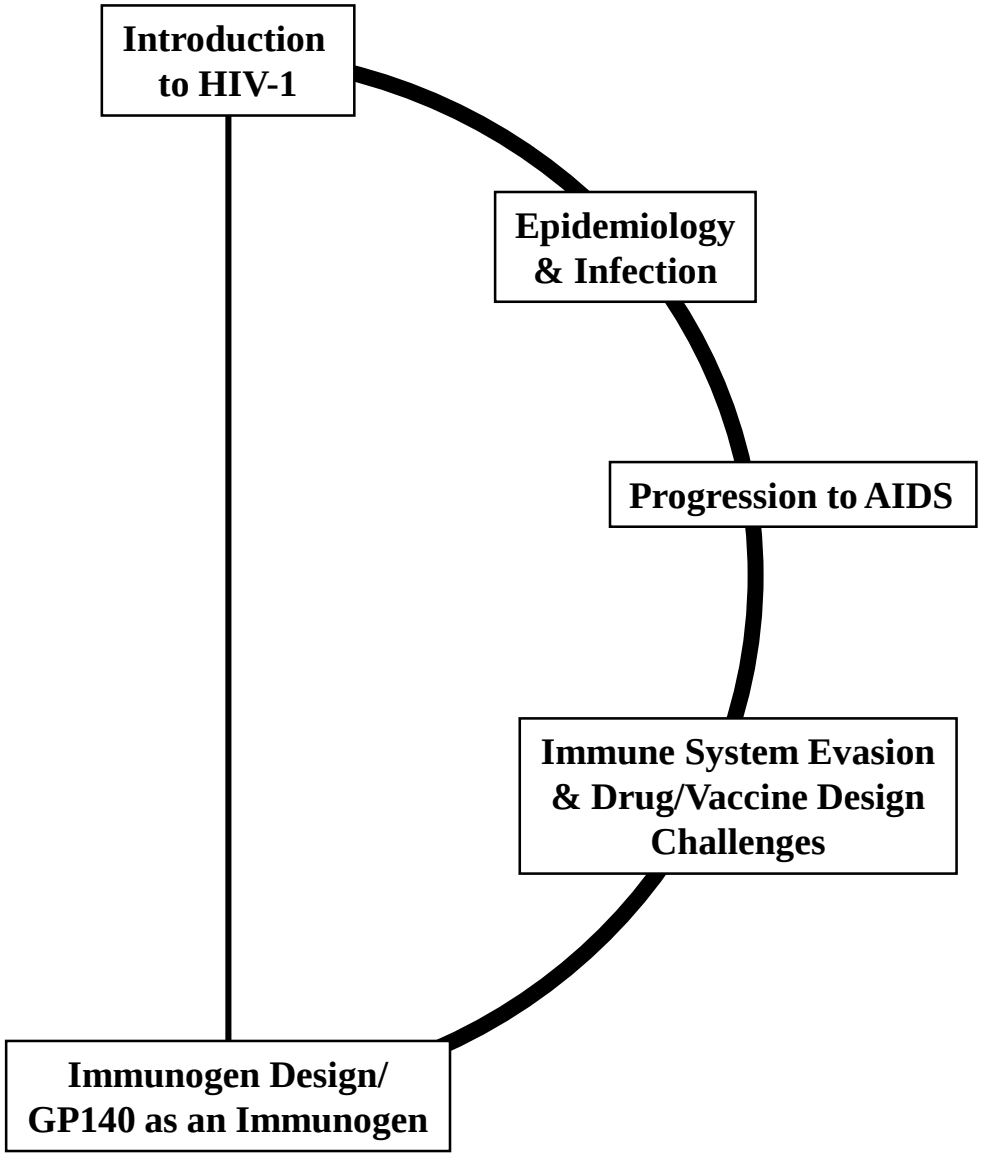
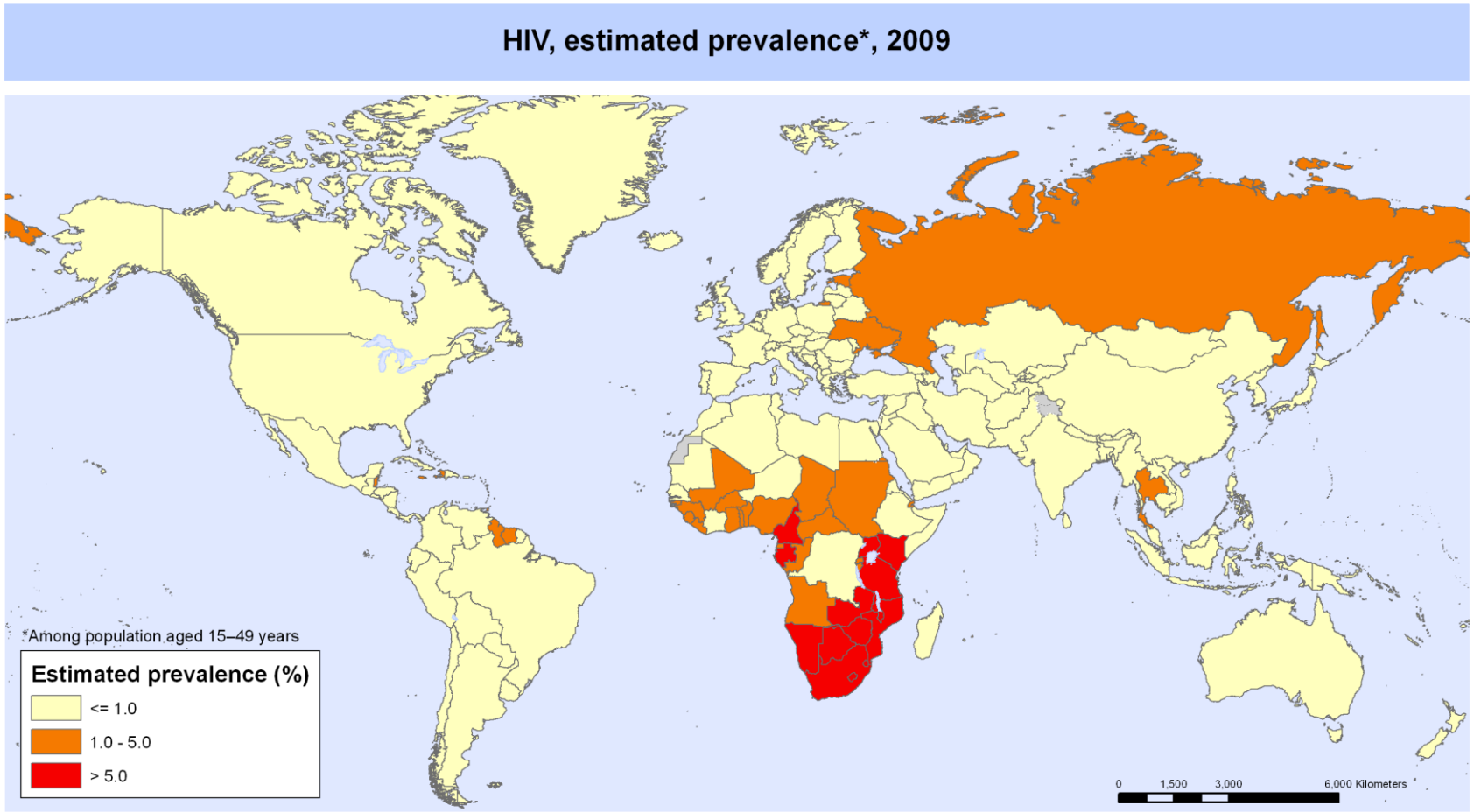
8.14.14

Today's Talk


Introduction to HIV-1
Epidemiology & Infection
Progression to AIDS
Immune System Evasion & Drug/Vaccine Design Challenges
Immunogen Design/ GP140 as an Immunogen

HIV/AIDS Global Impact / Epidemiology

Rapid Spread



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: WHO/UNAIDS
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

~3.3 x 10^7 people living with HIV

HIV/AIDS Global Impact / Epidemiology

- Rapid spread
- High death rates
- No cure from HIV infection
- No vaccination / intravenous prevention

People living with HIV/AIDS

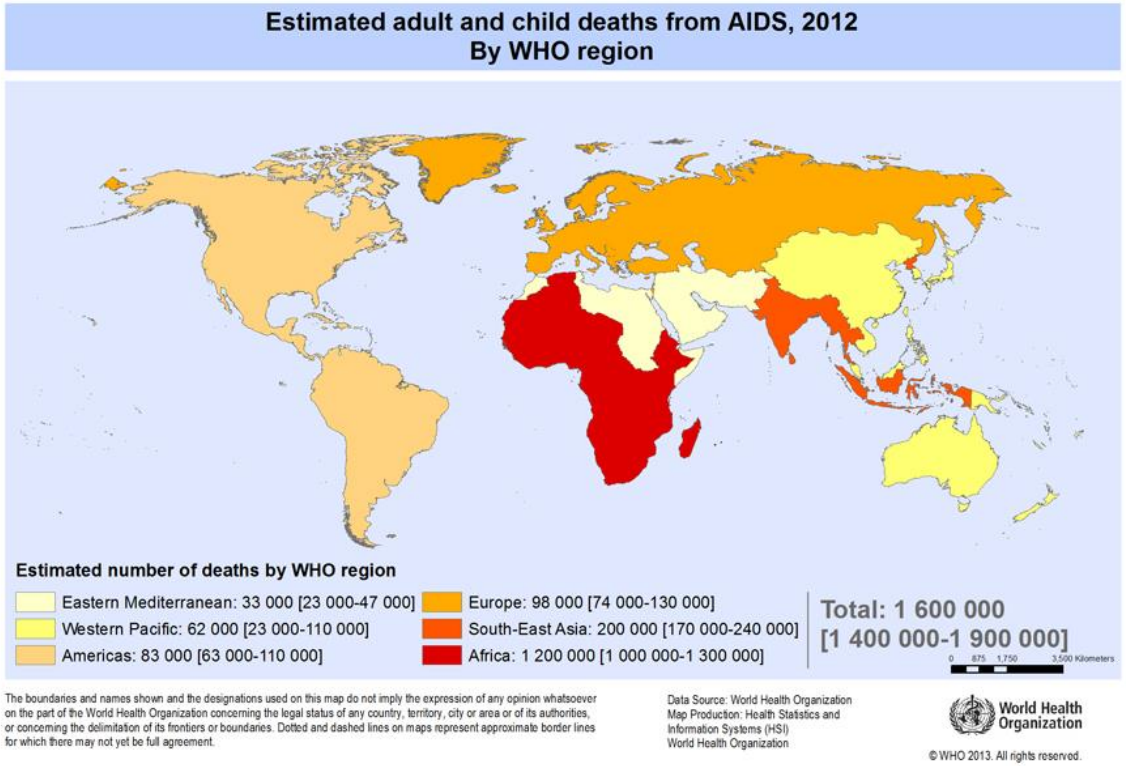
35.3 million

people living with HIV/AIDS worldwide in 2012

Mortality

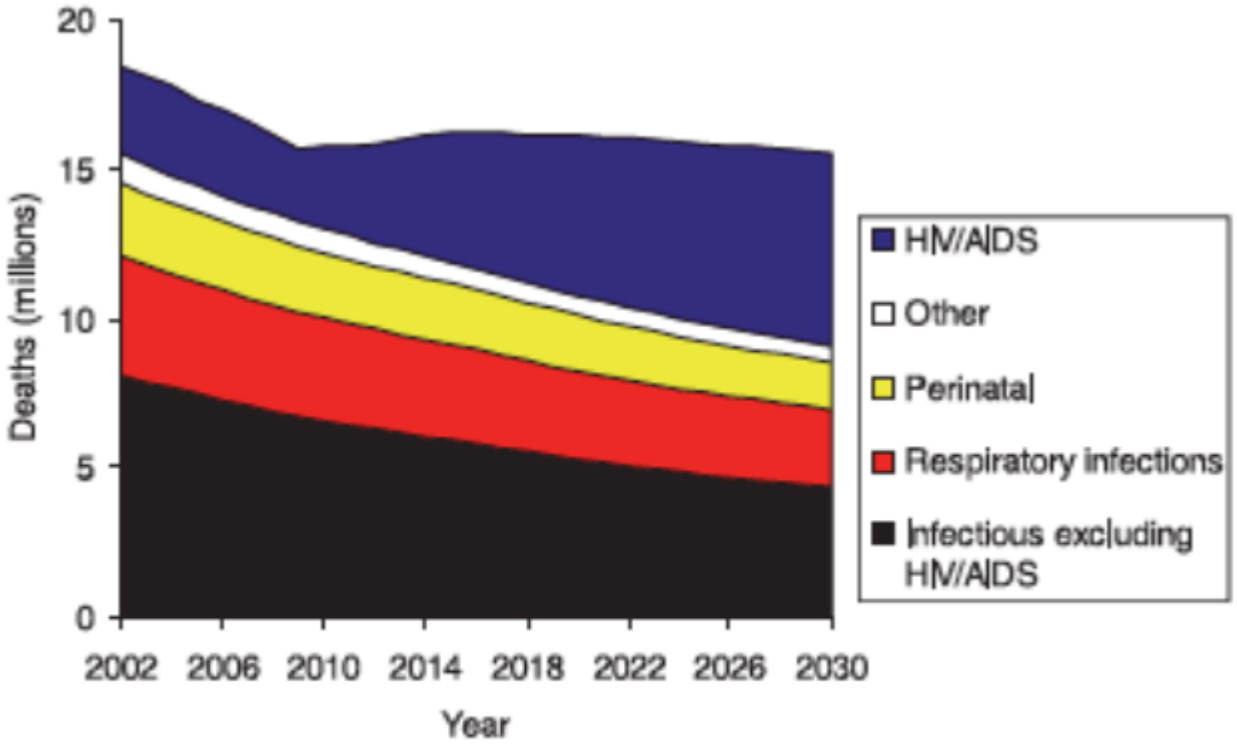
1.6 million

people died of AIDS-related illnesses worldwide in 2012



~3.9 x 10^7 people living with HIV

HIV/AIDS Global Impact / Epidemiology



Source: Mathers, C.D. and D. Loncar. Projections of Global Mortality and Burden of Disease from 2002 to 2030. Figure 3, PLoS Medicine, November 2006 3(11): e442. www.plosmedicine.org

HIV/AIDS Global Impact / Epidemiology

HIV → AIDS

1980s

Among all the cool technology 1980s….
There was a new epidemic!

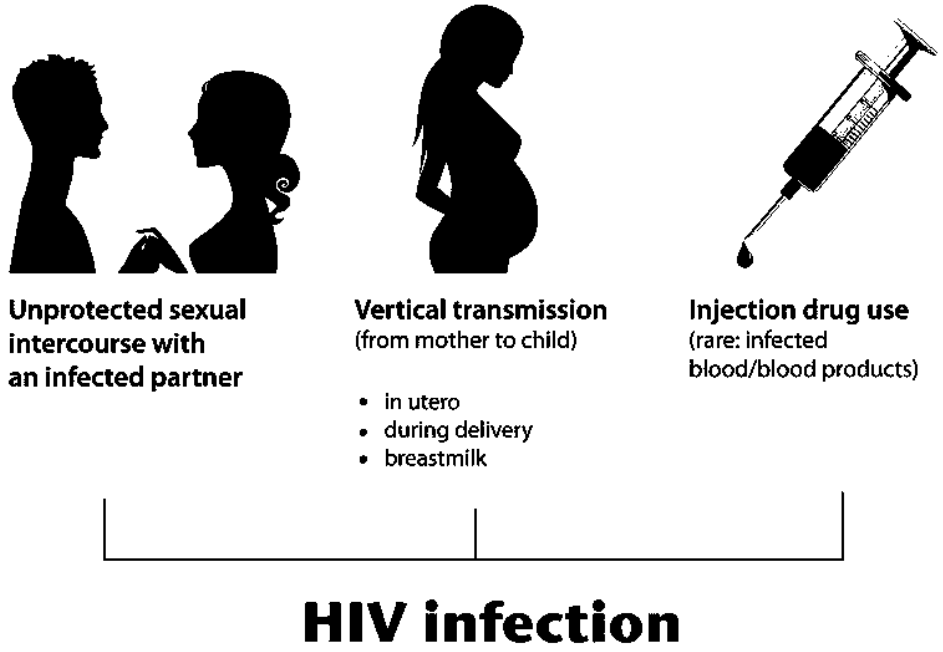
Early Recognition

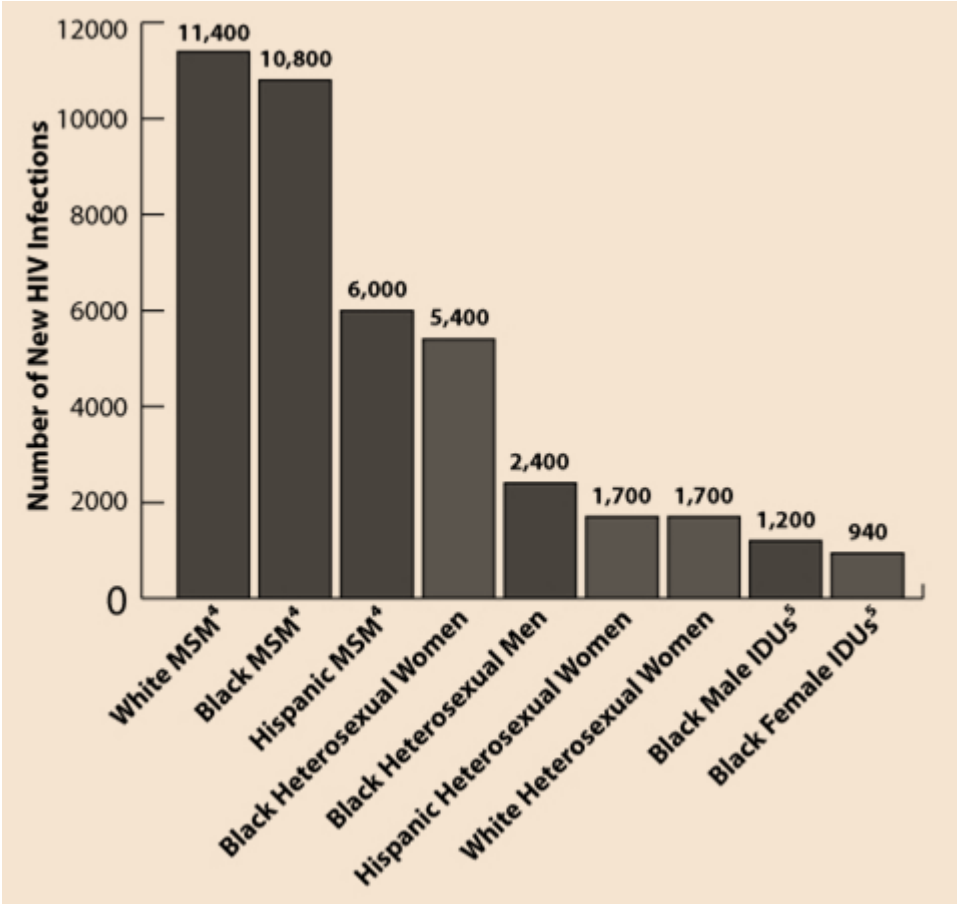
- Late 1970s and early 1980s
- Diminished count of CD4+ T-Lymphocytes
- Reduction of mitogen-induced proliferative capacity of lymphocytes
- A group of homosexual men were noted to be dying of normally benign opportunistic infections.
- Gallo et al. and Montagnier et al. reported the isolation of HIV-1 from patients with lymphadenopathy and AIDS in 1983 – later, a variant of HIV-1, HIV-2 was isolated in West Africa.

HIV/AIDS Global Impact / Epidemiology

Modes of Transmission

1) Unsafe sex with infected partner
2) Vertical transmission
3) Injection paraphernalia





HIV/AIDS Global Impact / Epidemiology

Rare Cases!

Transmission via saliva, through open wounds or mucosa.
Via Insect bite! (very rare)
A rare case of infection was reported for a new born, whose mother pre-chewed the food for her! One in a trillion chance! But it could happen. (Gaur et al. 2008)
The higher the viral load, the higher chances of contracting HIV

Table 1. Potentially Infectious Body Fluids

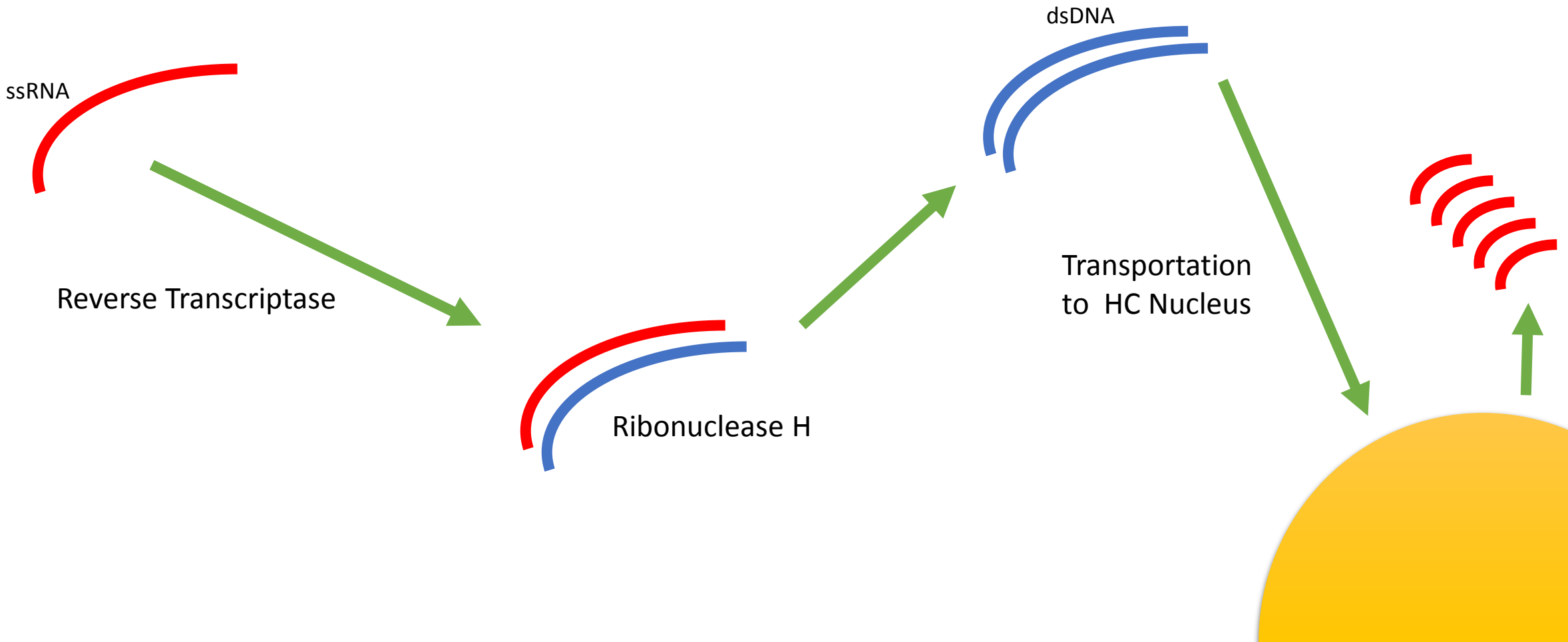
Potentially Infectious	Not Considered Infectious[a]
Blood	Feces
Tissue	Nasal secretions
Semen	Saliva
Vaginal secretions	Sputum
Visibly bloody body fluids	Sweat
Cerebrospinal fluid	Tears
Synovial fluid	Urine
Pleural fluid	Vomit
Peritoneal fluid	
Pericardial fluid	
Amniotic fluid	

[a] *Unless visibly bloody. Source: References 3, 7.*

Causative Agent of AIDS – Central Dogma?

Retroviruses – HIV

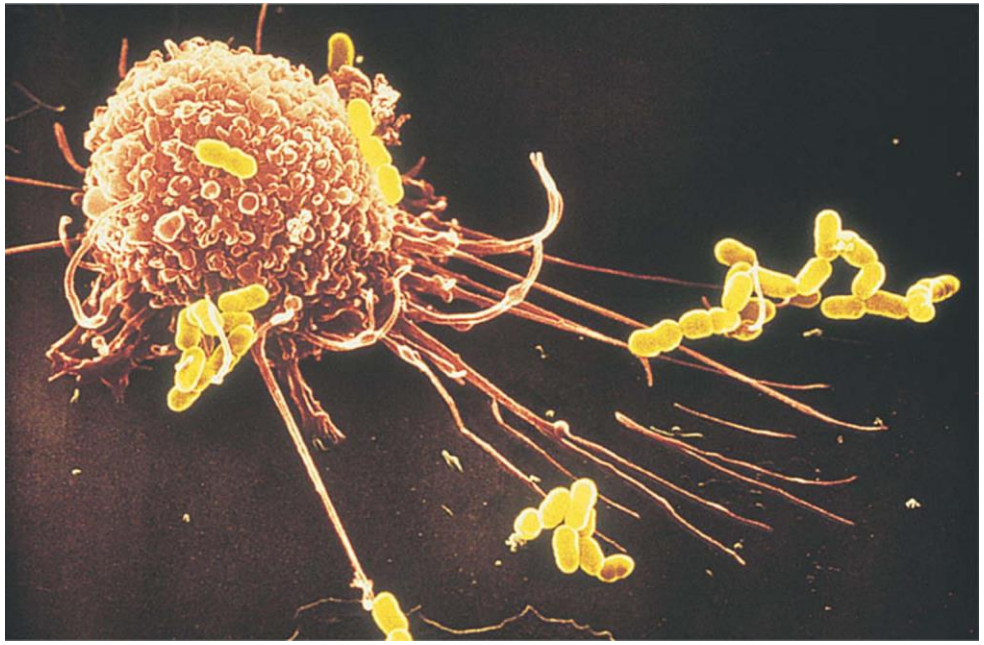
DNA → RNA → Protein!

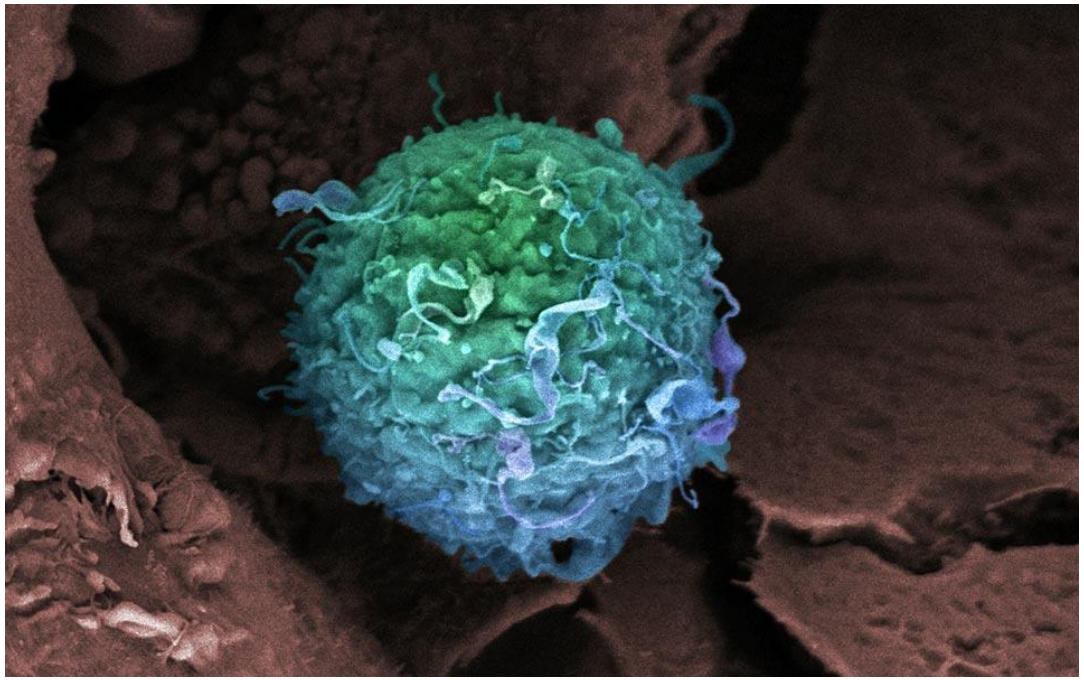


HIV/AIDS Global Impact / Infection

Virus Target

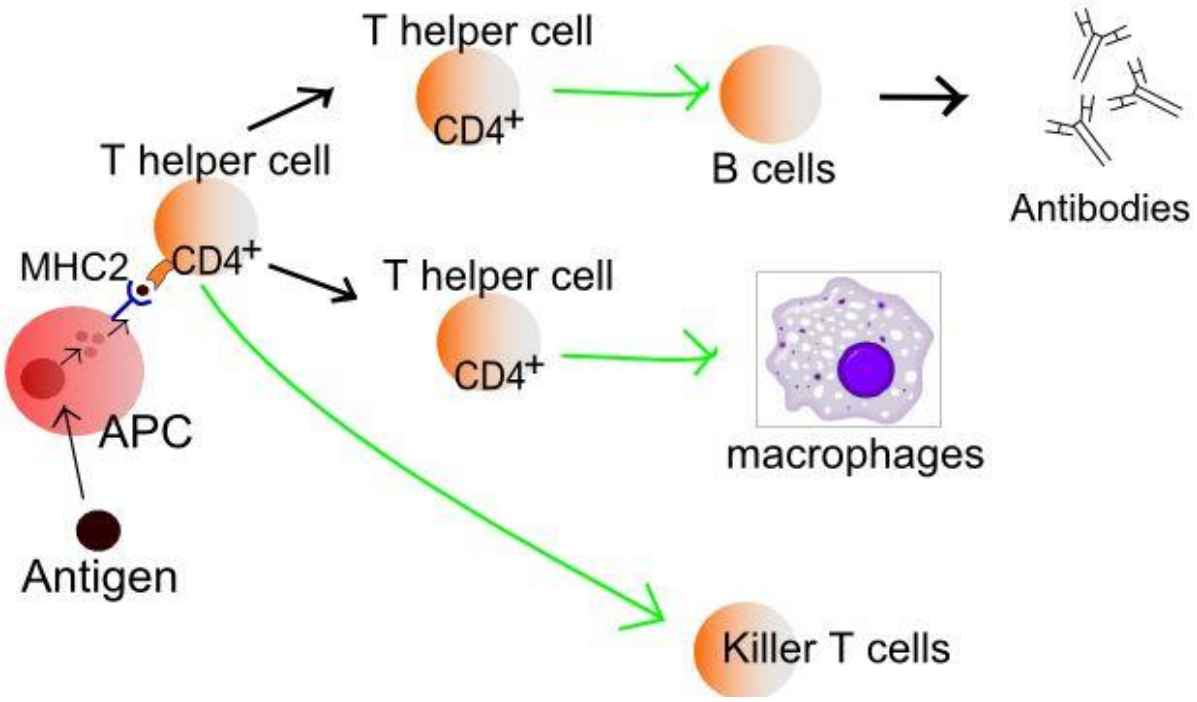
CD4 + T Cells and Macrophages/Monocytes

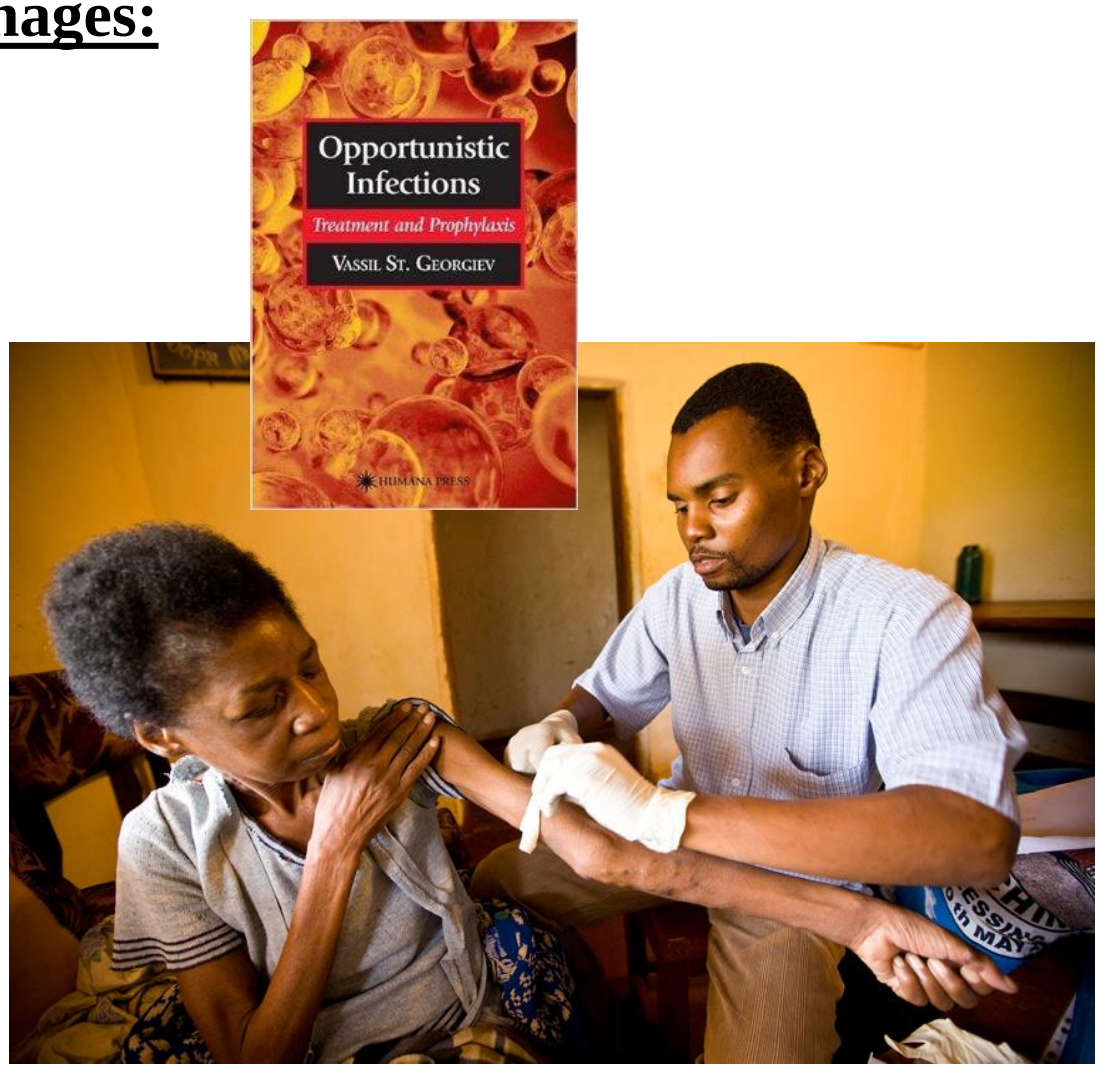




HIV/AIDS Global Impact / Impaired Immunity

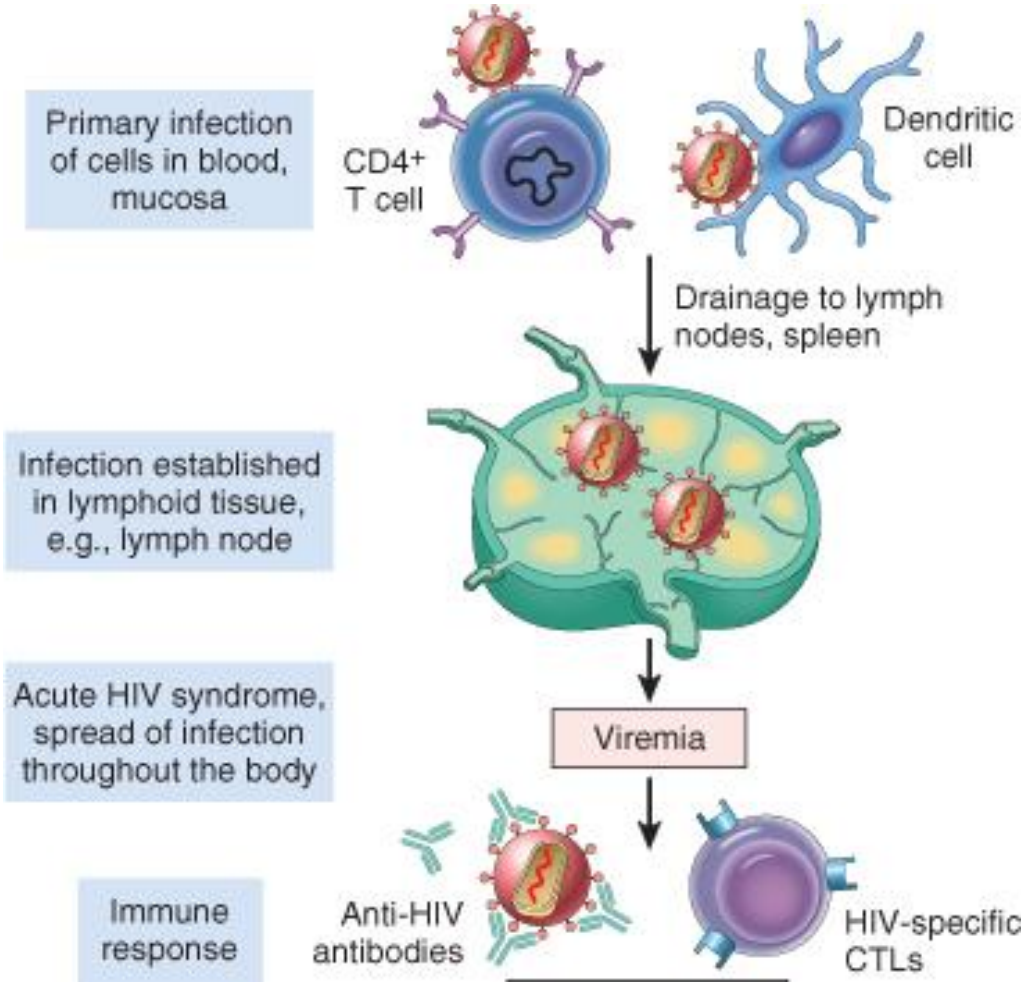
Role of CD4+ T Cells & Monocytes/Macrophages:





HIV/AIDS Global Impact / Infection

Viral Dissemination

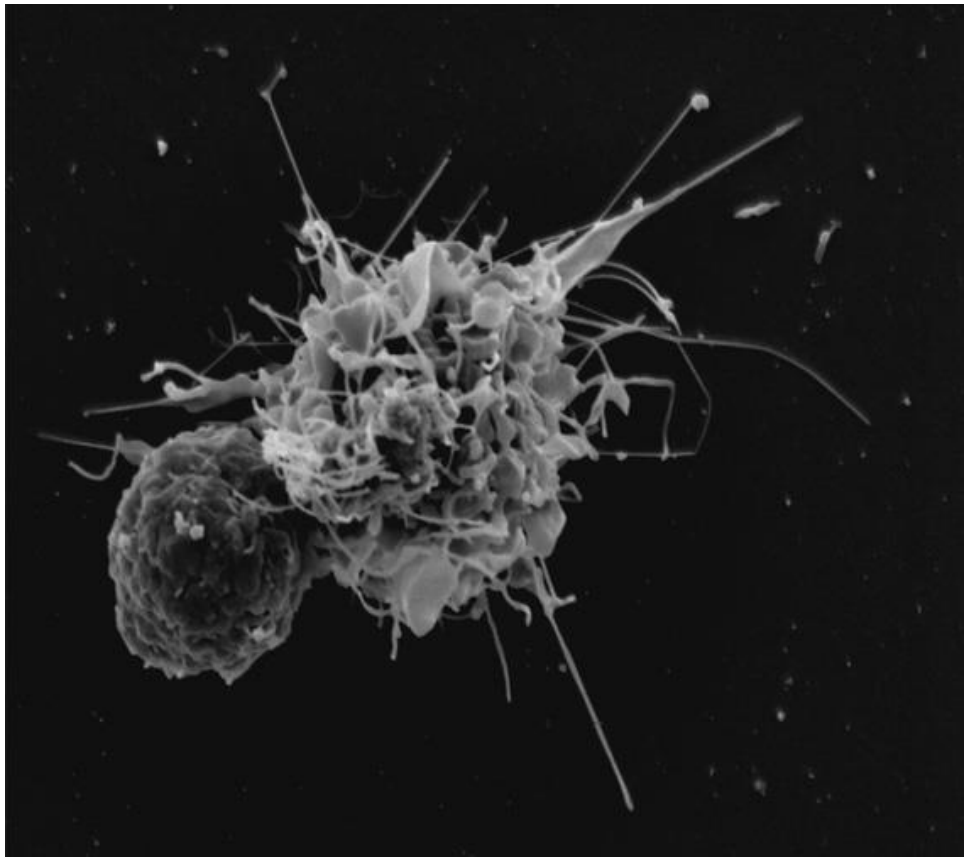


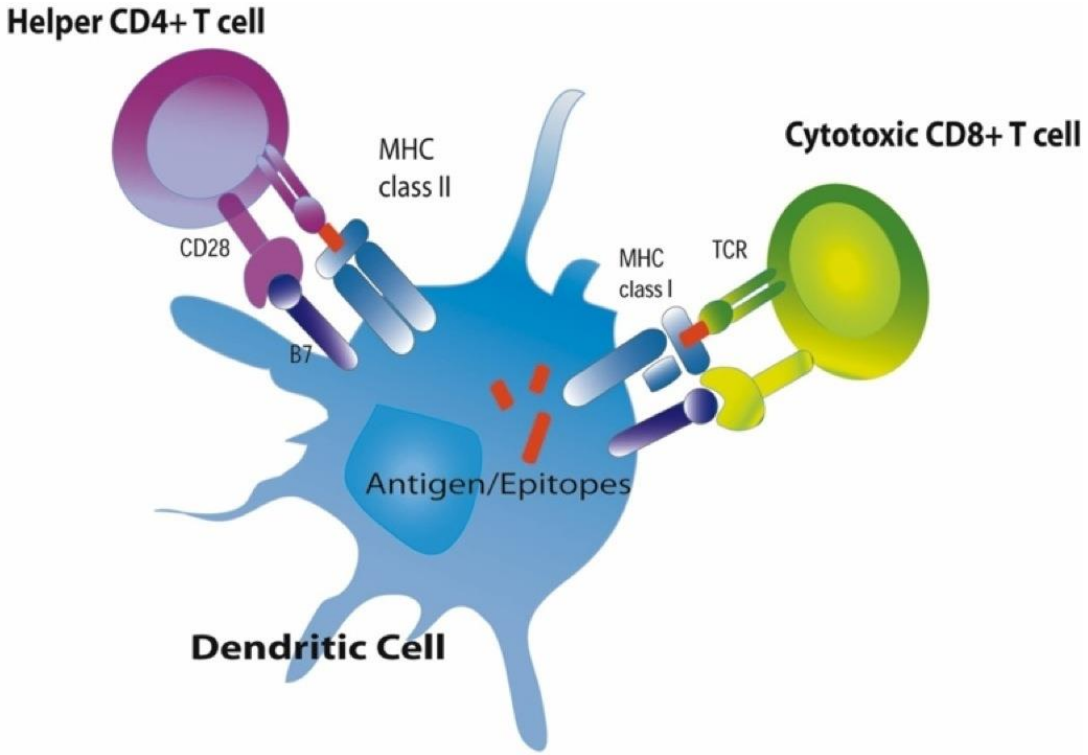
HIV/AIDS Global Impact / Infection

DC Mediated HIV-1 Transmission

DC

Dissemination



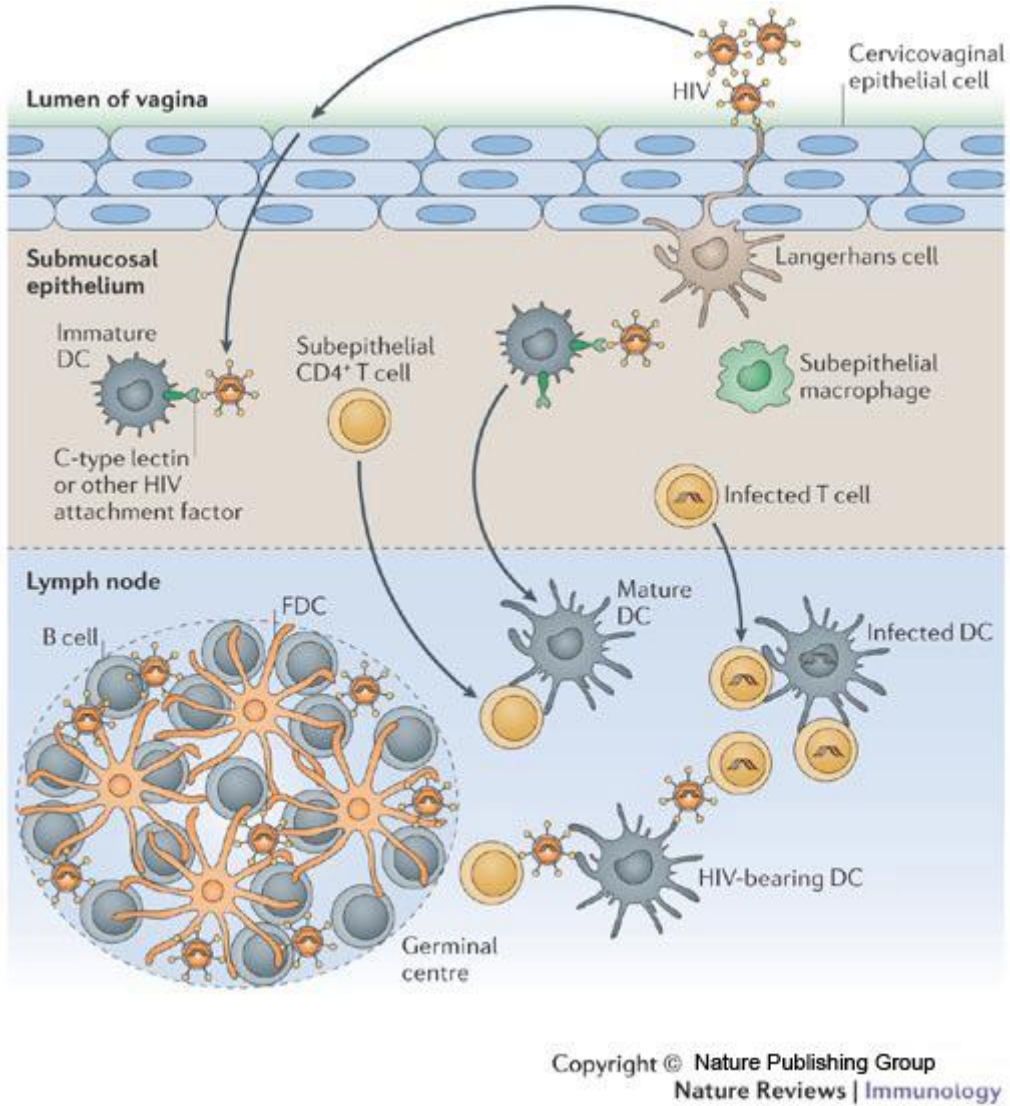


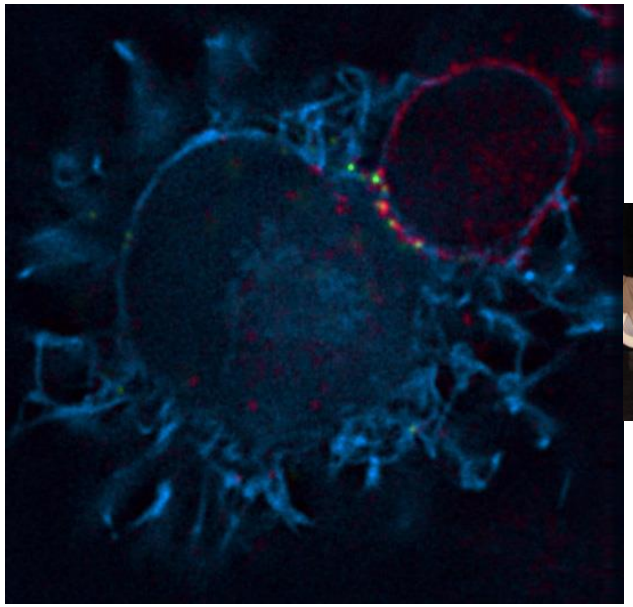
HIV/AIDS Global Impact / Infection

DC Mediated HIV-1 Transmission

DC

Dissemination





Causative Agent of AIDS - HIV

Human Immunodefiency Virus or HIV is a Retrovirus

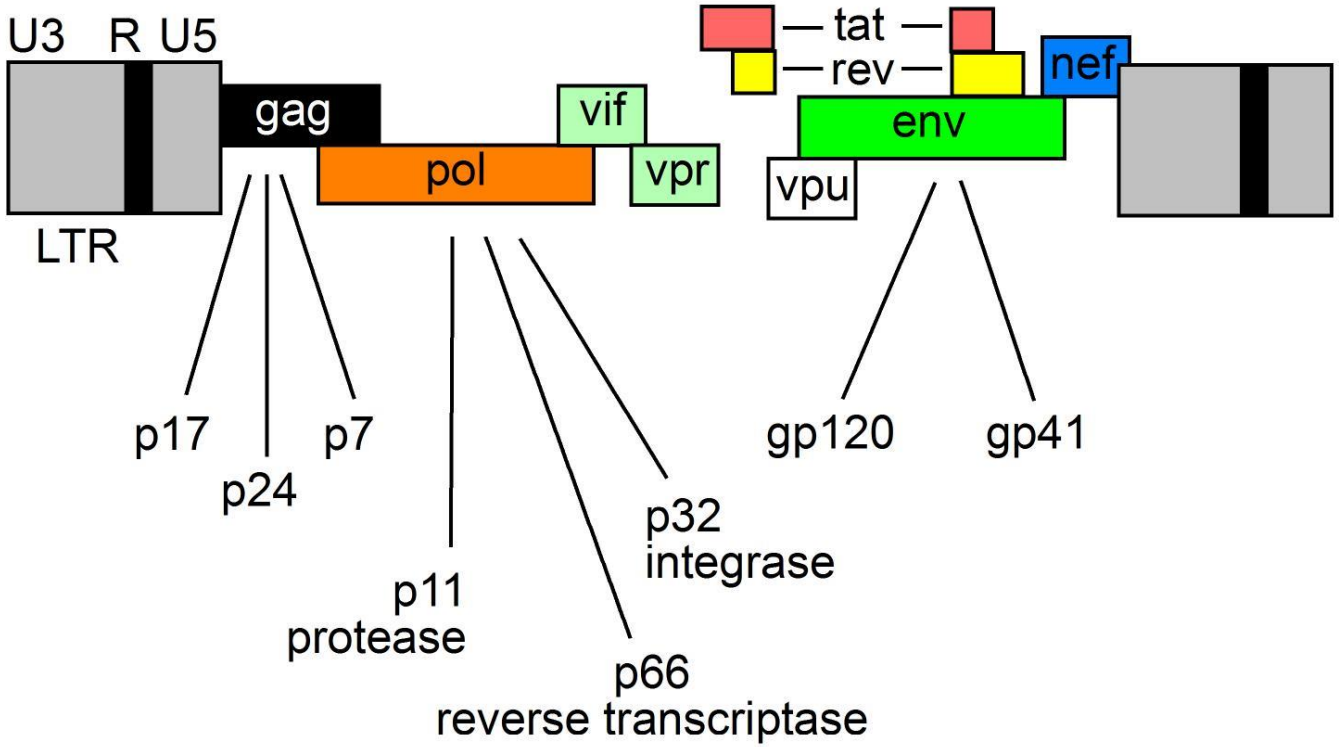
Retrovirus

- Unique Genome
- Host Dependent
- Encode for their own Enzymes
- Utilize Host Cell's Replication Machinery
- Genome consists of three major genes
  - GAG
    - group-specific antigen, capsid, matrix, and nucleic acid-binding proteins
  - POL
    - Polymerase, protease, and integrase
  - ENV
    - Envelope glycoproteins
  - At both ends of the genome are "long-terminal repeat (LTR) sequences.
    - Contain promoters, enhancers, and other gene sequences used for binding different cellular transcription factors.

Causative Agent of AIDS - HIV

HIV Genome

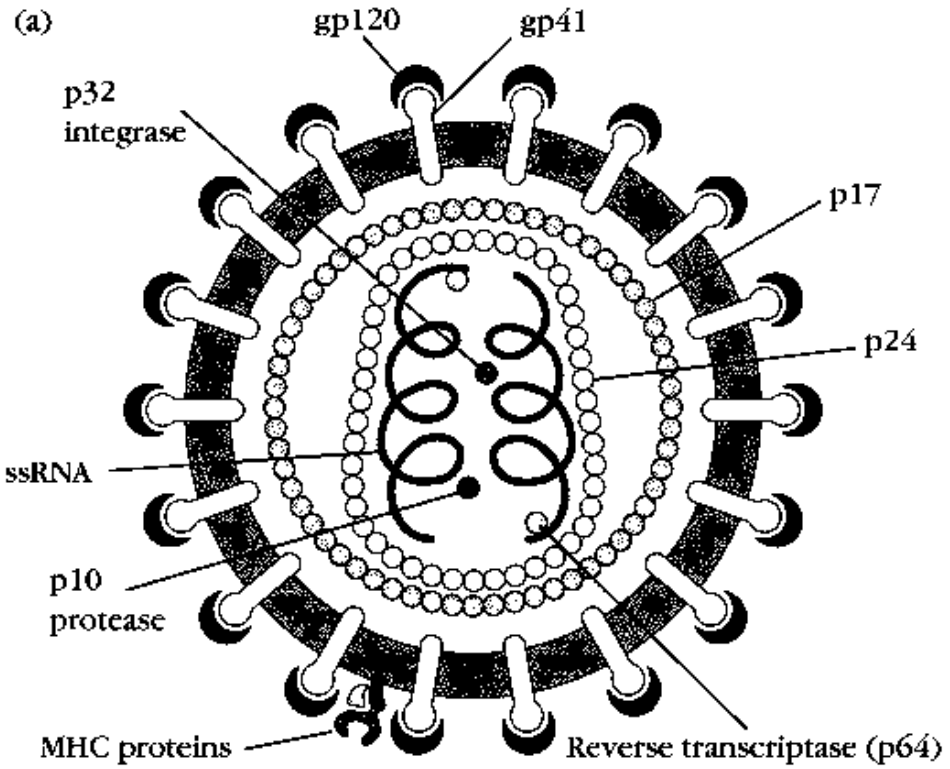
Accessory Proteins



Causative Agent of AIDS - HIV

HIV

- Diameter ~100 nm
- Enveloped – Host cell derived lipid bilayer
- The envelope contains viral glycoproteins
- Capsid (CA-p24)
  - Truncated Cone Shaped
    - Two copies of +RNA genome
      - Not directly infectious
    - Two copies of cellular transfer RNA (tRNA)
    - Genome codes of viral enzymes:
      - Reverse Transcriptase (RT)
      - Integrase (IN)
      - Protease (PT)

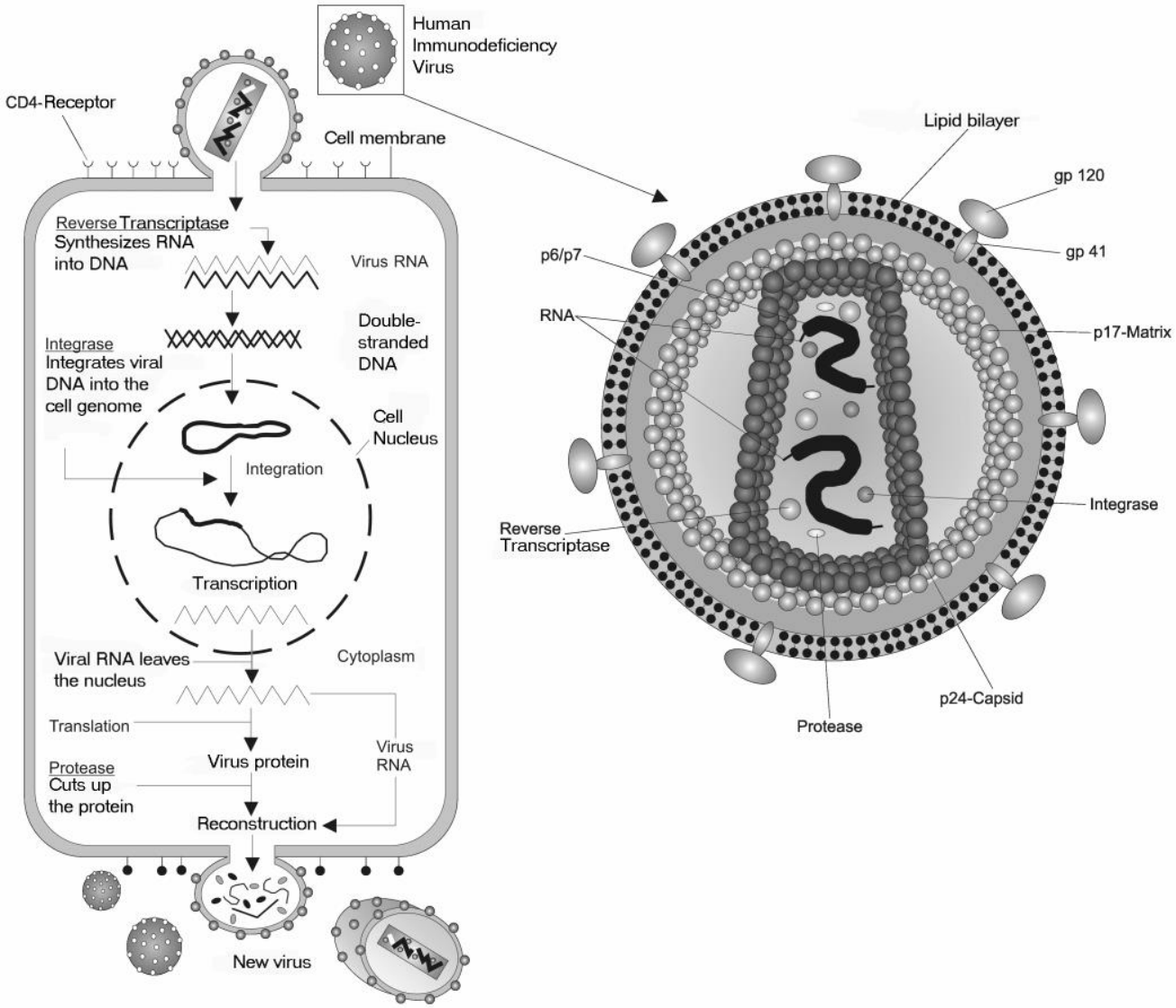


HIV – Life Cycle

v. load

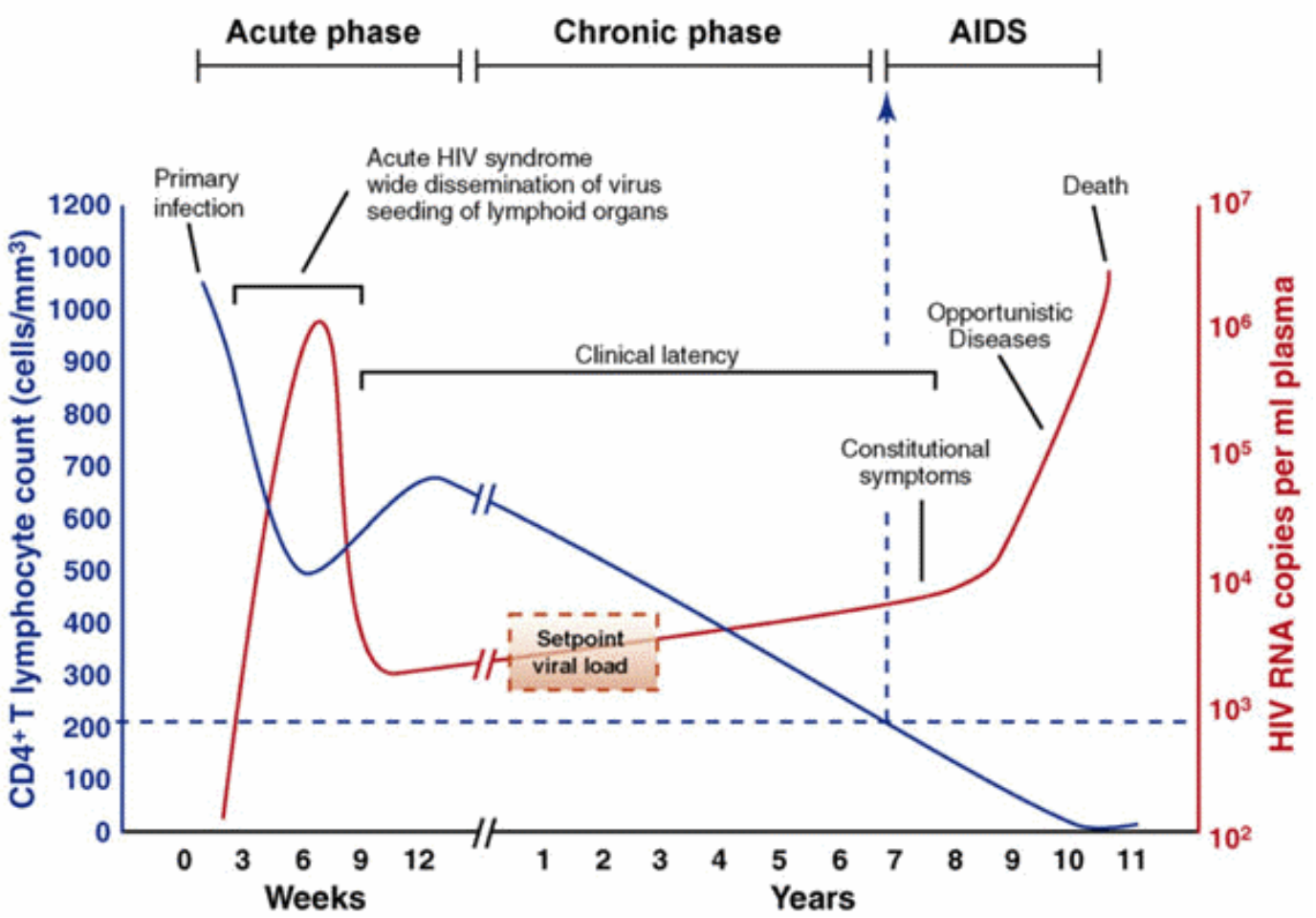
HIV-1 Surface Proteins

1) HIV Infection
2) Virology



HIV/AIDS Progression

Viral Load



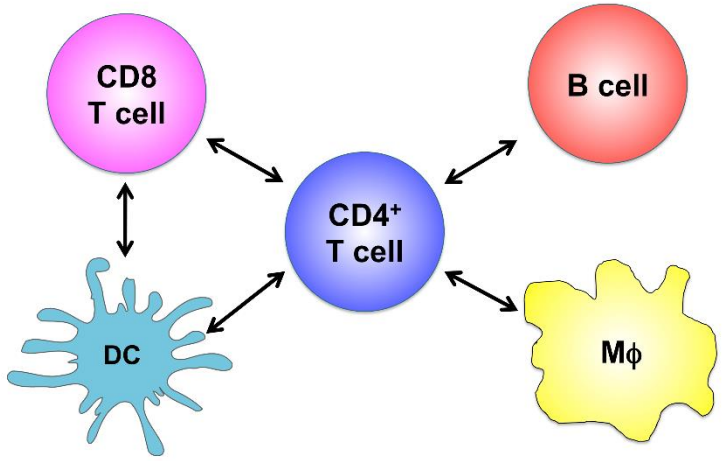
WHY CAN'T WE CURE AIDS?

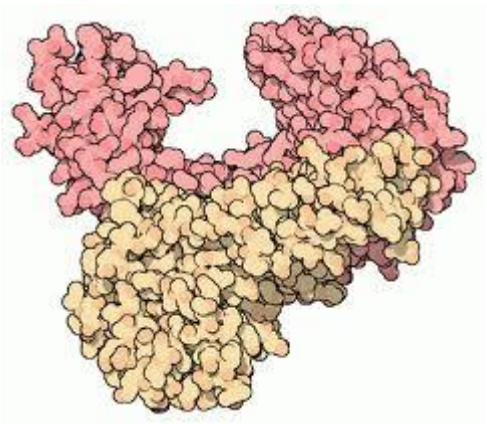
- HIV is able to evade the human immune system
  - HIV belongs to the Retroviruses group and to the Lentiviruses sub-group
    - Lentiviruses are known to have "latency" period – HIV can hide in the host cell's genome for a long time
- HIV posses an enzyme called Reverse Transcriptase, which does not have proof-reading capabilities
  - GP120 Genetic Drift
  - Therefore, every time it replicates the genome, it makes mistakes – variations
    - These variations are seen more in some parts of GP160 than others
      - For instance, the Variable Loops of GP120, are highly "dynamic" and unstable in terms of structural/biochemical consistency
- Glycosylation
- Limited Host Range of HIV-1 – Lack of model
- One can get infected with multiple strains of HIV at once!

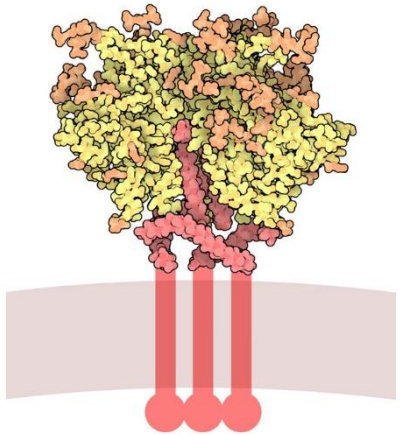
Variation due to Error-Prone Reverse Transcriptase

Evading the Human Immune System

Characteristic	Function
Infection of Lymphocytes and Macrophages	Inactivation of key element of immune defense
Inactivation of CD4 helper cells	Loss of activator of the immune system and delayed-type hypersensitivity
Antigenic drift of gp120	Evasion of antibody detection
Heavy glycosylation of gp120	Evasion of antibody detection



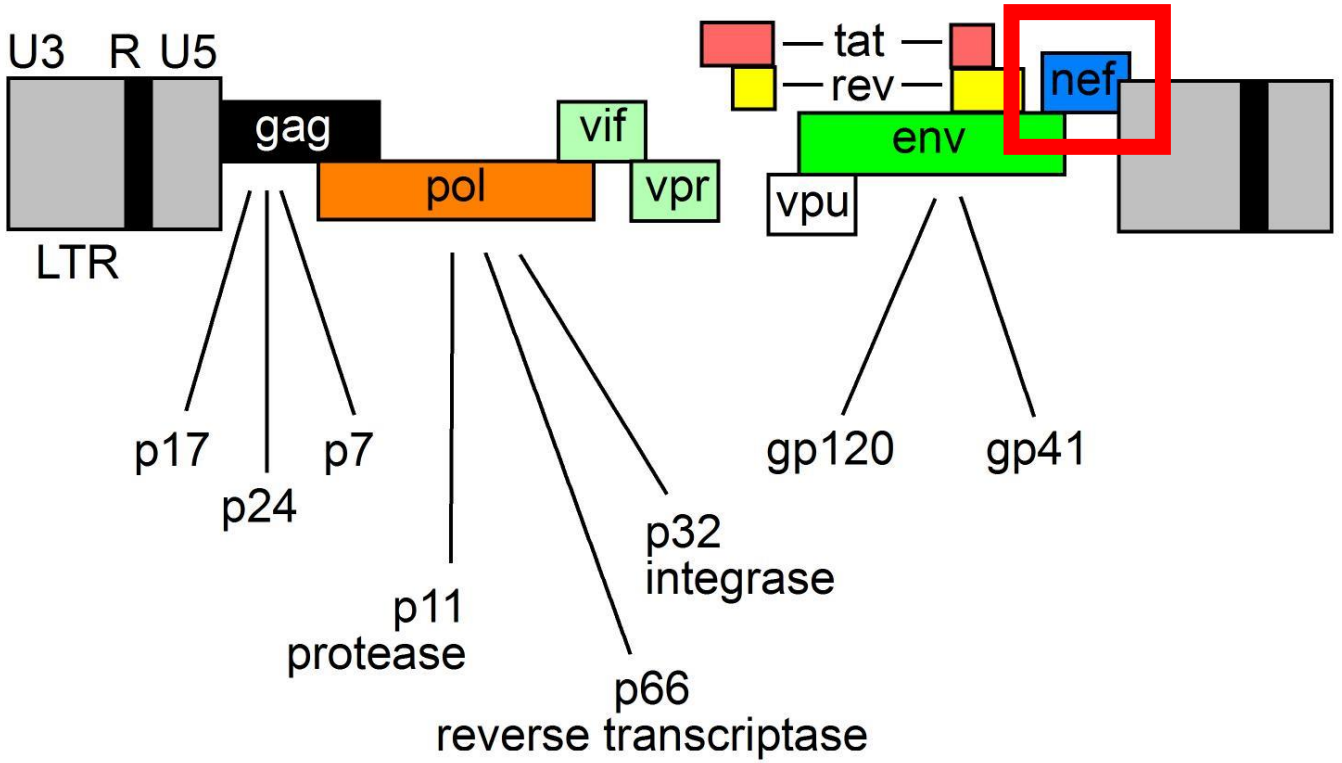




Causative Agent of AIDS - HIV

HIV Genome

Accessory Proteins



Causative Agent of AIDS - HIV

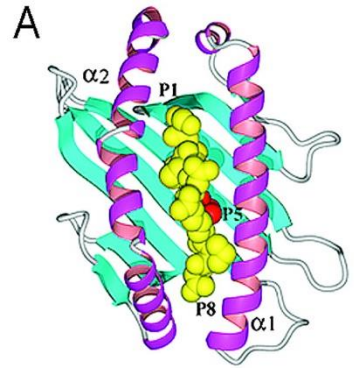
Limited host

MHC I & MHC II

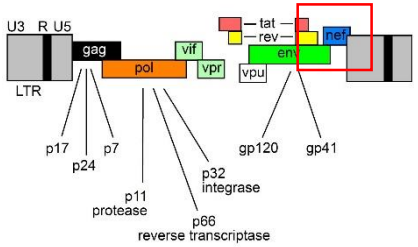
HIV nef –

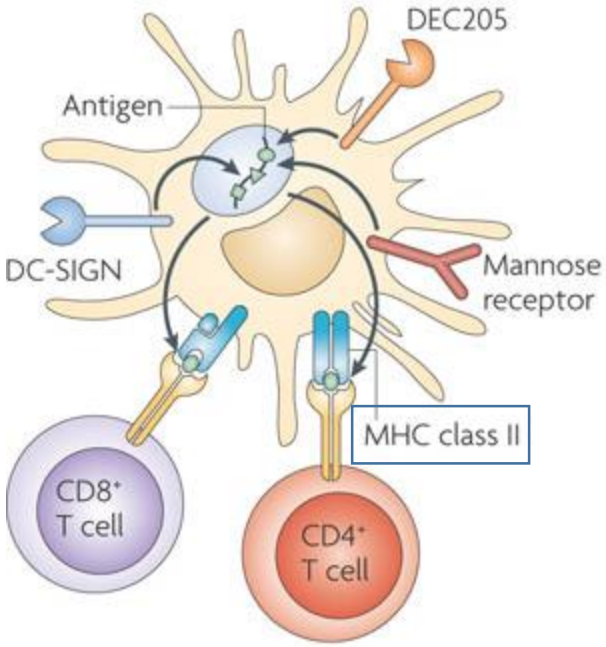
Reduction of cell surface CD4 and MHC I

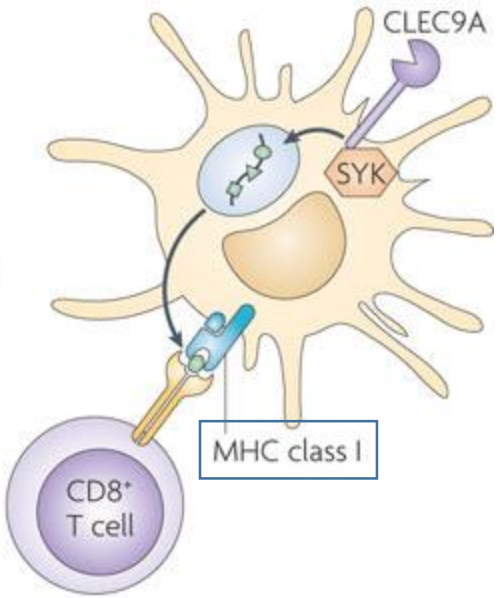
Primary function: alteration of cell activation signals; essential for progression to AIDS



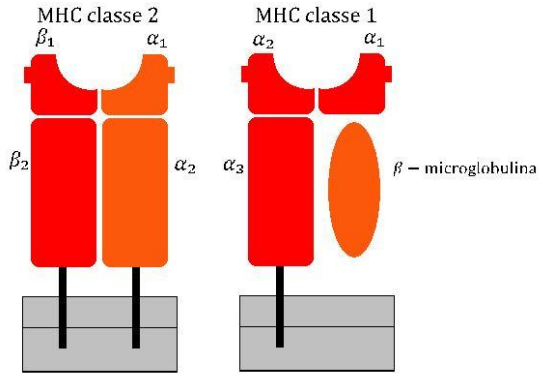
major histocompatibility complex
Or MHC







Nature Reviews | Immunology

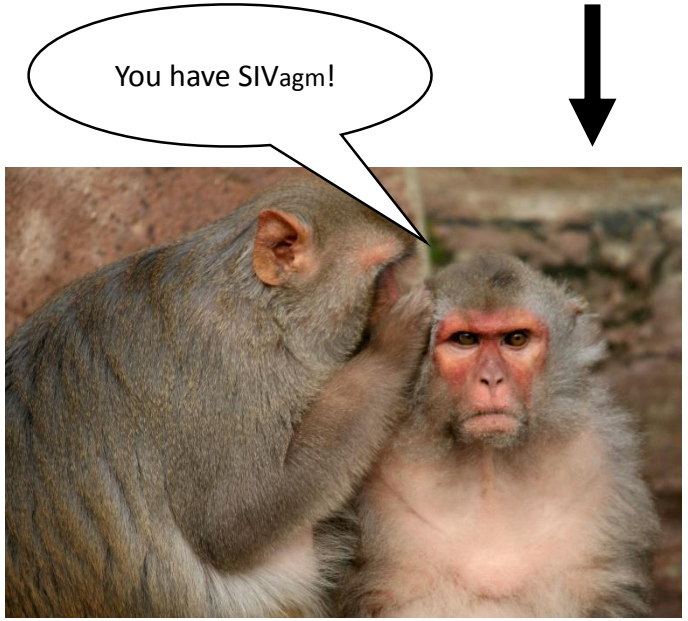


Causative Agent of AIDS – Vaccination/Drug

Lack Of Suitable Infection Model

<u>Viruses related to HIV-1:</u>

- Variants of Simian Immunodeficiency Virus, SIV – Cause no disease to normal host
    - Example:
        - **SIV$_{agm}$** – Carried by a high percentage of healthy African Green Monkeys
        - When injected into Macaques, causes severe immunodeficiency – often lethal.
    - Other animal retroviruses such as Feline and Bovine Immunodeficiency Virus, as well as the Mouse Leukemia virus only yield information concerning the general nature of retroviruses.
    - Only our closes relatives, chimpanzees support HIV-1 infection! (vaccination/drug?)
        - However they rarely develop AIDS

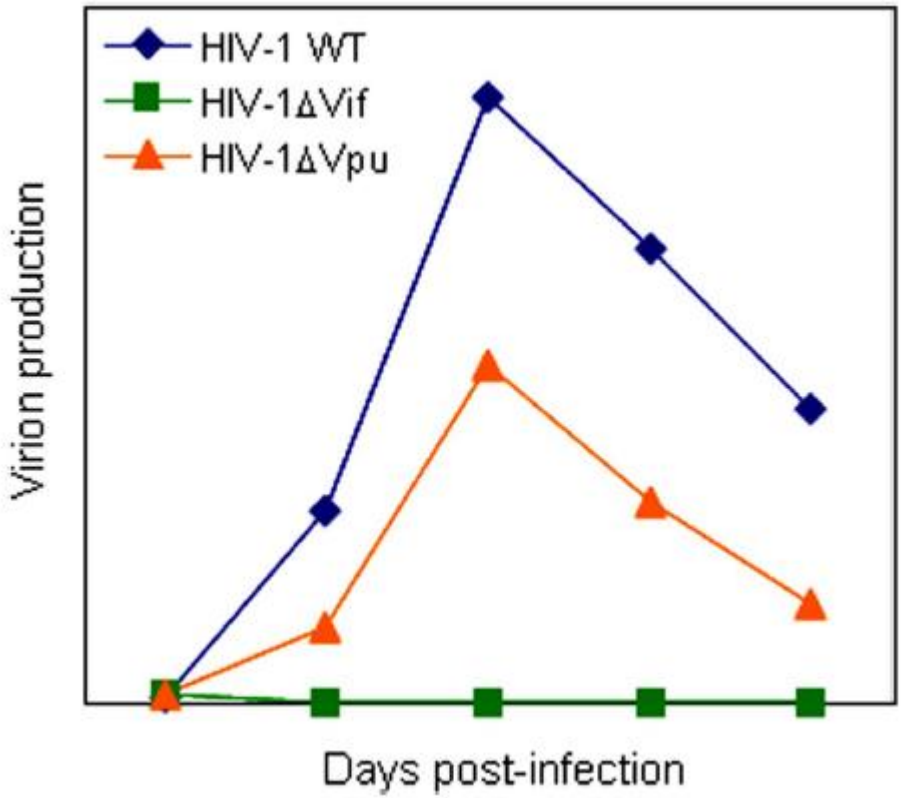


Reasons For Limited Host Range?

Causative Agent of AIDS – Limited Host Range

Lack Of Suitable Infection Model

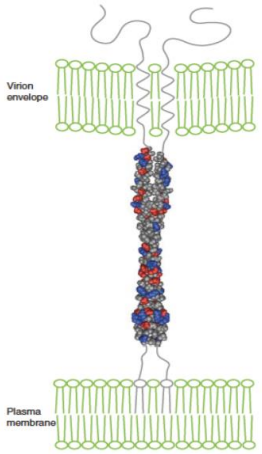
- Virion coded genomic proteins such as vif and vpu, and their interactions with host (humans) cell proteins are key!



Causative Agent of AIDS – Limited Host Range

Lack Of Suitable Infection Model

- Variation in host genes and cellular factors.
  - Human Tetherin and HIV-1 VPU



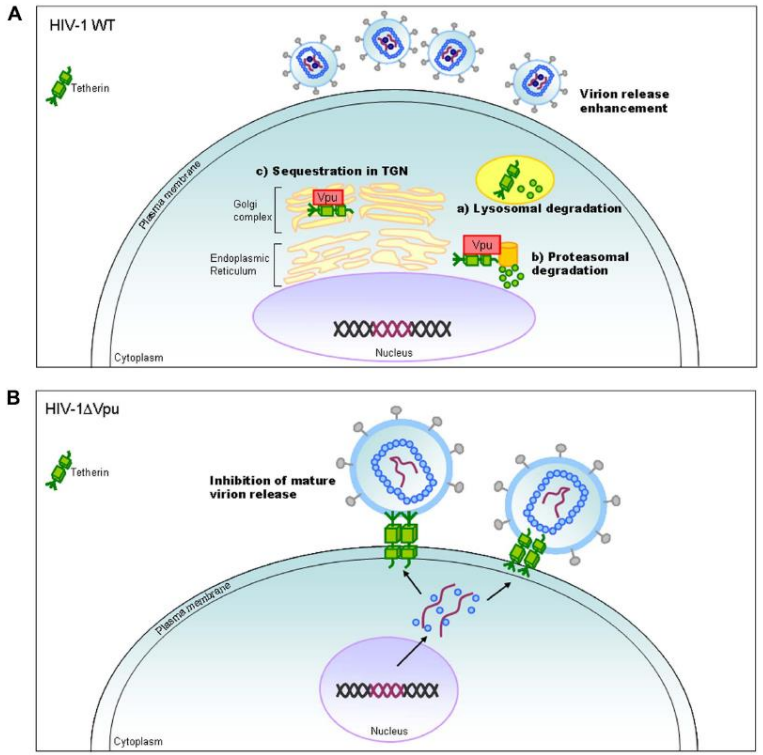
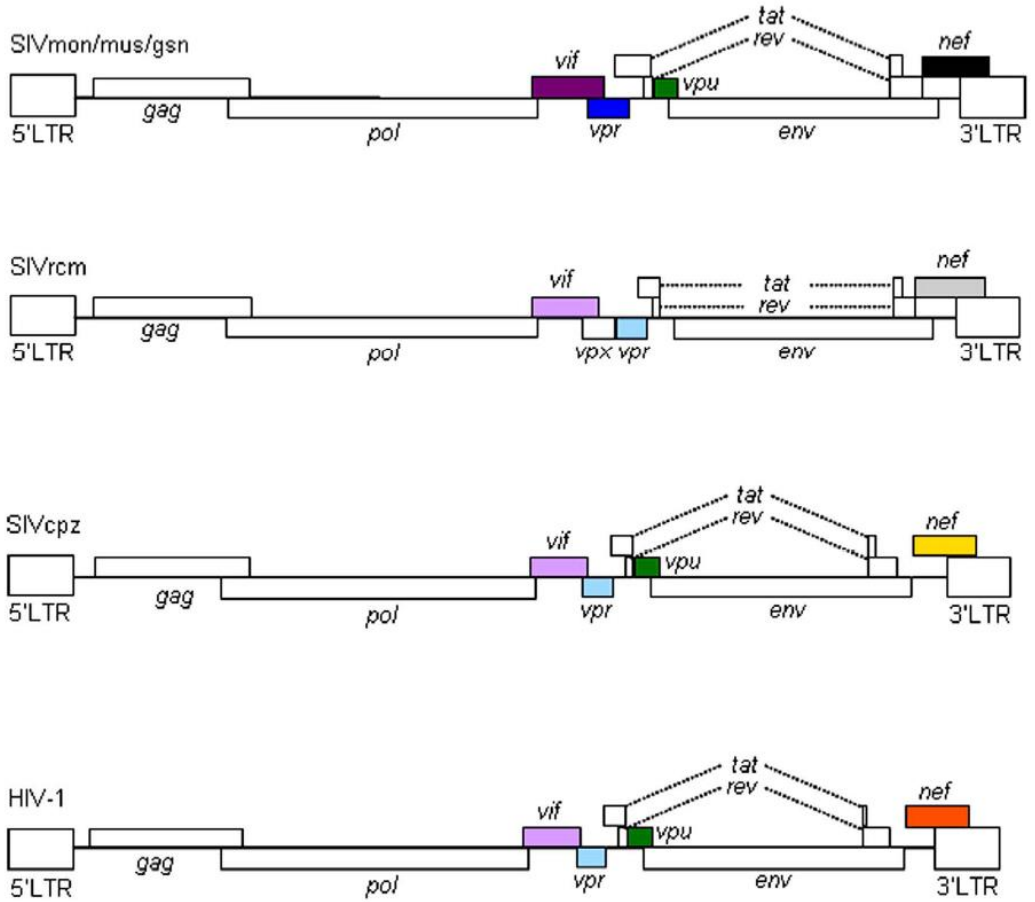


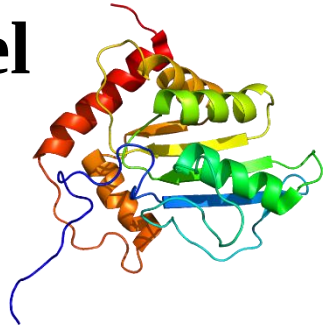
FIGURE 4 | HIV-1 replication and Tetherin. On the basis of results reported so far, the action mechanism of Vpu is depicted. Replication process for wild-type (WT) and ΔVpu mutant viruses are schematically shown on the basis of previously reported review articles (Tokarev et al., 2009; Douglas et al., 2010; Evans et al., 2010). TGN, trans-Golgi network.



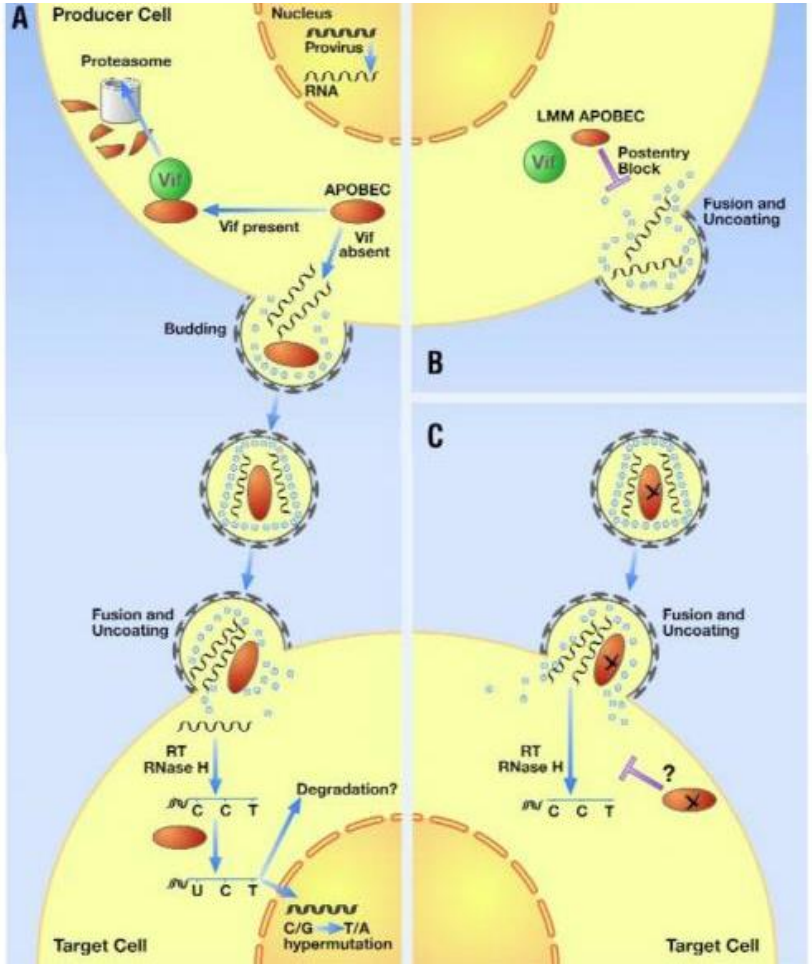
Causative Agent of AIDS – Limited Host Range

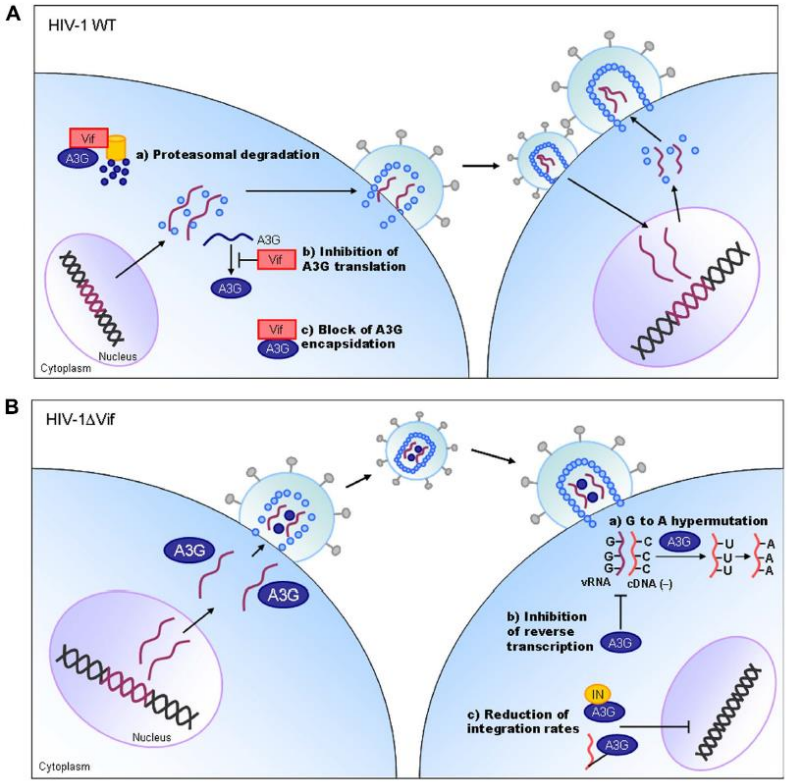
Lack Of Suitable Infection Model

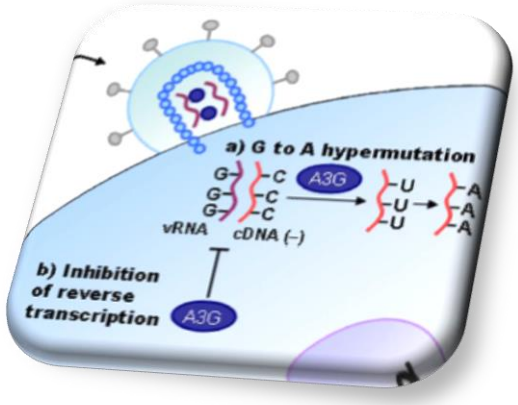
- Variation in host genes and cellular factors.
  - Human APOBEC3G (A3G) and HIV-1 VIF



Blocks viral replication in natural target cells
Causes a G → A hypermutation



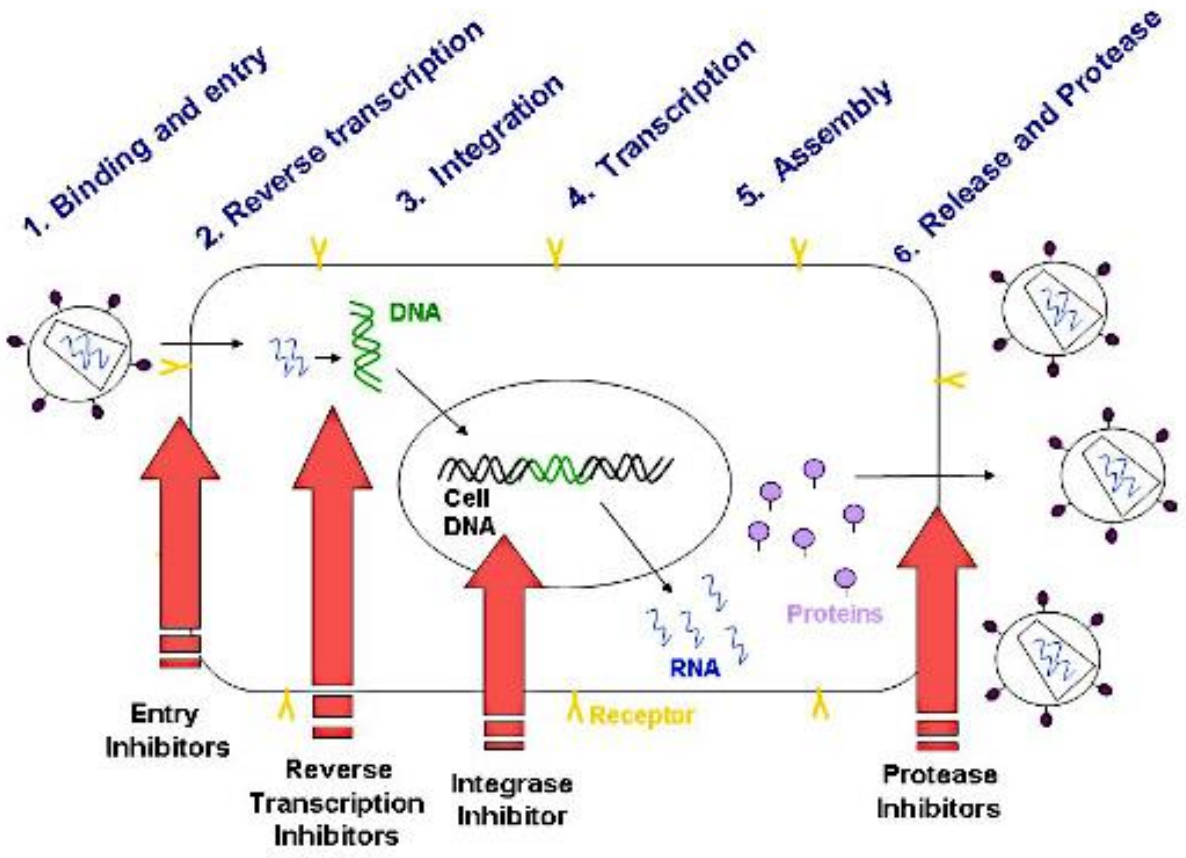




Causative Agent of AIDS – Lentiviruses

Human Immunodefiency Virus or HIV

Drug Targets



Many classes of drugs have been developed to attack HIV at different stages of its life cycle. Some examples (drug name is followed by its three-letter code and *trade name*):

Entry Inhibitors (EIs)

TNX 355	CD4 binding blocker
Maraviroc	chemokine receptor binding blocker
Enfuvirtide (ENF, *Fuzeon*)	gp41 zipping inhibitor

Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

Tenofovir (TDF, *Viread*)	adenosine monophosphate (A) analog

Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Zidovudine (AZT, *Retrovir*)	thymidine (T) analog
Lamivudine (3TC, *Epivir*)	cytidine (C) analog
Emtricitabine (FTC, *Emtriva*)	cytidine (C) analog
Abacavir (ABC, *Ziagen*)	guanine (G) analog
Didanosine (ddI, *Videx*)	adenosine (A) analog

Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Efavirenz (EFV, *Sustiva*)
Nevirapine (NVP, *Viramune*)

Integrase Inhibitors

Raltegravir (RGV, *Isentress*)
Elvitegravir

Protease Inhibitors (PIs)

Lopinavir (LPV) — given with ritonavir as *Kaletra*
Ritonavir (RTV, *Norvir*)
Fosamprenavir (908, *Lexiva*)
Atazanavir (ATV, *Reyataz*)

Reduction of Disease Severity and Progression

Anti-retrovirus Drugs

- Complications:
  - Resistance
  - Toxicity
  - Side Effects
  - Financial Aspect

Natural Selection!
HIV = evolutionary masterpiece

High replication rate!
Higher mutation rate!
Resistance build up to antiviral drugs have been linked to the error prone RT enzyme encoded by viral genome

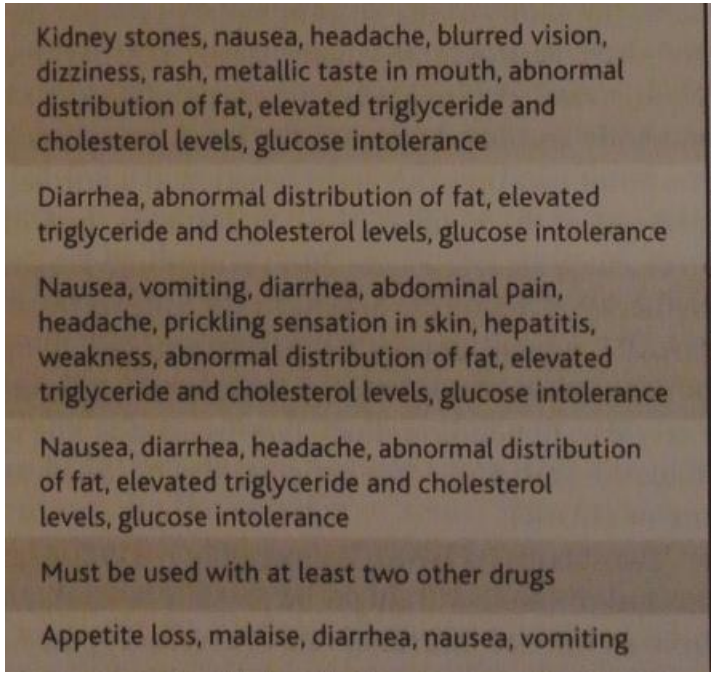
Overall, 80% of participants had at least one major resistance mutation to at least one drug from the three major classes of antiretrovirals – nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). Specifically, two-thirds carried resistance to an NRTI, 50% to an NNRTI and 33% to a protease inhibitor.

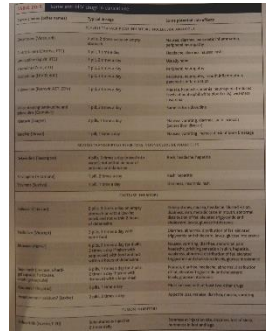
De Luca A et al. *Declining prevalence of HIV-1 drug resistance in antiretroviral treatment-exposed individuals in Western Europe*. J Infect Dis, online edition, 2013.

Accumulation of chemicals in patient's body

Toxic Buildup From ART – Killed by what's saving you…

Mitochondrial Toxicity
Pancreatitis
Peripheral Neuropathy
Lactic Acidosis
Metabolic Complications such as:
Dyslipidemia or Dysglycemia
Bone Metabolism and Lipodystrophy

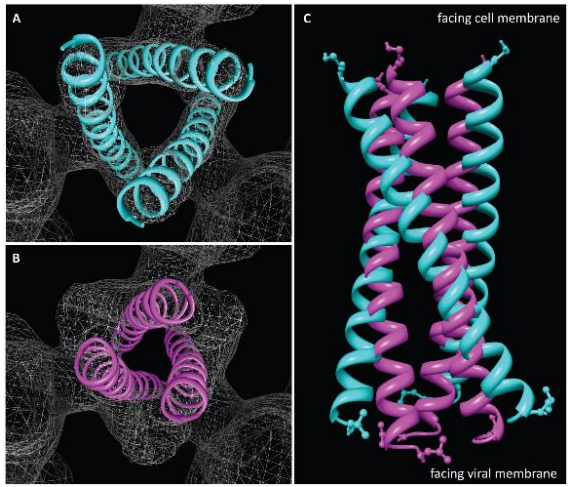


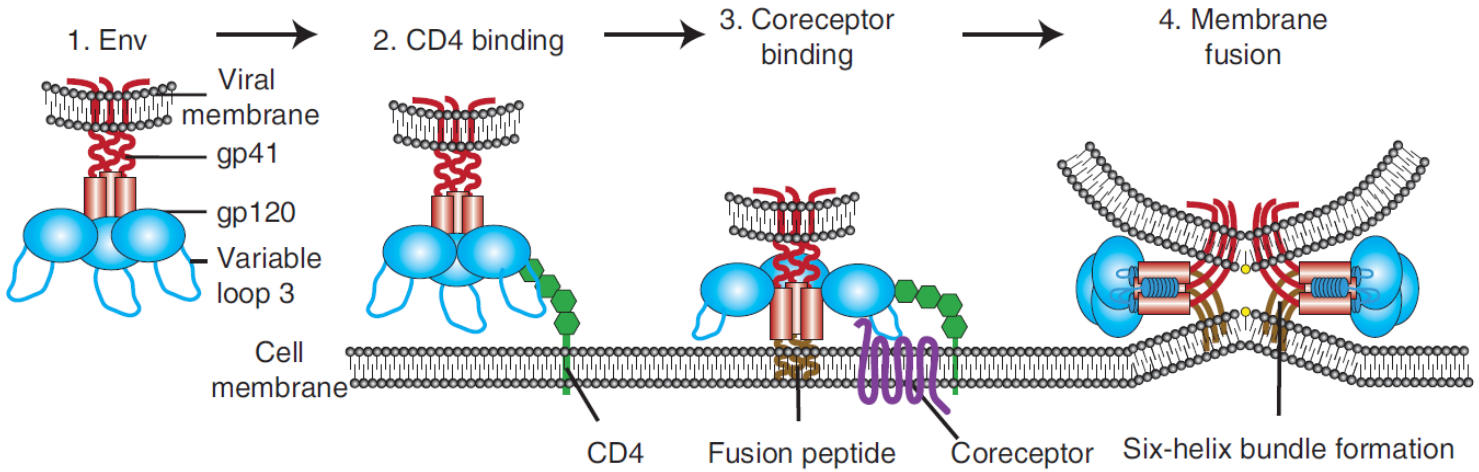


Anti-HIV-1 Drug T20

T20 Blocks Viral Entry

- GP41
  - Extra cellular domain contains Fusion Peptide (FP) and 2 helical regions –
    - FP is highly hydrophobic and glycine rich
      - Post FP activity – HR1 and HR2 undergo conformational → 6-helix bundle → membrane fusion



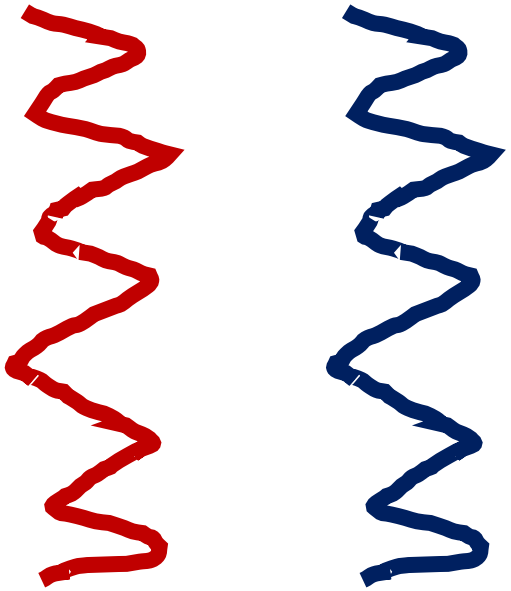


Overview of viral entry mechanism

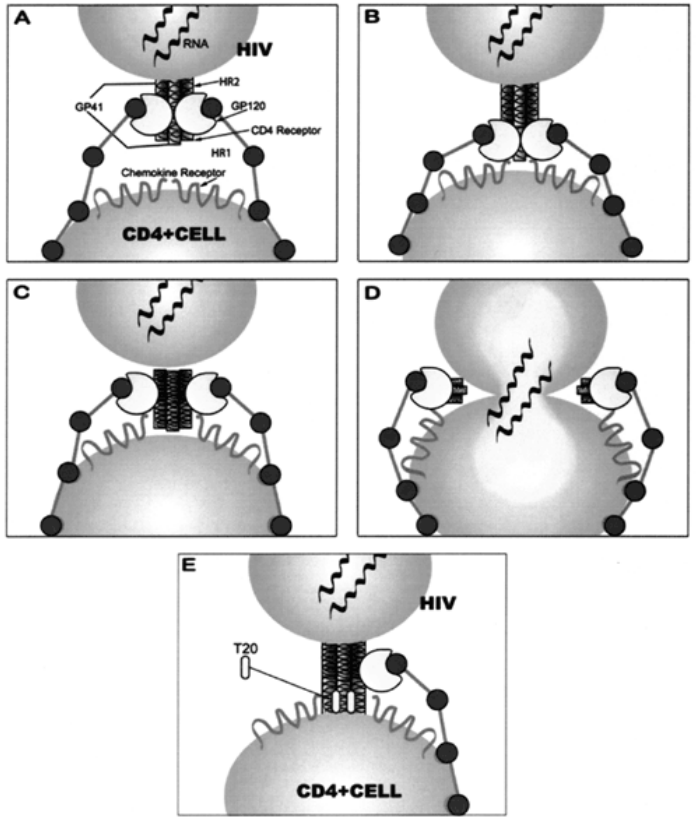
Anti-HIV-1 Drug T20

T20 Blocks Viral Entry

- T20 or ENF
  - 36 AA Seq. corresponding HIR2 domain in GP41
  - Furthermore, T20 binds the HR1 domain of GP41 to prevent the formation of 6-helix bundle – impeding the translation of GP41 into a fusion active state.



Blocking Fusion
By clocking the formation
of FP formation



Have we eradicated the virus from the human body? No more AIDS?

Causative Agent of AIDS – Immunogen Design

The Focus On HIV-1 Env

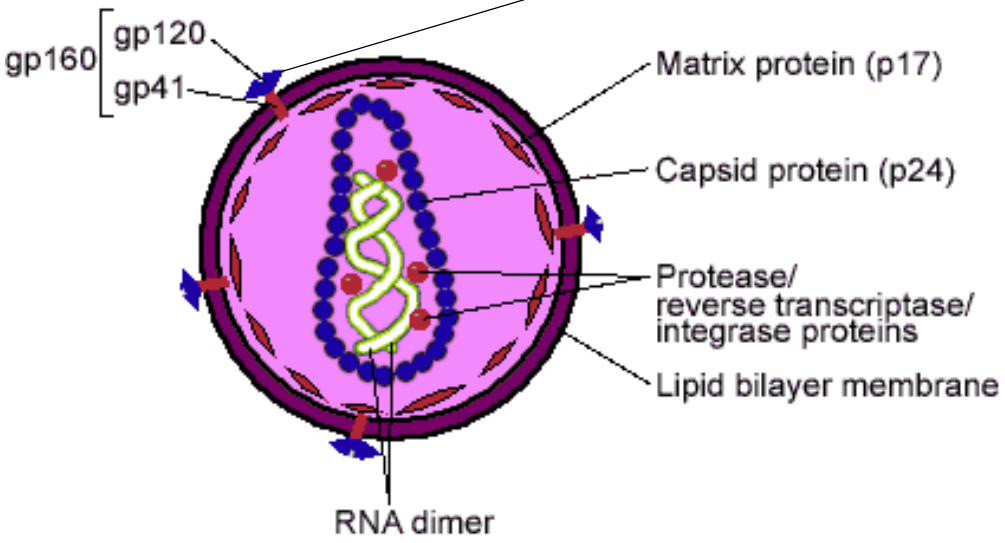
Revealing Structural Details

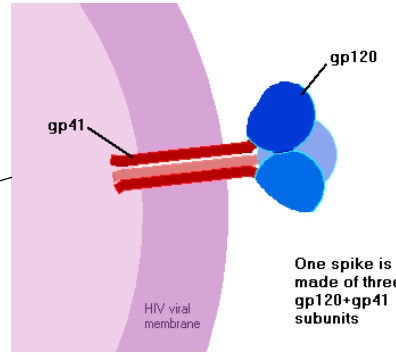
Crucial For Drug/Vaccine Design

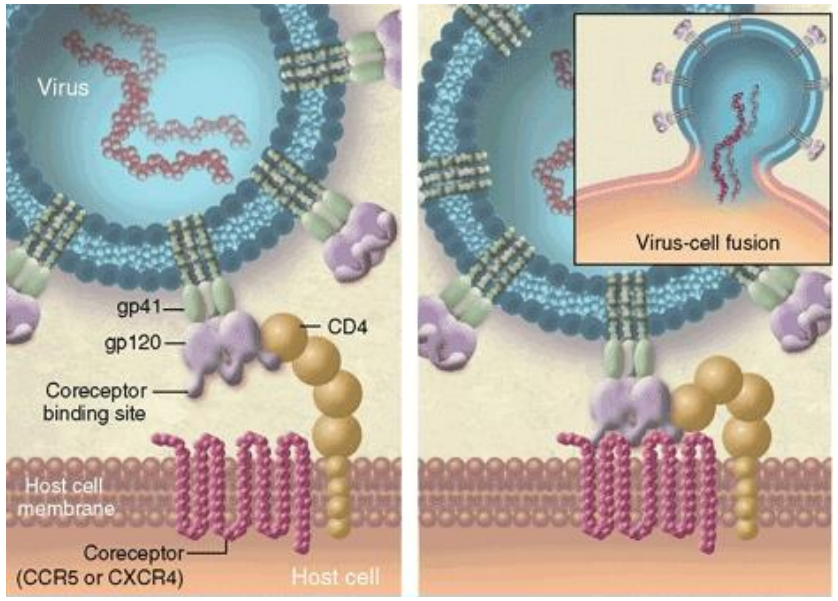
Bio-Medical Image Analysis – Background

HIV-1 Surface Proteins

1) HIV Env – Viral Entry
2) Structural Variability
3) Vaccine Development





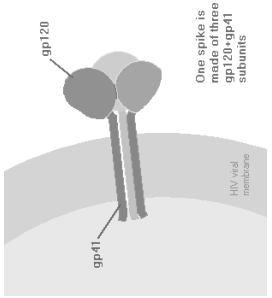


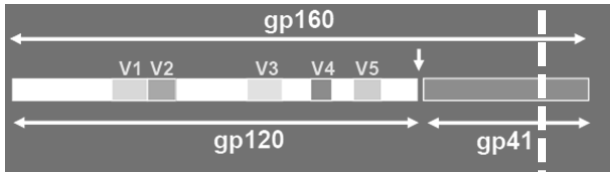
Host CD4+ T-Lymphocytes

Characterization of Env – GP140 as an Immunogen!

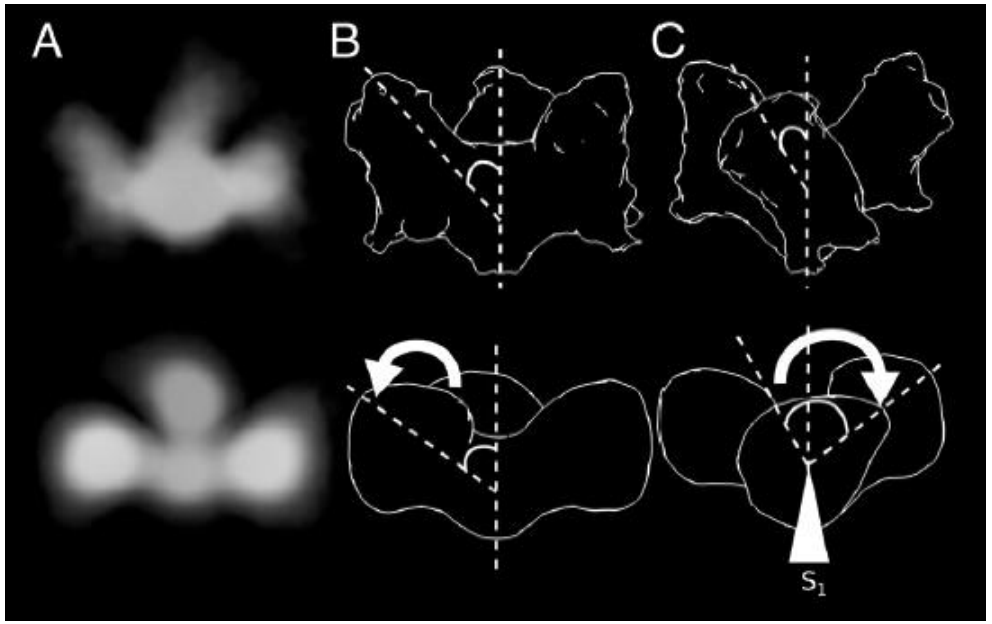
HIV-1 Surface Proteins

1) HIV Env – Viral Entry
2) Structural Variability
3) Vaccine Development





GP140 a soluble form of GP160, with truncated trans-membrane domain



Moscoso et al. 2010

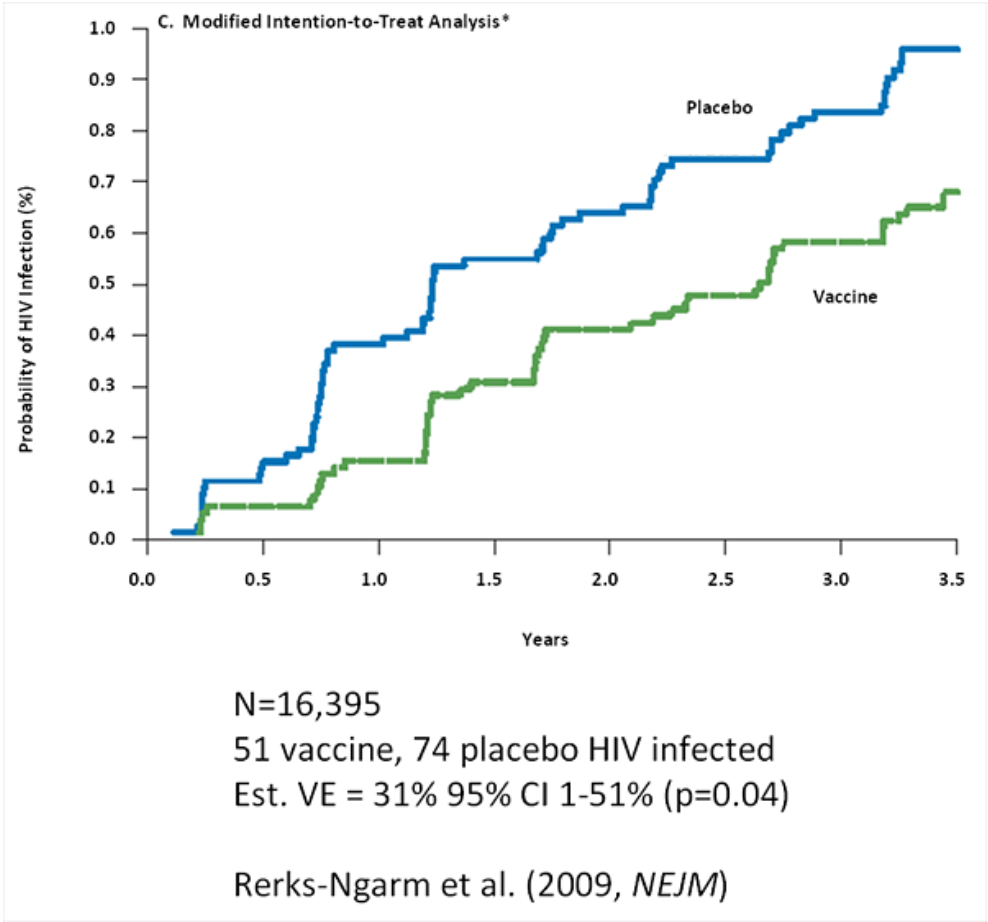
Immunogen Design – GP120

Env Derived Immunogen

- GP120 – Monomeric – Vaccine Candidate RV144
  - With efficacy of 31%
  - Failure to induce bNAbs

Failure?

- GP140 – trimeric Immunogen
  - Induces bNAbs
  - Debate on cleavage site presence between gp41 and gp120 subunits.
  - Controversial deletion of V2



N=16,395
51 vaccine, 74 placebo HIV infected
Est. VE = 31% 95% CI 1-51% (p=0.04)

Rerks-Ngarm et al. (2009, *NEJM*)

Immunogen Design – Immune System Evasion

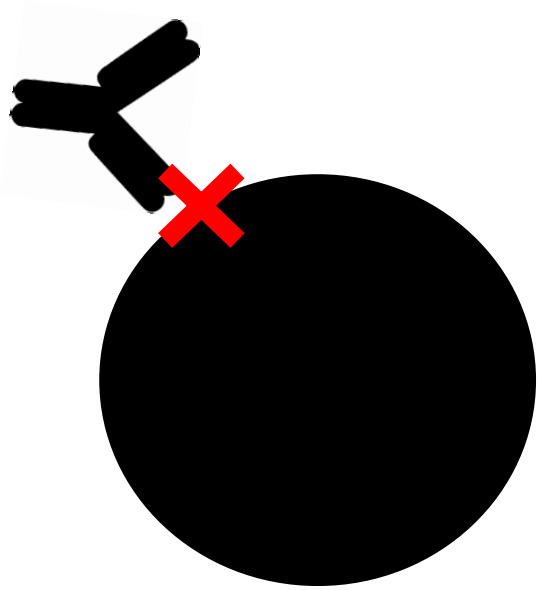
Formidable Barrier to Ab Efficacy

Major Consideration - Env Derived Immunogen

Enforcing Nano-imaging & Structural Studies

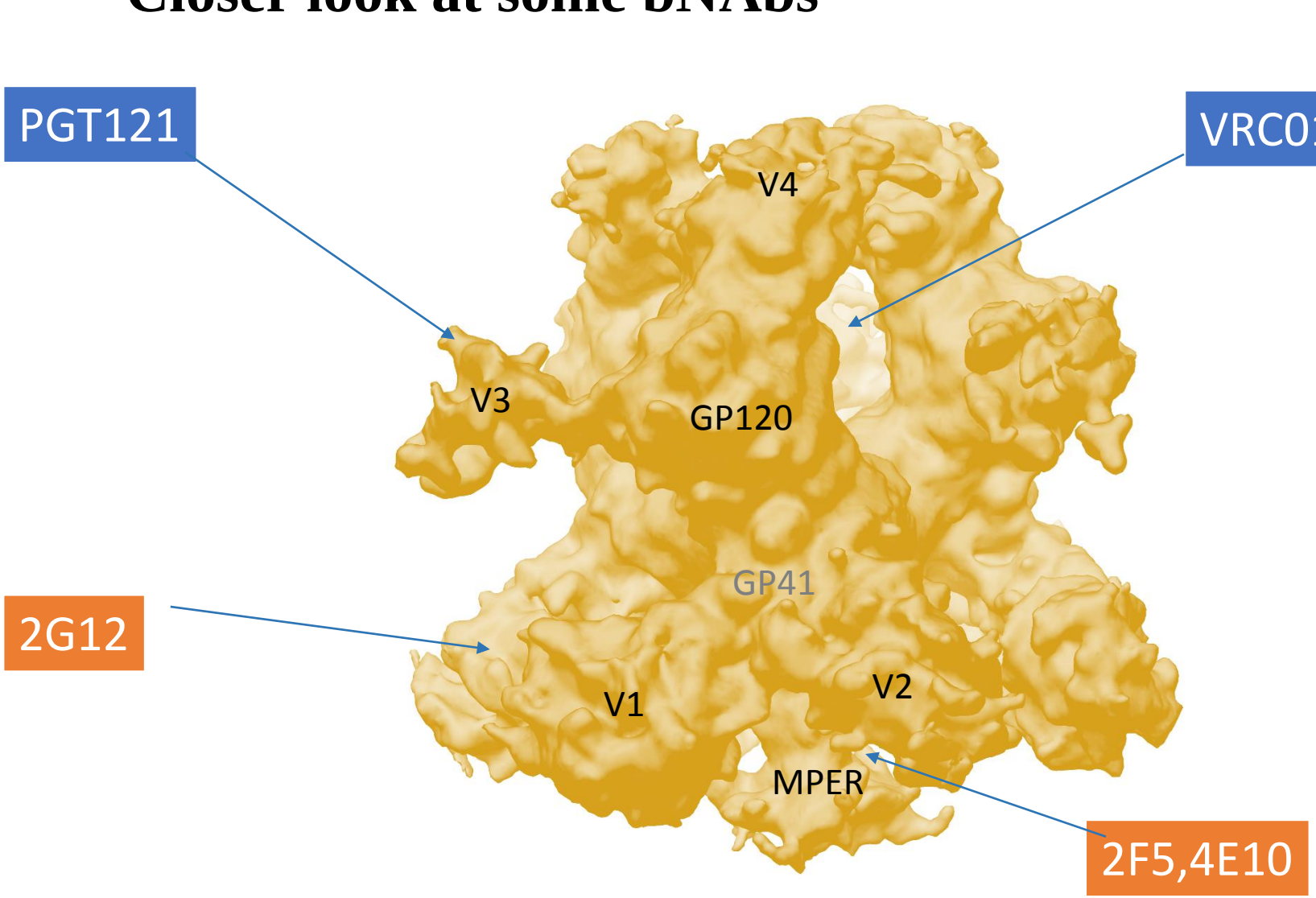
Abrogation of the impact of bNAbs

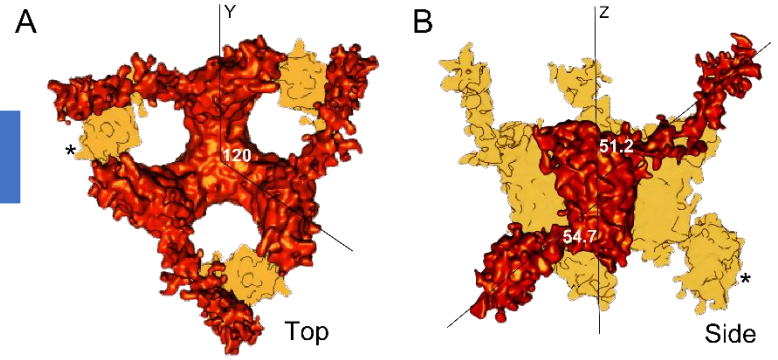
- **AA Seq. Variation**
  - V1,V2,V3
  - Error-prone RT
- **Glycosylation**
  - The “Fence” – “Viral Defense”!?
- **Steric Exclusion**
  - CD4BS
    - Example: Common Cold Picornavirus
  - CRBS
  - MPER
- **Metastability and Adaptation**
  - Native, CD4B, CRB, and Intermediate
    - Example: Dissociation of GP120
- **Heteroligation of B-cells**
  - Leading to the dearth of high avidity antibody production



Immunogen Design

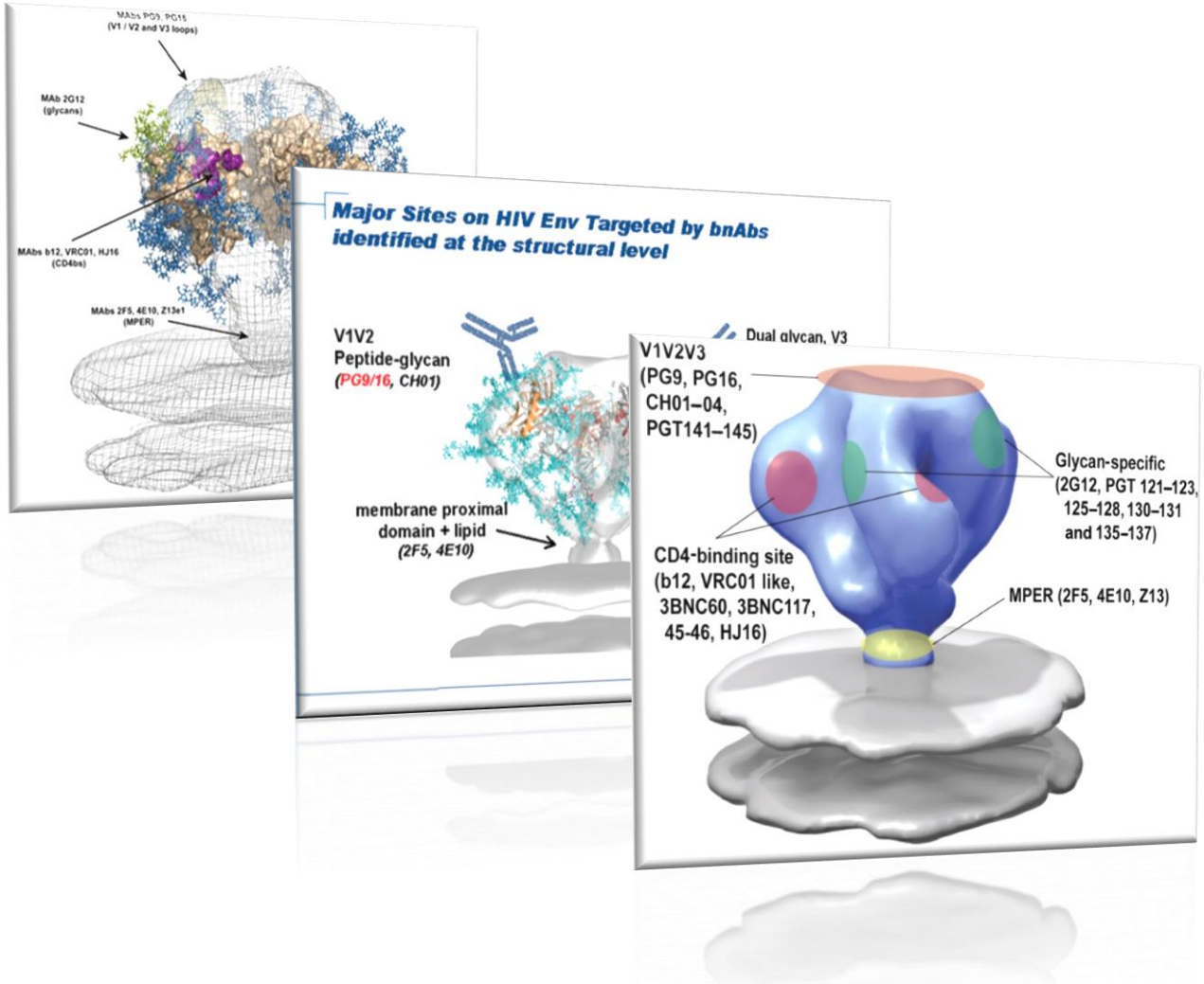
Closer look at some bNAbs

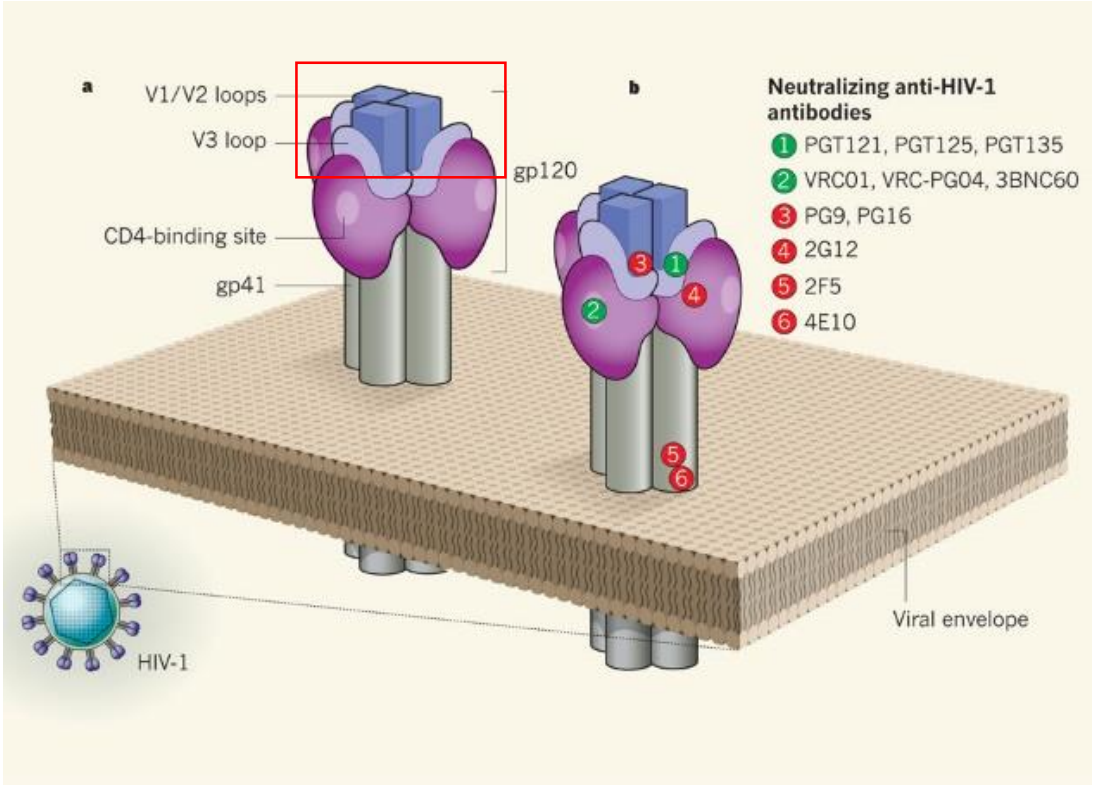




Immunogen Design

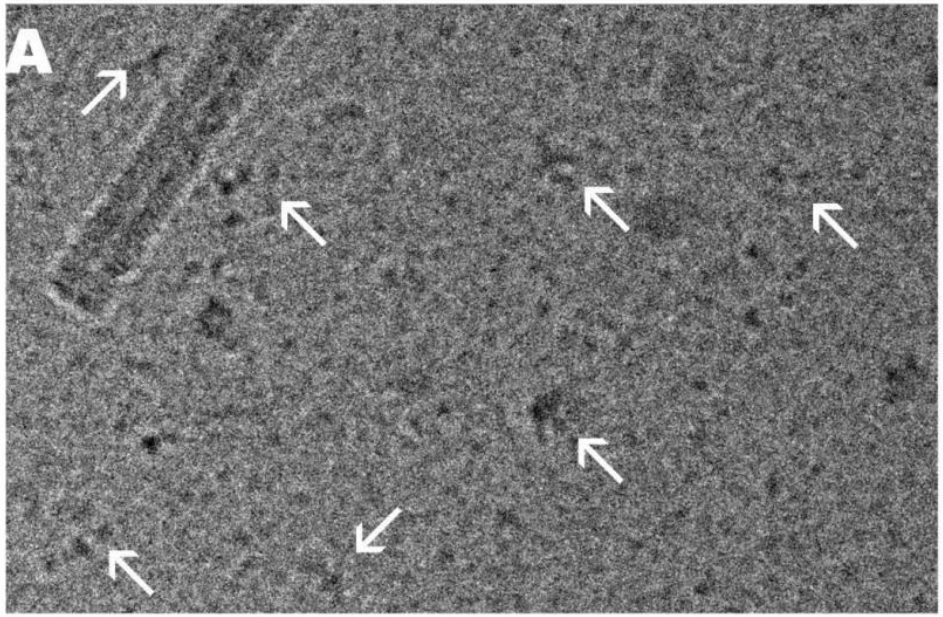
Locations Of Variable Loops!

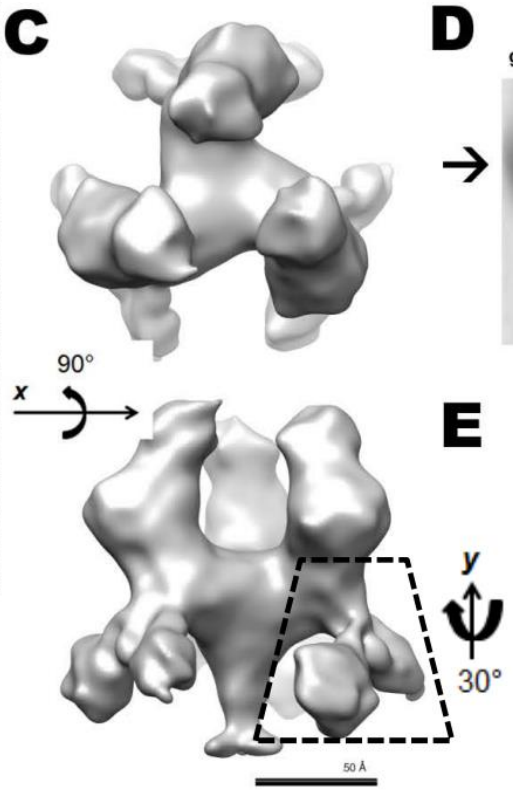


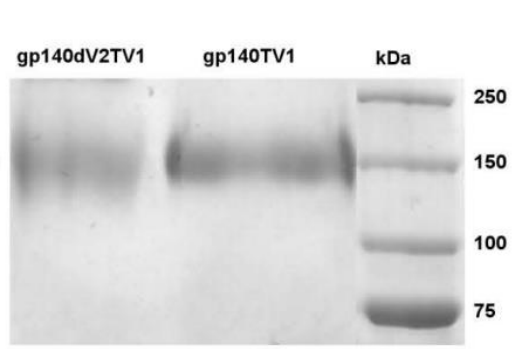


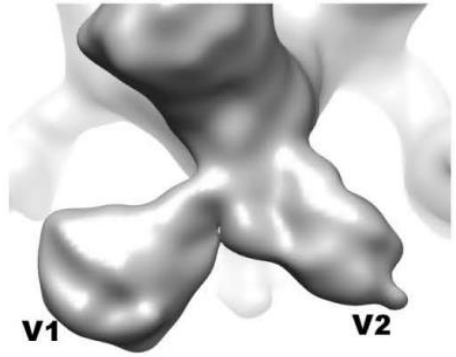
Immunogen Design / Structural Biology

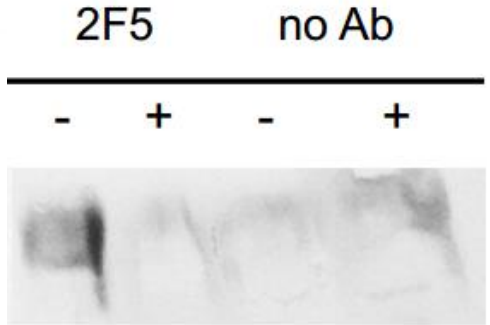
4° location of variable loop 2





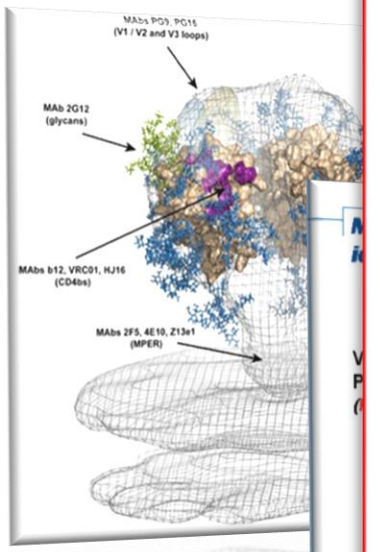


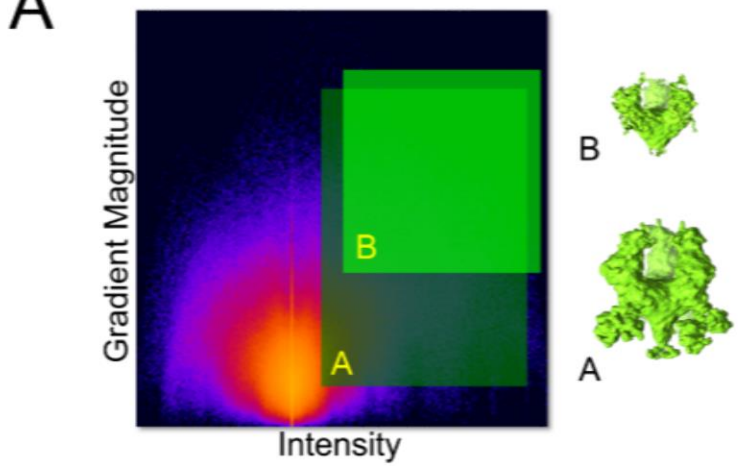


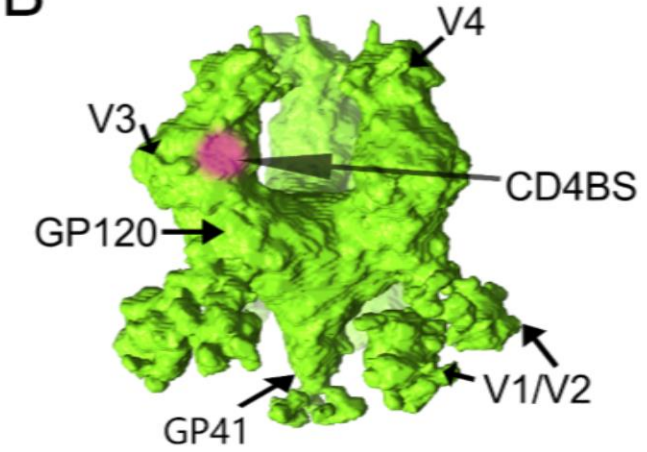


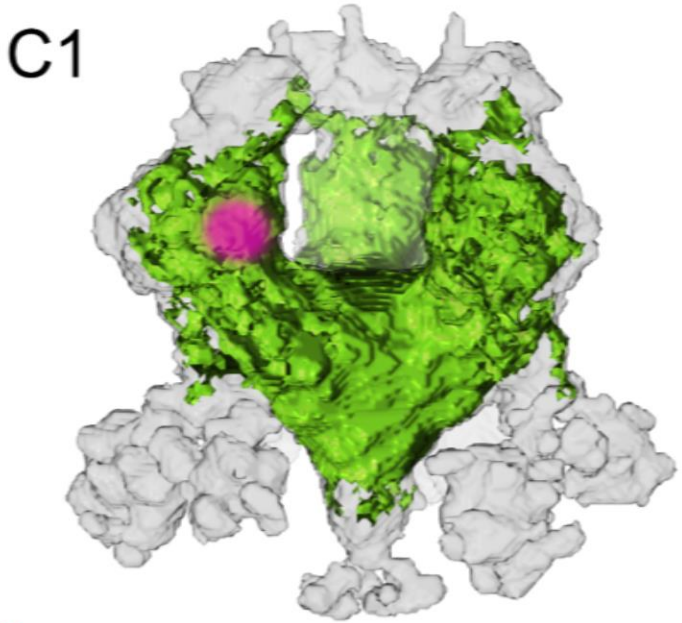
Immunogen Design

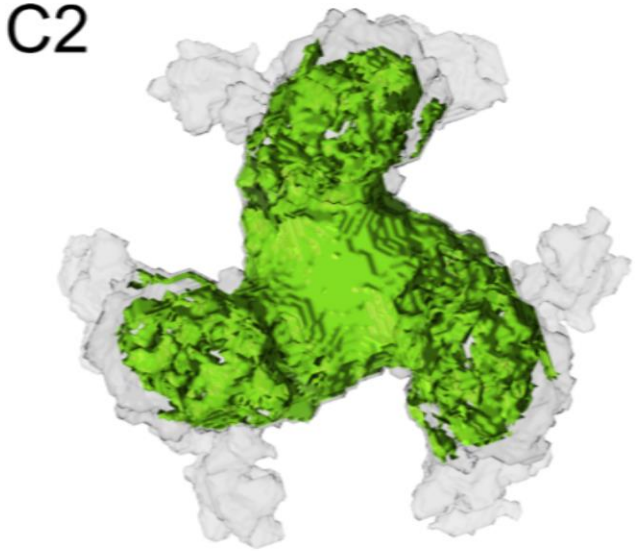
Locat

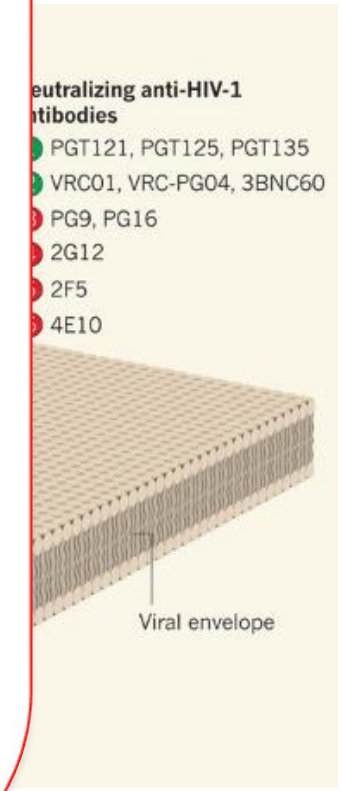




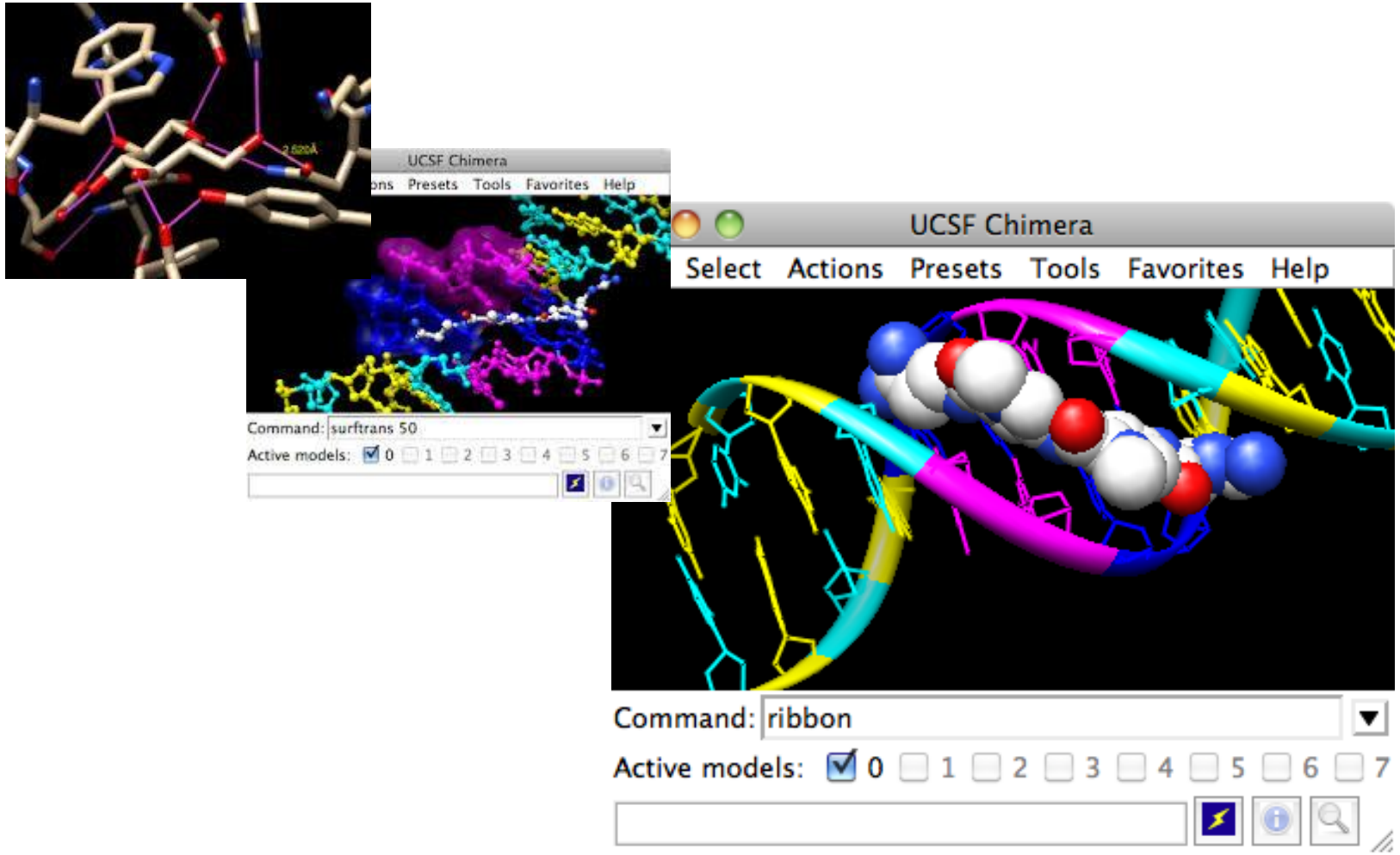








Quick demonstration of Chimera



- Structure-based studies enable us to further investigate possible routes towards effective, highly potent, and high Ab (or bNAbs) avidity vaccine development.
- In discussion of “NanoMedicine”, multiple approaches, weather biochemical, structural, or computational, can be combined to generate effective results, and draw more accurate conclusion.
- Furthermore, 3D analysis of reconstructed structures will enhance our understanding of the inter and intra molecular interaction, as well as structural elements, which will ultimately help us facilitate drug delivery and drug design.

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