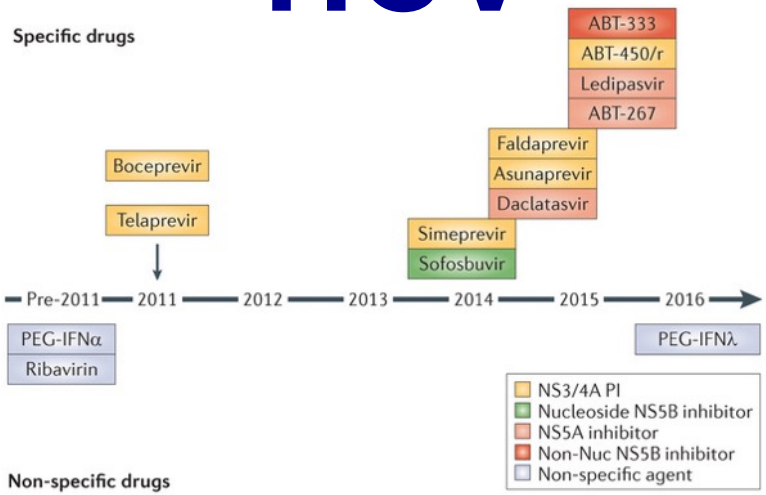
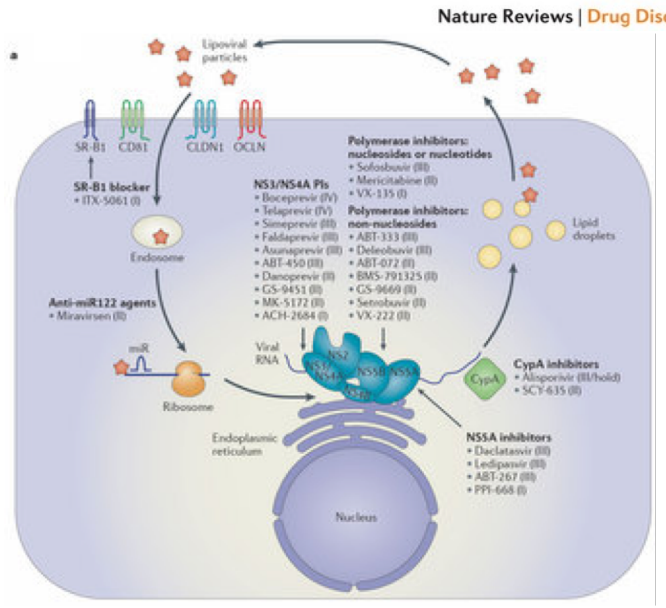
VIROLOGY

Research, Development and Applications of Antiviral Agents 2

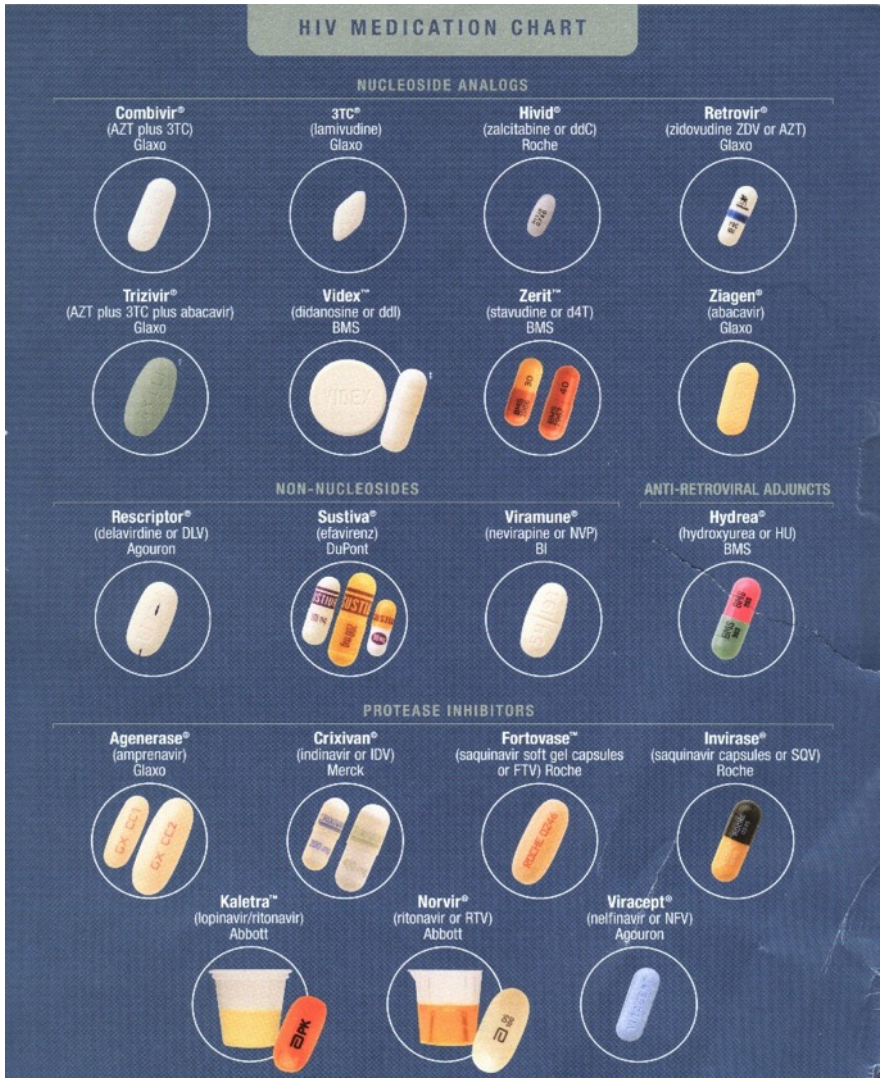
Two Success Stories

HCV





HIV



Hepatitis C Virus

Flaviviruses (Hepatitis C)

Virus	Vector	Disease
Hepacivirus • Hepatitis C virus	None	Hepatitis

Epidemiology

Transmission
- Blood
- Sex

At risk or risk factors
- IV drug users
- Health care workers

Distribution
- Worldwide
- No seasonal incidence

Vaccines or antiviral drugs
- Currently 7 FDA-approved antivirals
- No vaccines

Pathogenesis

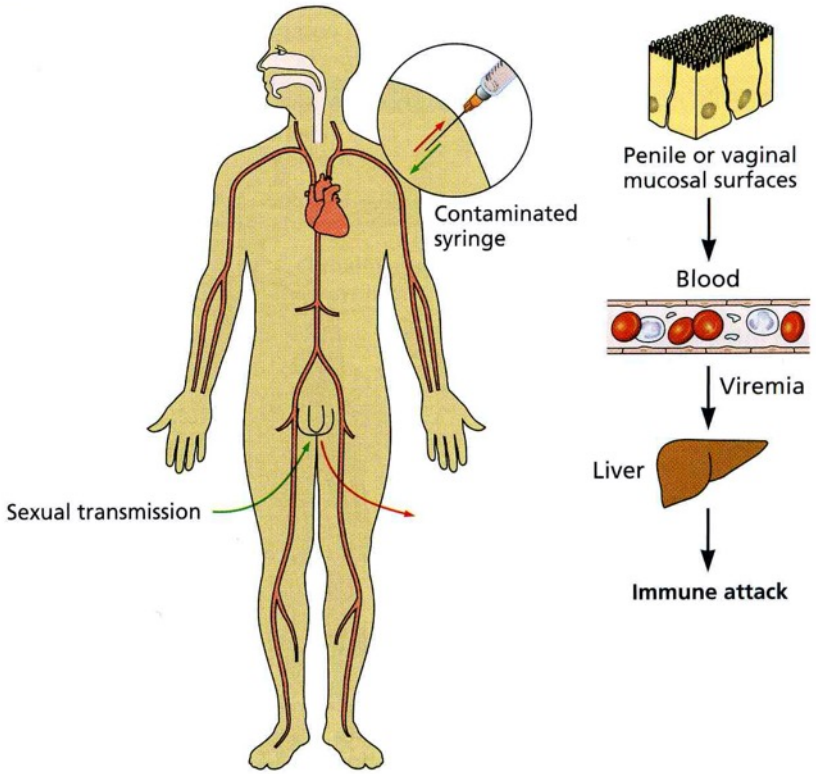
Viruses are noncytolytic and chronic

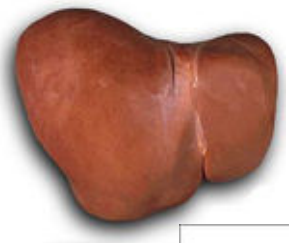
Disease caused by ongoing immune response

Liver cancer can result from chronic cirrhosis

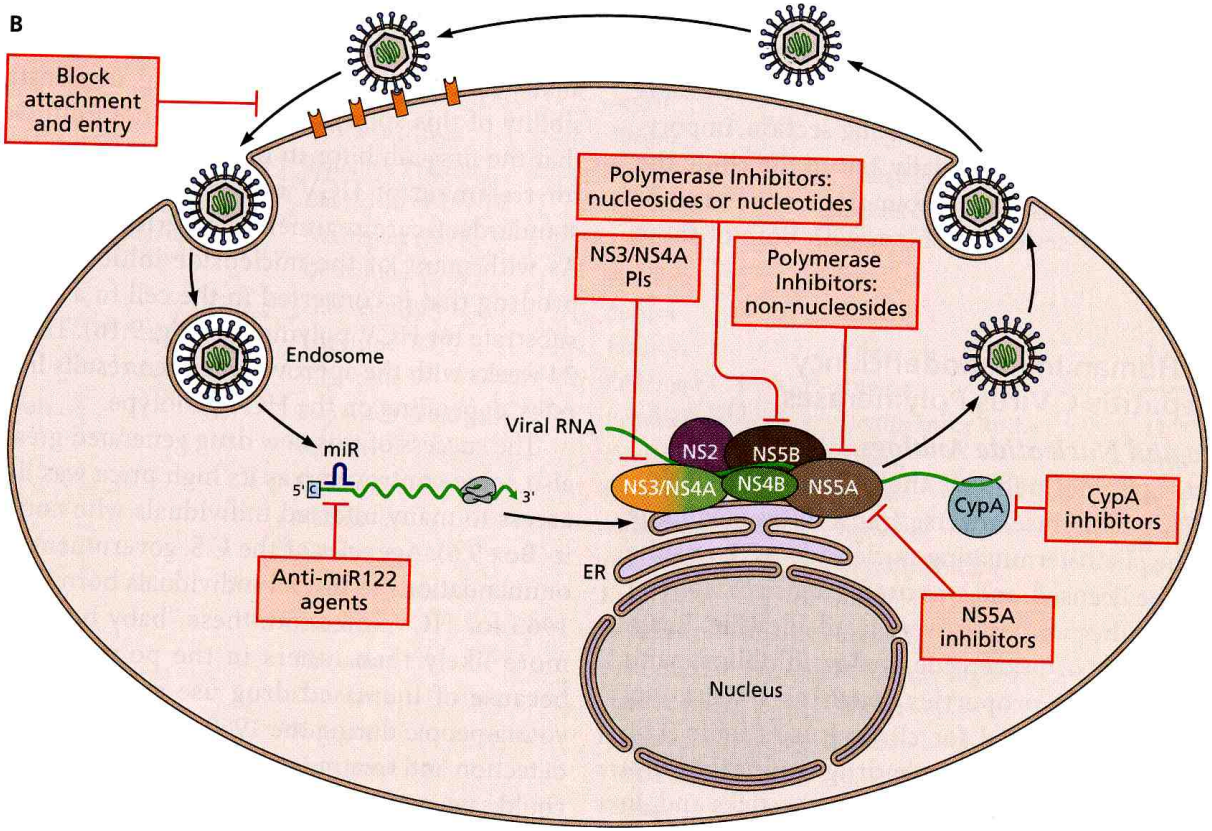
Human Infections

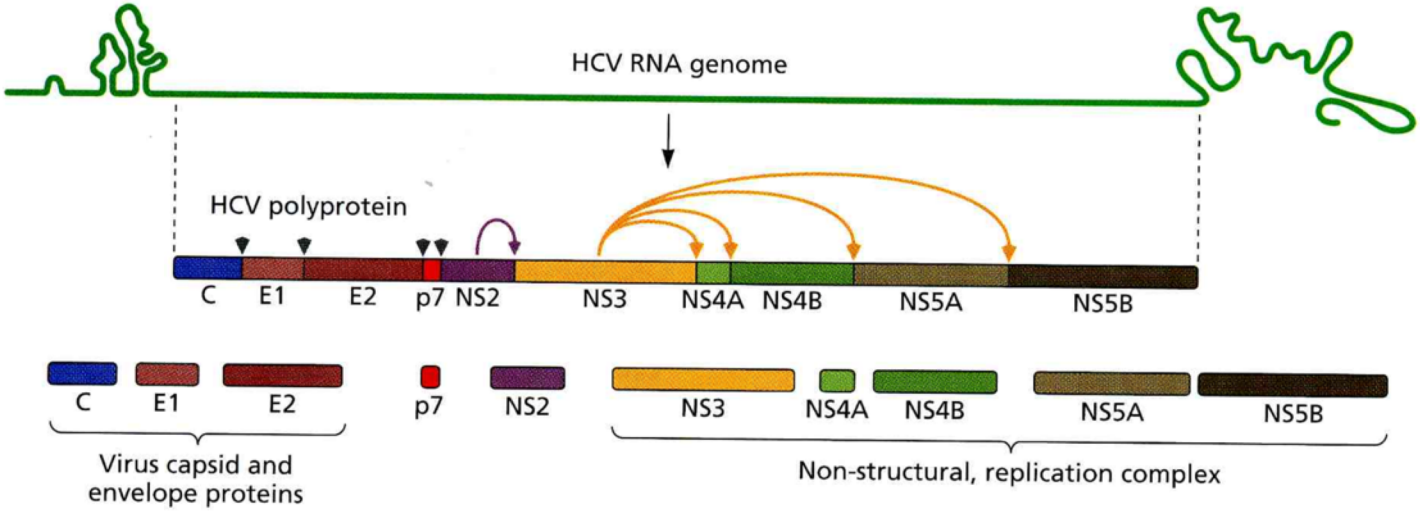
Persons living with hepatitis C
Worldwide: 130 million–150 million
In United States: 3.2 million



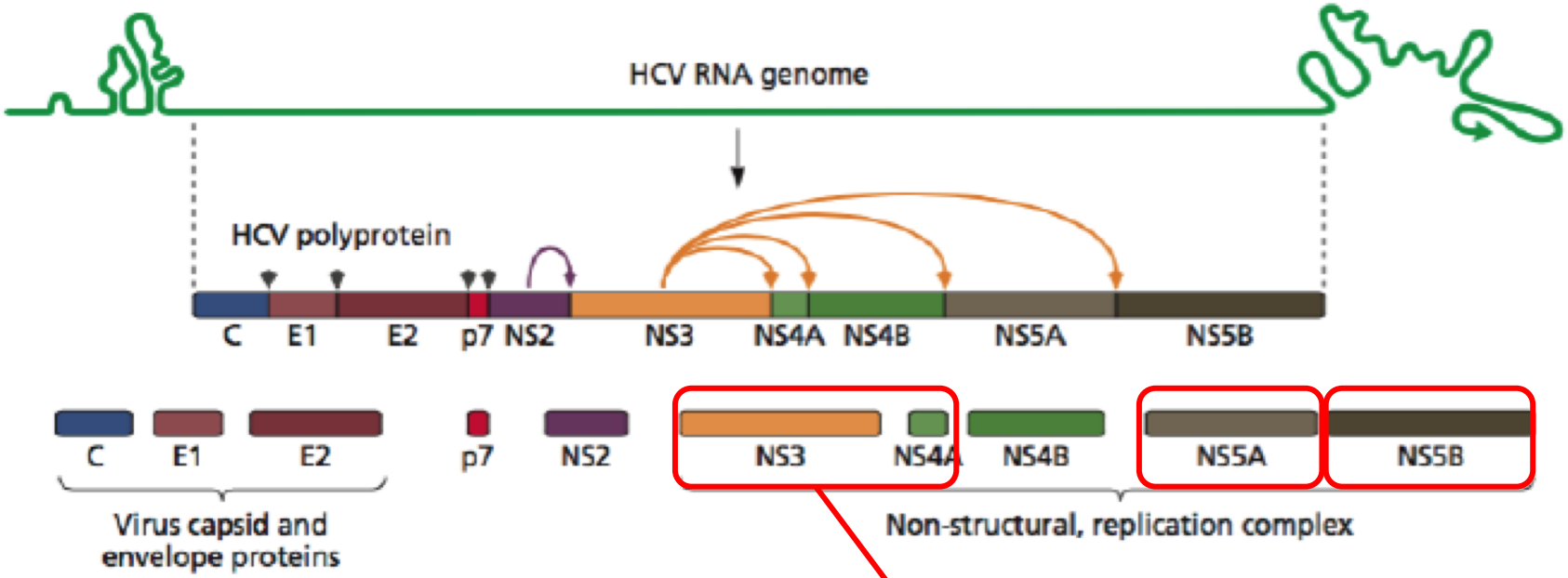


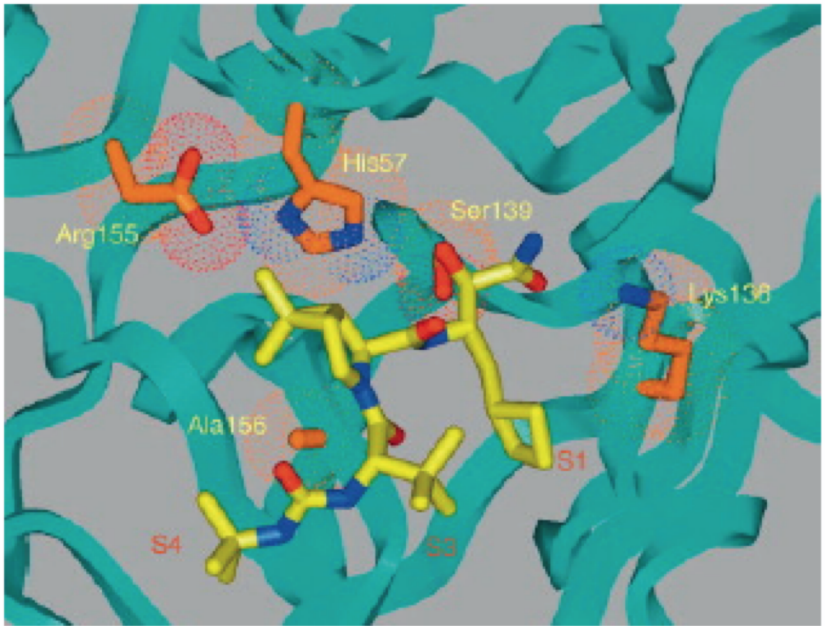
HCV replication cycle

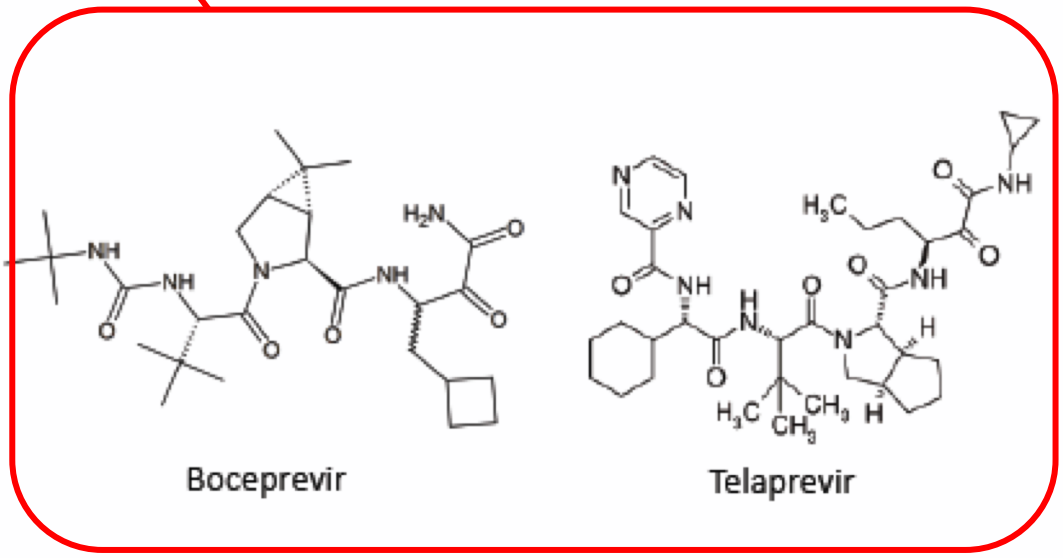




New HCV drugs



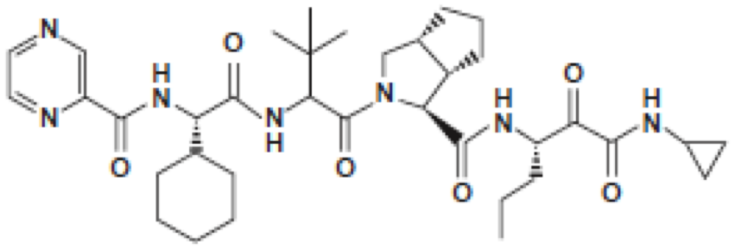




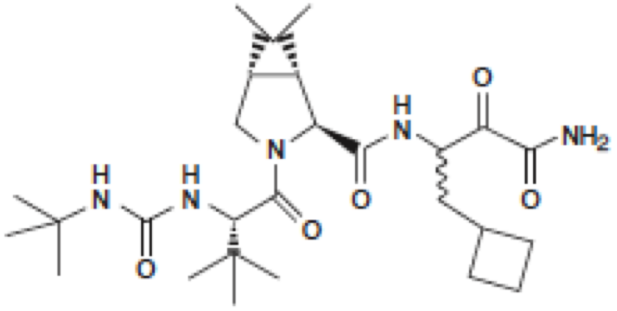
Examples of drugs targeted against HCV

Target	Generic name	Brand name	Developer	Date approved/ Trial phase
Polymerase (NS5B)	Sofosbuvir	Sovaldi	Gilead Sciences	2013
Nucleoside	Mericitabine		Roche	II
Nonnucleoside	Deleobuvir		Boehringer Ingelheim	III
	ABT-333		Abbott	III
RNA binding (NS5A)	Ledipasvir		Gilead Scienes	III (filed)
	Daclatasvir		Bristol-Myers Squibb	III
	ABT-267		Abbott	III
Protease (NS3/4A)	Telaprevir	Incivek	Vertex/Johnson & Johnson	2011
	Boceprevir	Victrelis	Merck	2011
	Simeprevir	Olysio	Janssen/Tibotec/Medivir	2013
	Faldaprevir		Boehringer Ingelheim	III
	Vaniprevir		Merck	III
	Samatasvir		Idenix	II
Combinations	Sofosbuvir + ledipasvir		Gilead Sciences	III
	Faldaprevir + deleobuvir		Boehringer Ingelheim	III
	Simeprevir + samatasvir + TMC647055/r		Janssen	II
	ABT-450/r + ABT-267 and ABT-333		Abbott	II
	MK-8742 + MK-5172		Merck	II

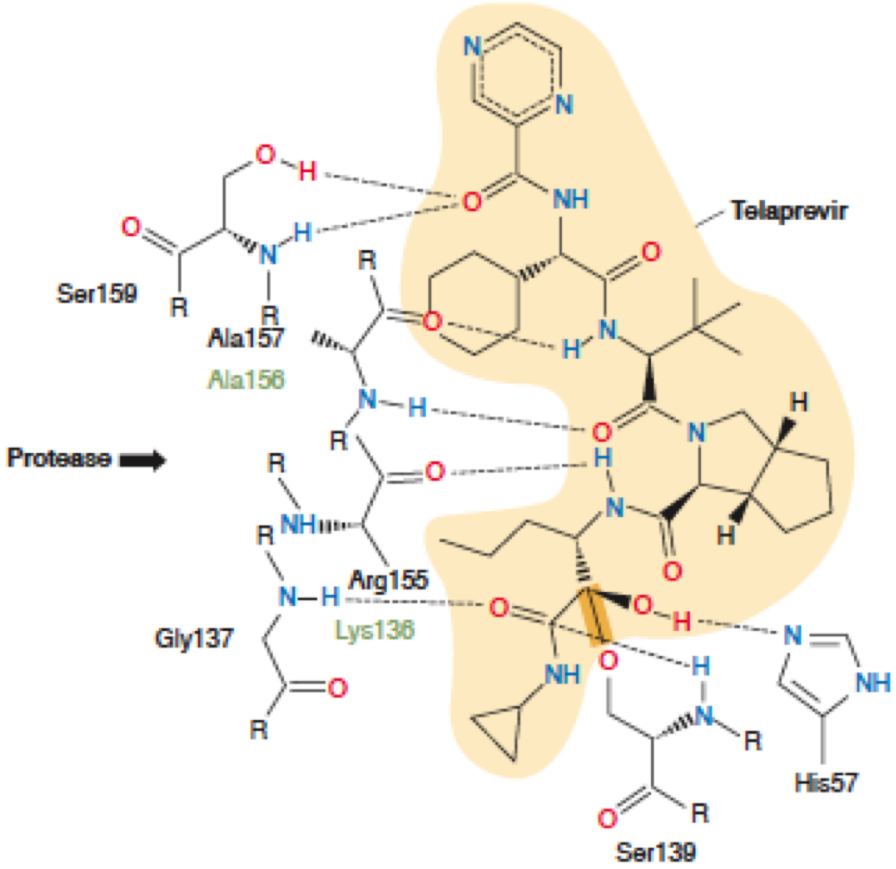
New HCV drugs: protease inhibitors



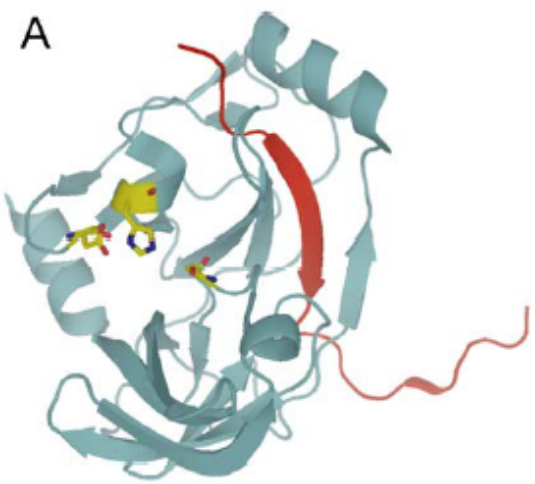
Telaprevir

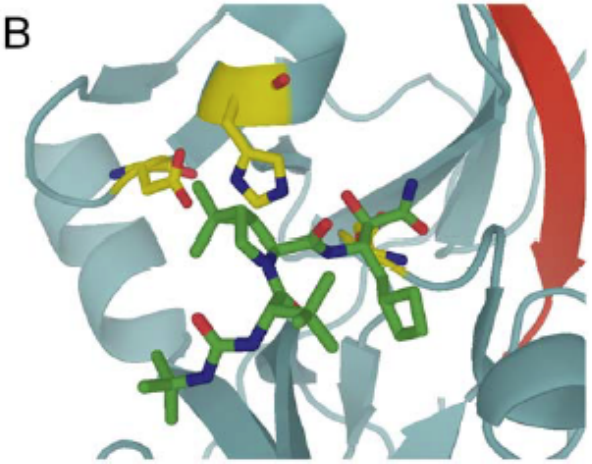


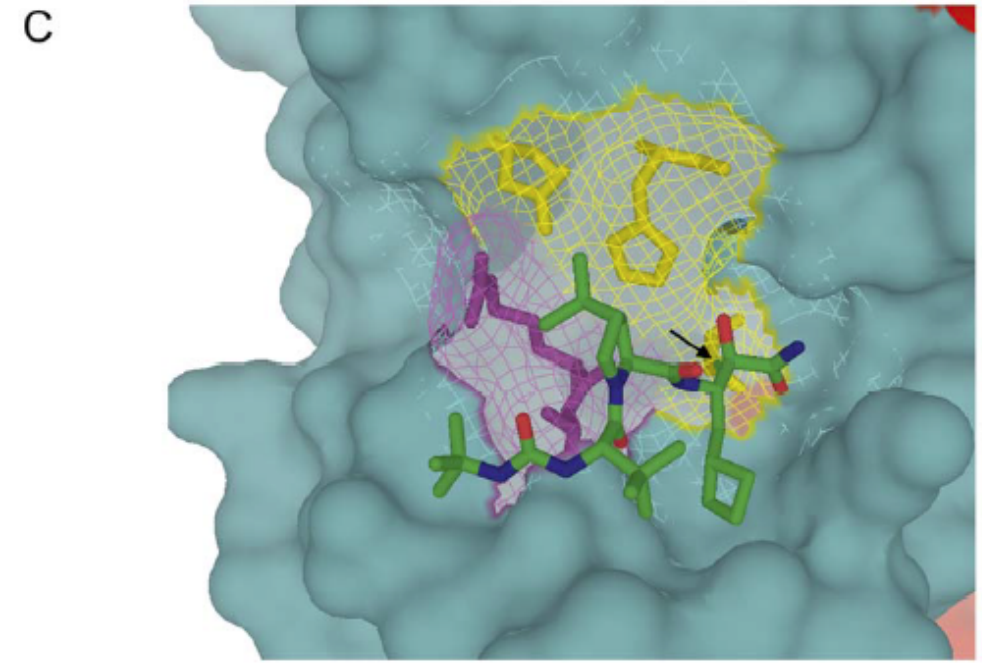
Boceprevir



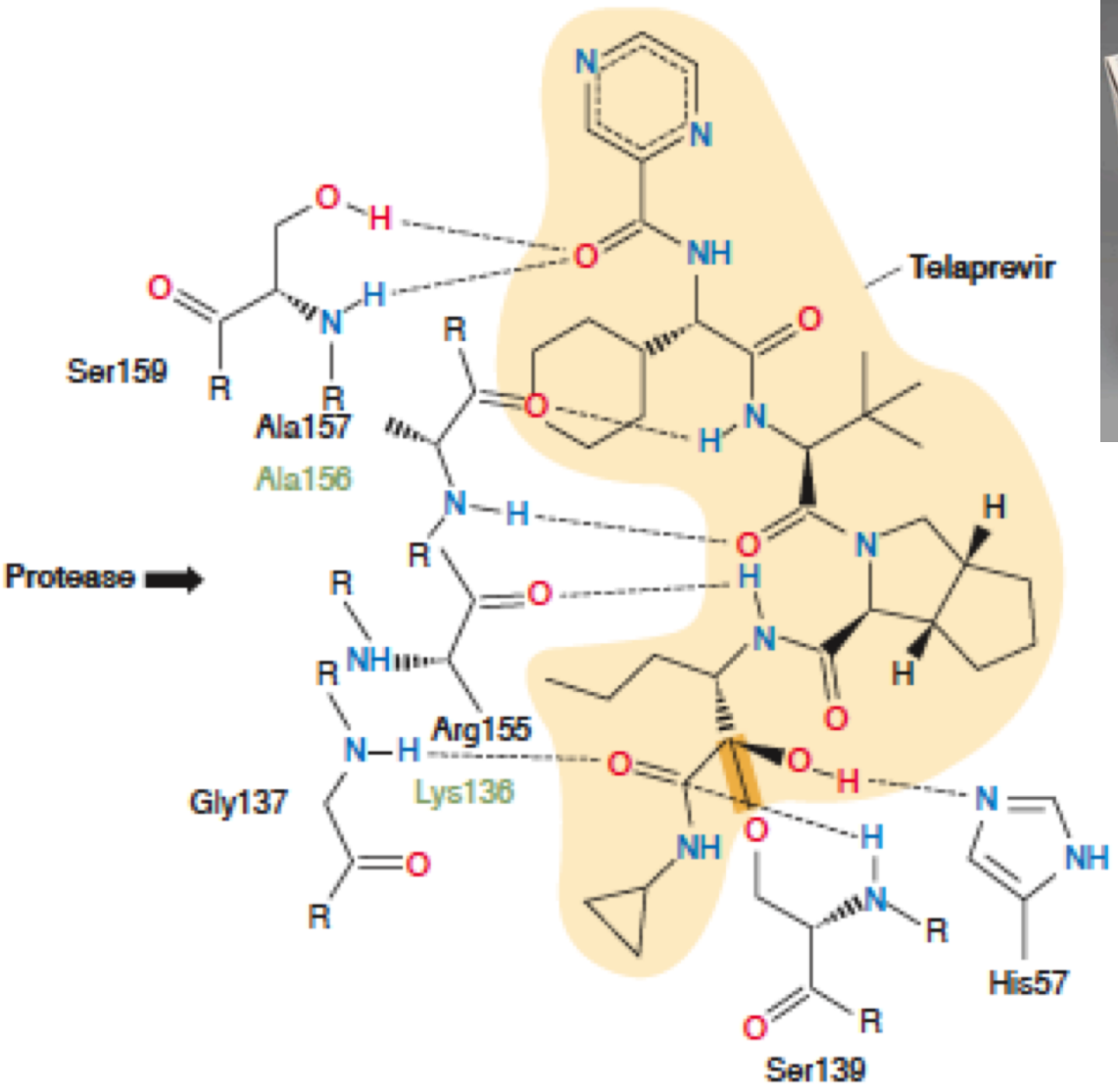
New HCV drugs: boceprevir bound to NS3/4A protease



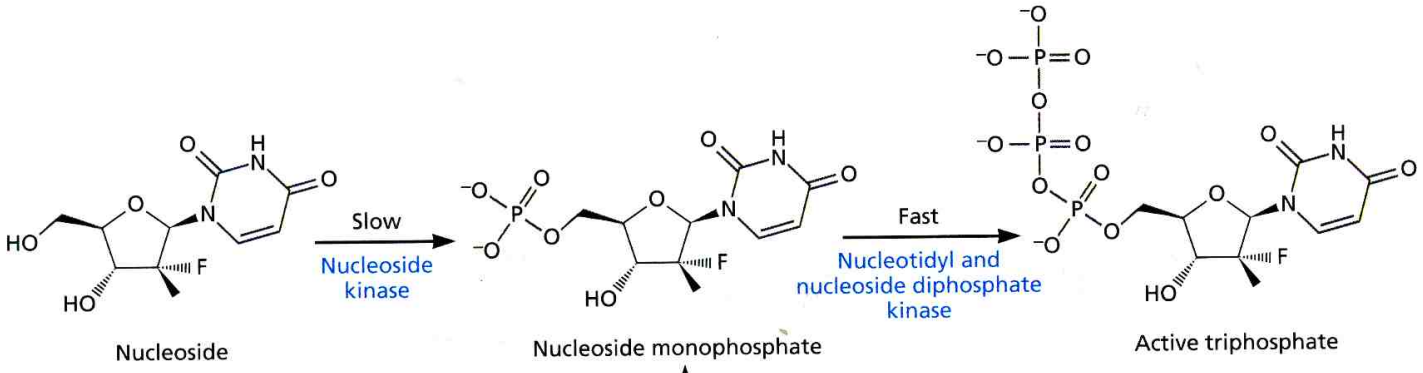


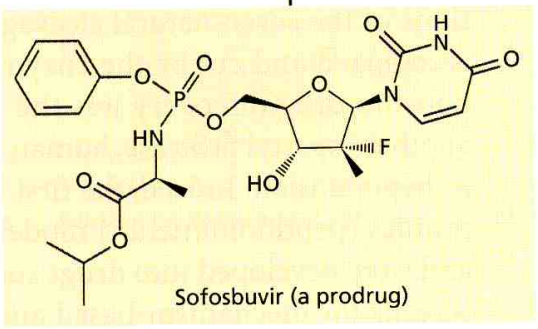


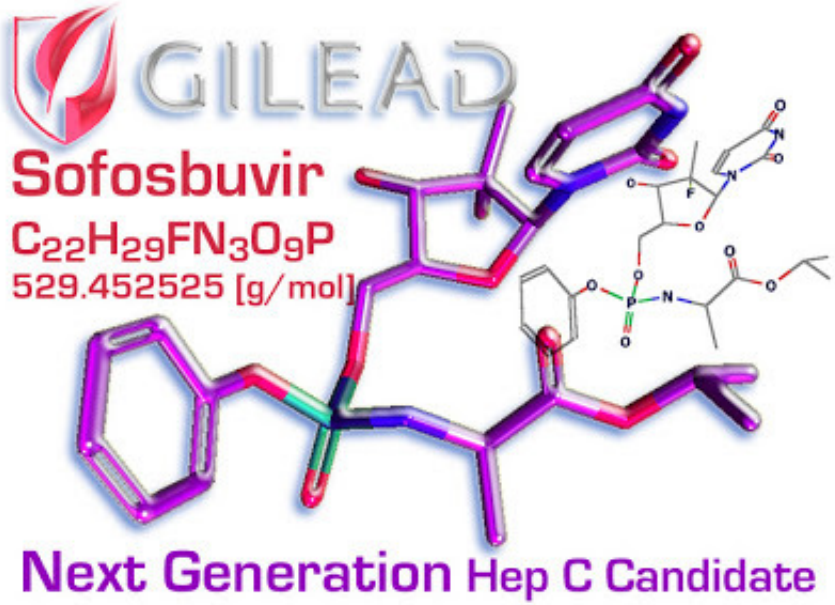
New HCV drugs: telaprevir bound to NS3-4A protease



New HCV drugs: the prodrug sofosbuvir (sovaldi), its structure and activation







HIV and AIDS

Human immunodeficiency virus types 1 and 2

Virus	Disease
Deltaretrovirus	
• Human T-lymphotropic virus type 1	Adult T-cell leukemia Tropical spastic paraparesis
• Human T-lymphotropic virus type 2	Hairy-cell leukemia
• Human T-lymphotropic virus type 5	Malignant cutaneous lymphoma
Lentivirus	
• **Human immunodeficiency virus types 1 and 2**	Acquired immune deficiency syndrome

Epidemiology

Transmission
- Virus in blood
  - Transfusions, needle sharing among drug users, needle sticks in health care workers, tattoo needles
- Virus in semen and vaginal secretions
  - Anal and vaginal intercourse
- Perinatal transmission
  - Intrauterine and peripartum transmission; breast milk

Distribution of virus
- Ubiquitous
- No seasonal incidence

At risk
- Intravenous drug users
- Homosexuals and heterosexuals with many partners
- Prostitutes
- Newborns of virus-positive mothers

Vaccines or antiviral drugs
- No vaccines
- Antiviral drugs
  - Nucleoside analog reverse transcriptase inhibitors (e.g., azidothymidine, dideoxycytidine)
  - Nonnucleoside reverse transcriptase inhibitors (e.g., nevirapine, delavirdine)
  - Protease inhibitors (e.g., saquinavir, ritonavir)

Disease mechanisms

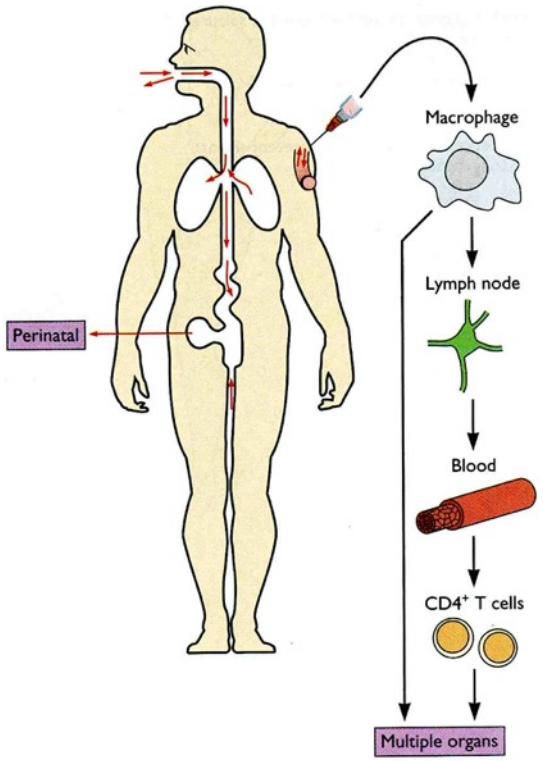
Infects mainly $CD4^+$ T cells and macrophages

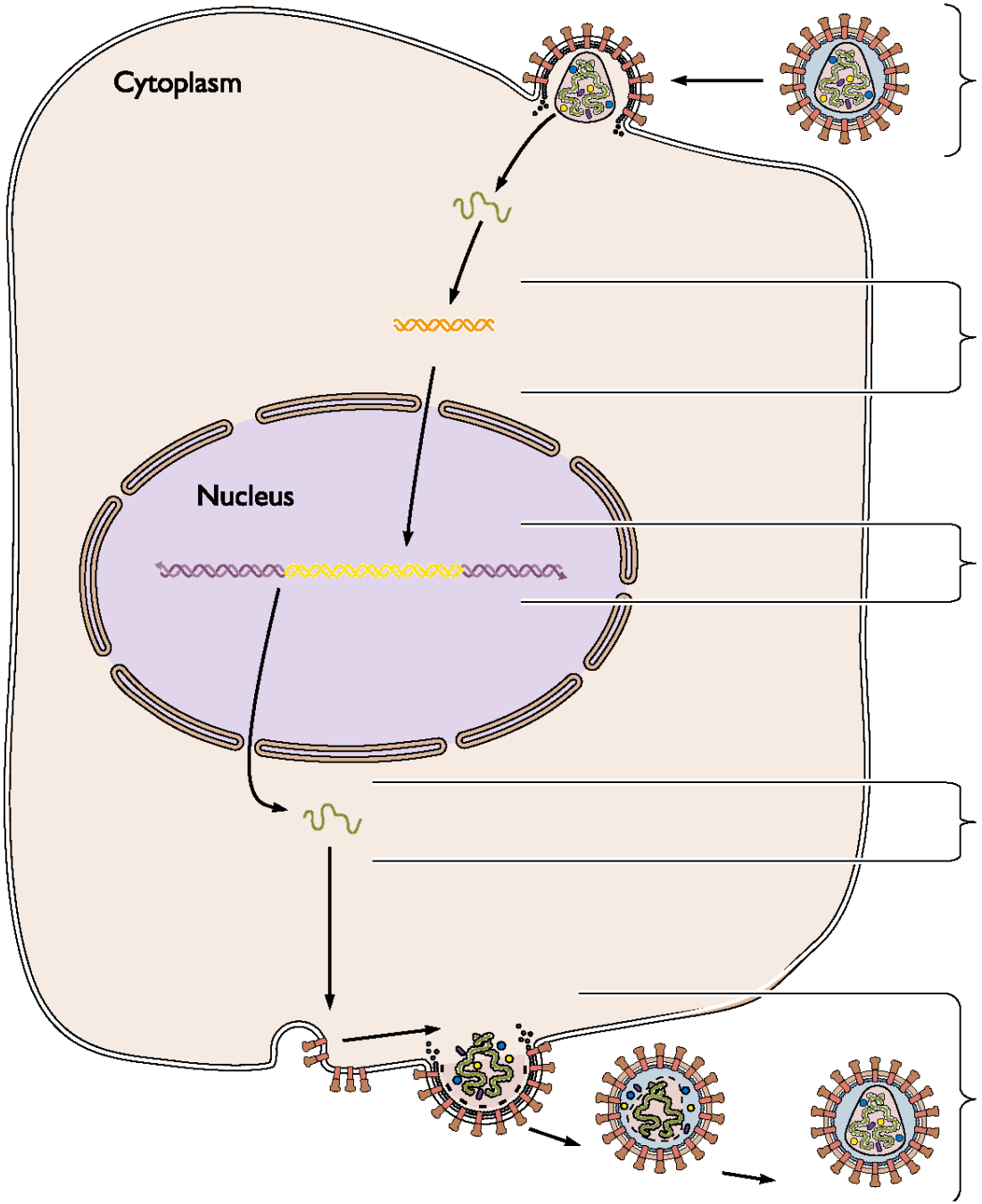
Lyses $CD4^+$ T cells, persistently infects macrophages

Infection alters T-cell and macrophage function; immunosuppression leads to secondary infection and death

Infects long-lived cells, establishing reservoir for persistent infection

Infected monocytes spread to brain, causing dementia





Cell attachment, fusion, and entry
- CD4 derivatives
- Polymers
- Plant lectins
- Bicyclams
- Chemokine analogs

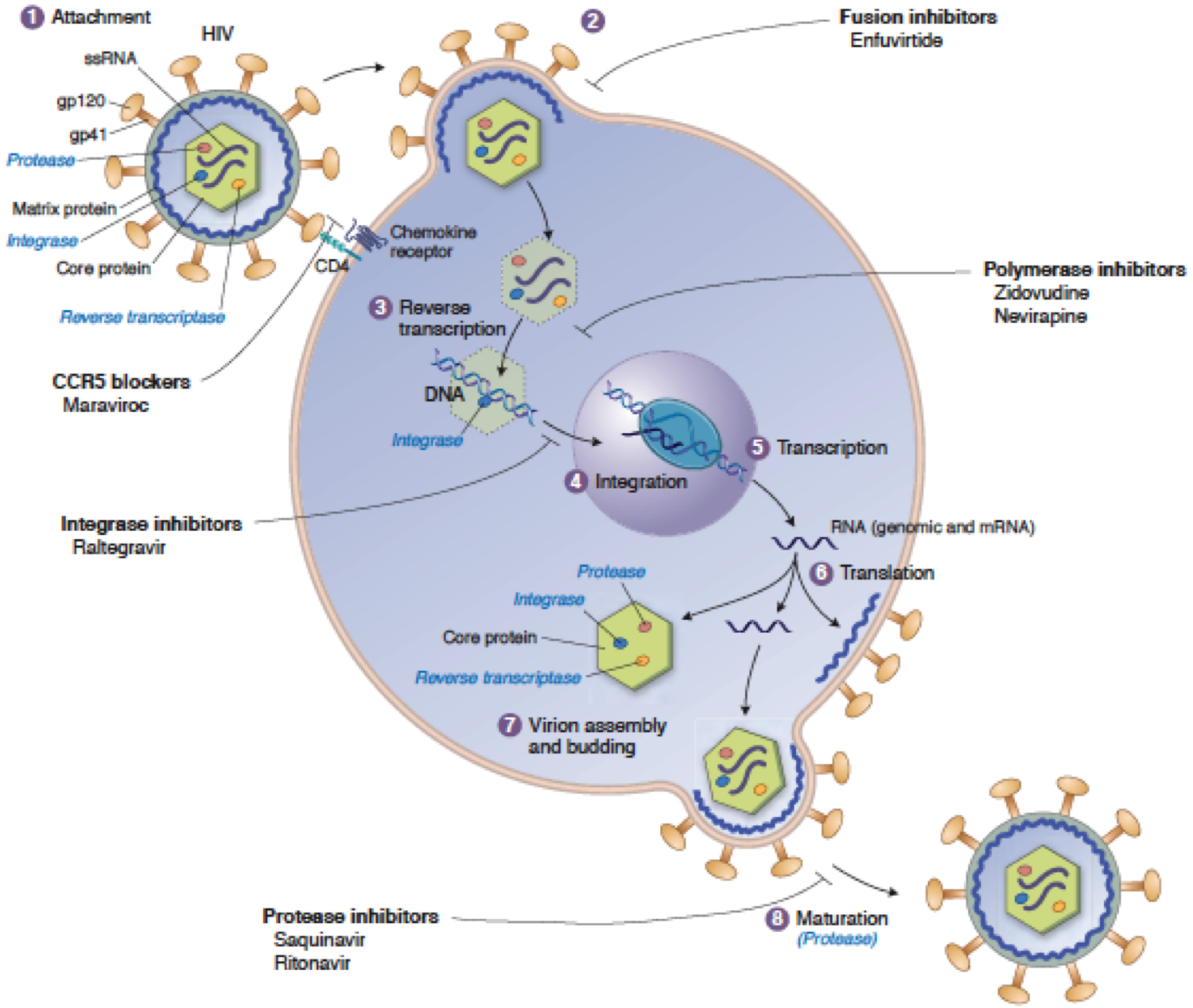
Reverse transcription
- Nucleoside analog reverse transcriptase inhibitors: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, acyclic nucleoside phosphonates
- Nonnucleoside reverse transcriptase inhibitors: nevirapine, delavirdine, loviride, efavirenz

Integration
- Integrase inhibitors

Transcription and posttranscriptional processing
- Tat inhibitors

Virion packaging and budding
- Protease inhibitors: saquinavir, ritonavir, indinavir, nelfinavir
- Nucleocapsid inhibitors

Stages of HIV blocked by different classes of antiviral drugs



BOX 9.6 DISCUSSION

A heroic effort: 19 new drugs, 3 targets, 9 companies, and 15 years

We must never forget the daunting task that faced the scientific and medical community in the 1980s when HIV was first identified and every infection was a death sentence. There was no experience with such infections in the clinics, and the drug hunters had nothing in the pipeline that was proven to be effective against retroviruses. In fact, there were few scientists with any experience at all with lentiviruses. Yet as the data in this table demonstrate, a truly heroic effort was mounted over the first 15 years of the pandemic, but it took time, money, and unprecedented cooperation.

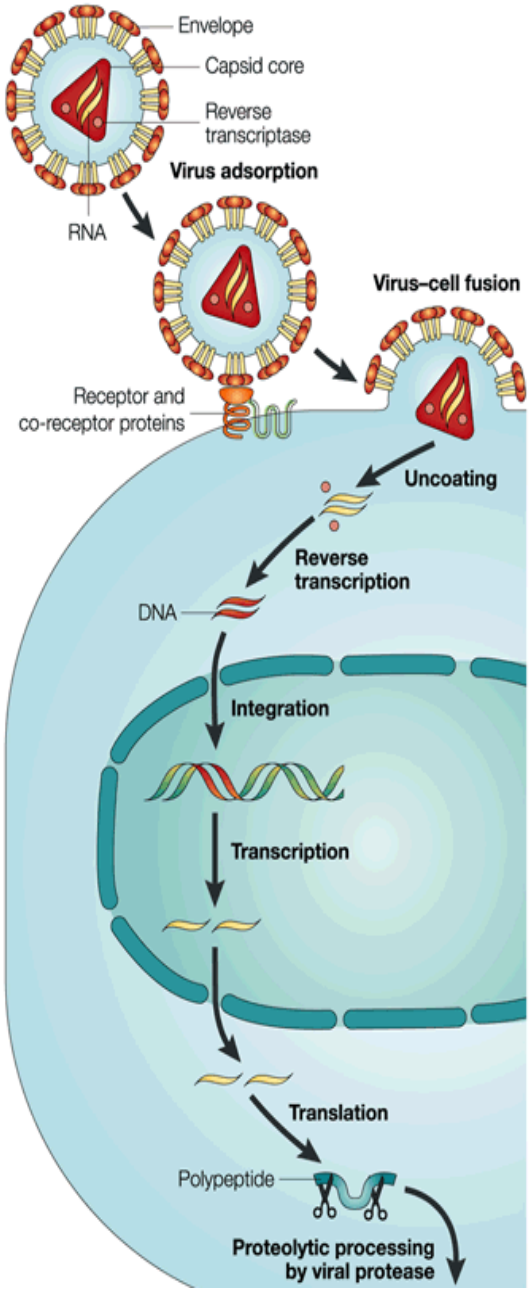
Target or mechanism	Generic name	Brand name	Manufacturer	Yr approved
Nucleoside reverse transcriptase inhibitors	Zidovudine (AZT, ZDV)	Retrovir	GlaxoSmithKline	1987
	Didanosine (ddI)	Videx	Bristol-Myers Squibb	1991
	Zalcitabine (ddC)	Hivid	Roche	1992
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	1994
	Lamivudine (3TC)	Epivir	GlaxoSmithKline	1995
	AZT/3TC	Combivir	GlaxoSmithKline	1997
	Abacavir (ABC)	Ziagen	GlaxoSmithKline	1998
	AZT/3TC/ABC	Trizivir	GlaxoSmithKline	2000
	Tenofovir (TDF)	Viread	Gilead	2001
Nonnucleoside reverse transcriptase inhibitors	Nevirapine	Viramune	Roxane	1996
	Delavirdine	Rescriptor	Agouron	1997
	Efavirenz	Sustiva	Dupont	1998
Protease inhibitors	Saquinavir (hard gel)	Invirase	Roche	1995
	Saquinavir (soft gel)	Fortovase	Roche	1997
	Ritonavir	Norvir	Abbott	1996
	Indinavir	Crixivan	Merck	1996
	Nelfinavir	Viracept	Agouron	1997
	Amprenavir	Agenerase	GlaxoSmithKline	1999
	Lopinavir/ritonavir	Kaletra	Abbott	2000
Summary				
Three enzyme targets	**16 unique compounds**	**19 approved drugs**	**9 companies**	**15 years**

Approved drugs targeted against HIV enzymes

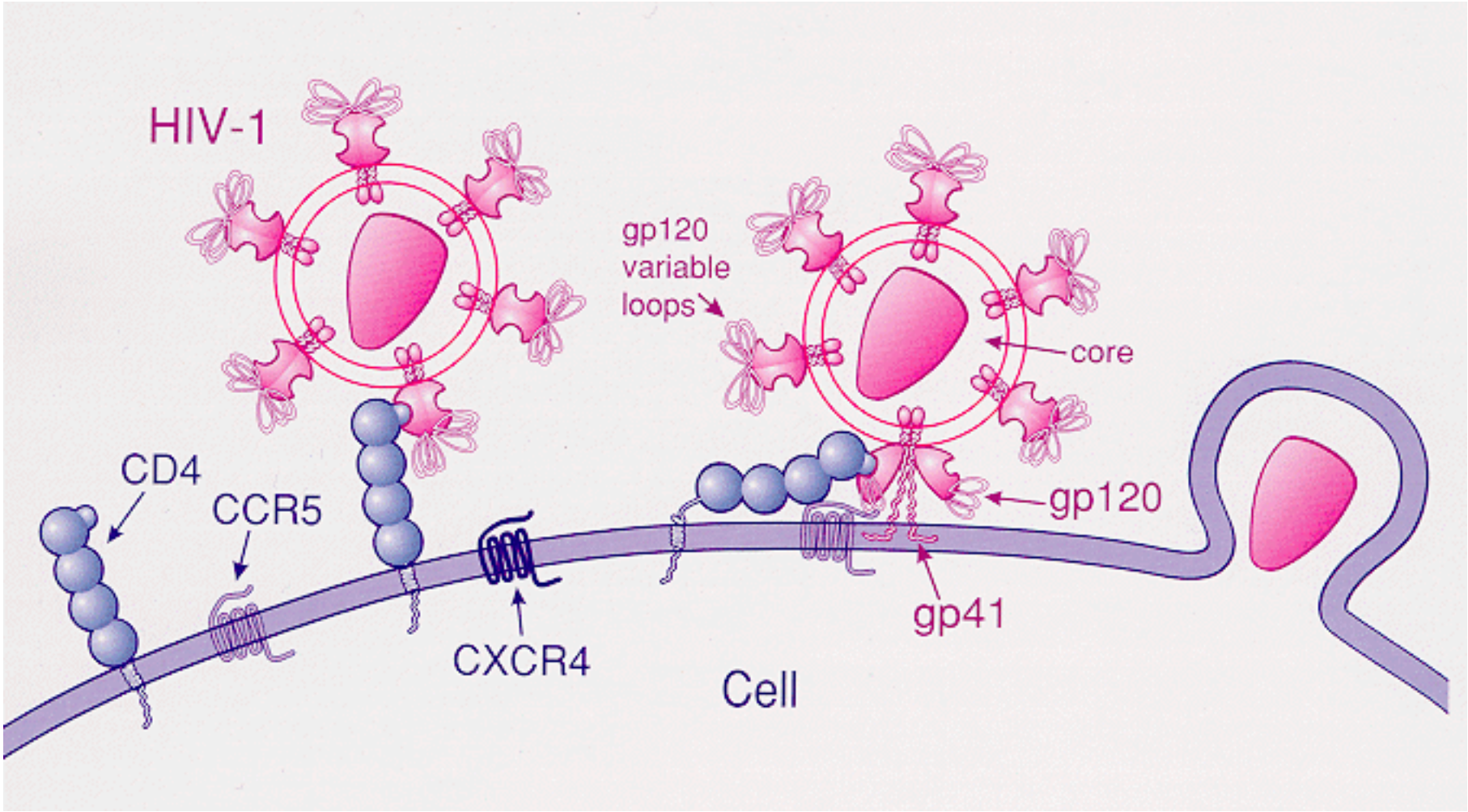
Target	Generic name	Brandname	Manufacturer	Year
Reverse transcriptase	Zidovudine (AZT)	Retrovir	GlaxoSmithKline	1987
Nucleos(t)ide inhibitors	Didanosine (ddI)	Videx	Bristol-Myers Squibb	1991
	Zalcitabine (ddC)	Hivid	Hoffmann-La Roche	1992
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	1994
	Lamivudine (3TC)	Epivir	GlaxoSmithKline	1995
	Abacavir (ABC)	Ziagen	GlaxoSmithKline	1998
	Tenofovir (TDF)	Viread	Gilead Sciences	2001
	Emtricitabine (FTC)	Emtriva	Bristol-Myers Squibb	2003
Nonnucleoside inhibitors	Nevirapine (NVP)	Viramune	Roxane	1996
	Delavirdine (DLV)	Rescriptor	Pfizer	1997
	Efavirenz (EFV)	Sustiva	DuPont	1998
	Etravirine (ETR)	Intelence	Tibotec	2008
	Rilpivirine	Edurant	Tibotec	2011
Protease	Saquinavir (hard gel)	Invirase	Hoffmann-La Roche	1995
	Ritonavir	Norvir	Abbott	1996
	Indinavir	Crixivan	Merck	1996
	Nelfinavir	Viracept	Agouron	1997
	Amprenavir	Agenerase	GlaxoSmithKline	1999
	Lopinavir/ritonavir	Kaletra	Abbott	2000
	Atazanavir	Revataz	Bristol-Myers Squibb	2003
	Tipranavir	Aptivus	Boehringer Ingelheim	2005
	Darunavir	Prezista	Tibotec	2006
Integrase	Raltegravir	Isentress	Merck	2007
	Elvitegravir	Vitekta	Gilead Sciences	2012
	Dolutegravir	Tivicay	GlaxoSmithKline	2013
Combinations	TDF/FTC/EFV	Atripla	Bristol-Myers Squibb/ Gilead Sciences	2006
	TDF/FTC/rilpivirine	Complera	Gilead Sciences	2011
	TDF/FTC/elvitegravir + cobicistat	Stribild	Gilead Sciences	2012

Druggable targets in HIV replication cycle

Adsorption
Entry

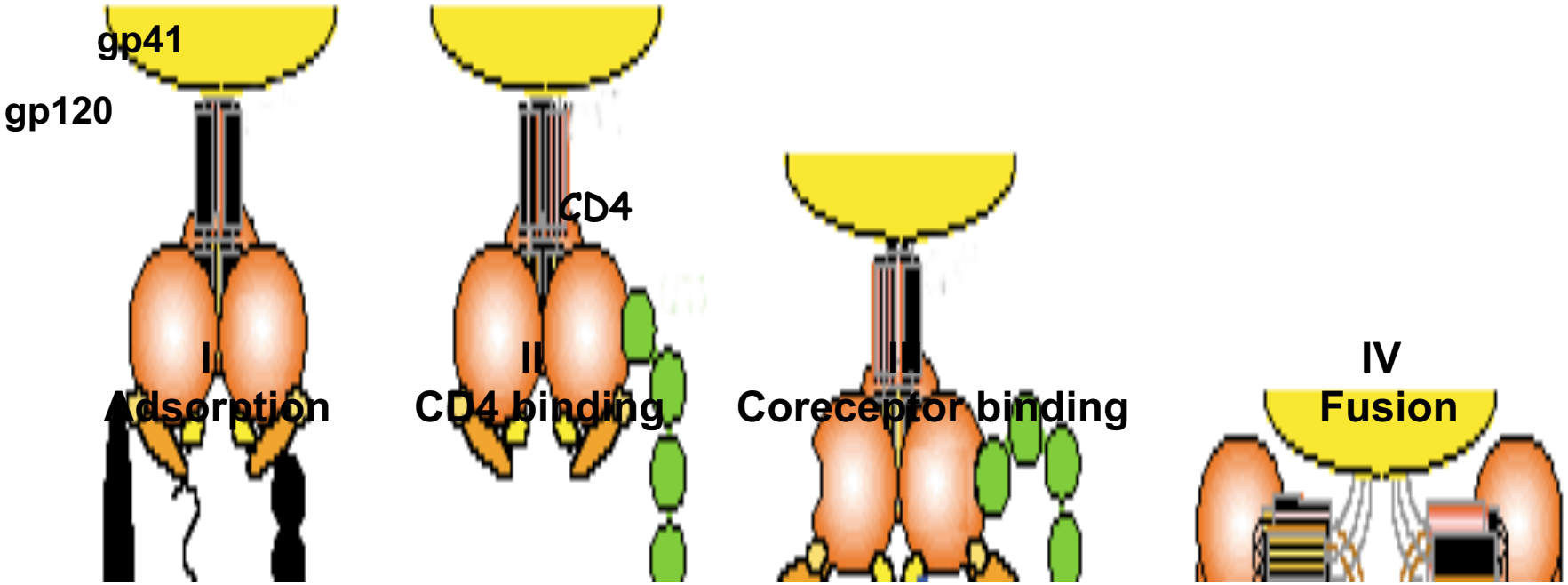


HIV adsorption and virus-cell fusion

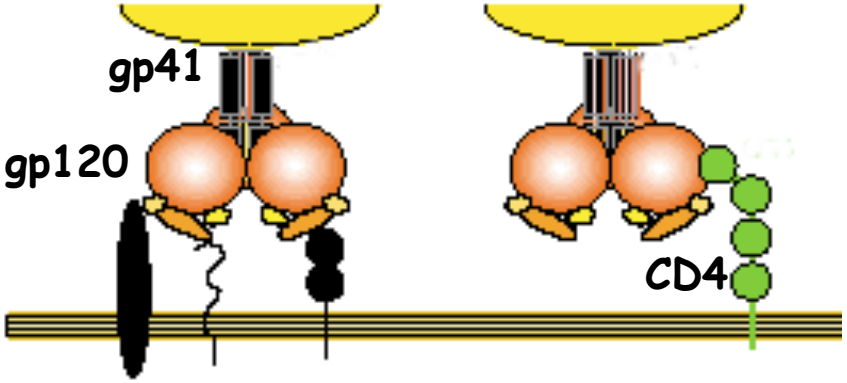


HIV adsorption and virus-cell fusion

- ✓ The viral receptor is constituted by a trimer of the **gp41/gp120** heterodimer



HIV adsorption: binding inhibitors



CD4

It is the high affinity cell receptor on:

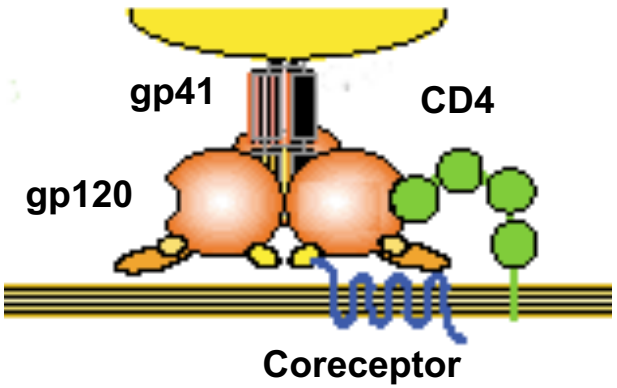
T helper lymphocytes, macrophages, dendritic cells

✓ **Anionic polymers**

✓ **sCD4-IgG**

Tetravalent fusion protein that binds and neutralizes HIV virions before their binding to cellular CD4

HIV binding to co-receptors



- ✓ **HIV coreceptors are chemokine cell receptors**
- ✓ **They belong to the G-coupled 7TMDs receptor superfamily**
- ✓ **The CD4-gp120 interaction promotes gp120 binding to coreceptors**

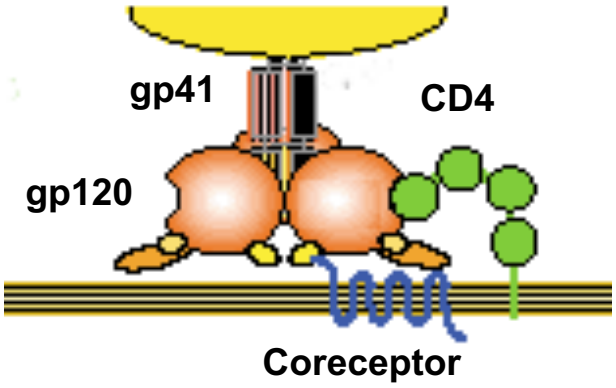
CXCR4

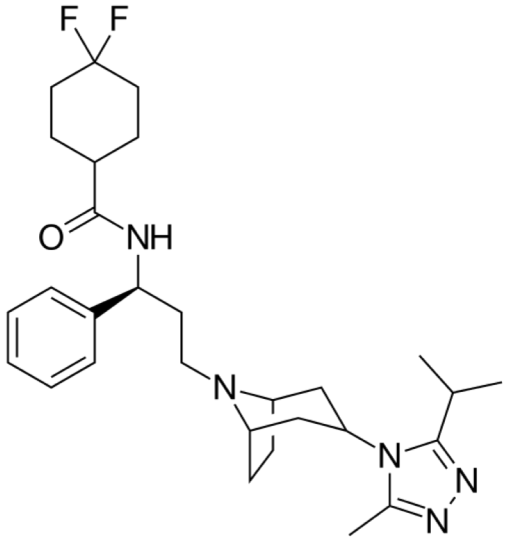
Coreceptors for T-tropic strains of HIV (X4)

CCR5

Coreceptor for M-tropic strains of HIV (R5)
di HIV

Inhibitors of binding to co-receptors: maraviroc





- ✓ **Maraviroc** (brand-named **Selzentry**) is a antiretroviral drug in the CCR5 receptor antagonist class and it is also classed as an entry inhibitor
- ✓ Maraviroc is a negative allosteric modulator of the CCR5 coreceptor. It binds to CCR5, thereby blocking the gp120 from associating with the coreceptor. Thus, HIV is then unable to enter human macrophages.
- ✓ FDA approved in 2007
- ✓ However, CCR5 inhibitors may drive evolution of R5 strains towards more virulent X4 strains that use CXCR4

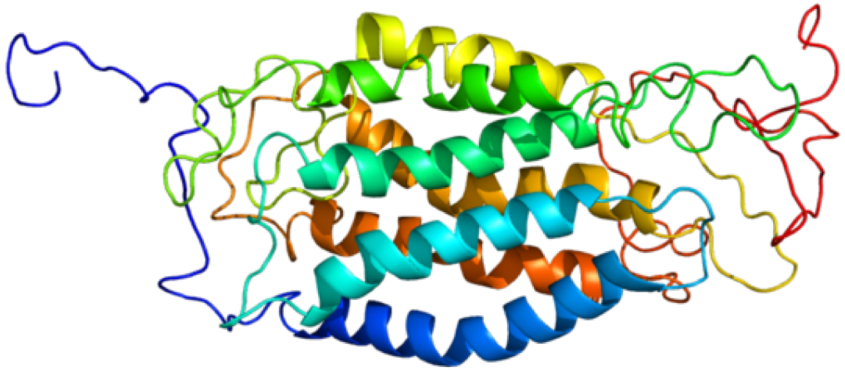
The co-receptor story

- ✓ **In the early 90s it became clear that some individuals were resistant to the infection despite repeated exposure and that some HIV-positive survived for a lot of time** ⇒ **long term survivors**

(Cao et al., *New England J. Of Medicine*, 332:201-208, 1995)

- ✓ **In 1996 HIV co-receptors CCR5 and CXCR4 were identified**

(Alkhatib et al., *Science* 272:1952-1955, 1996; Feng et al., *Science* 272:872-877, 1996)

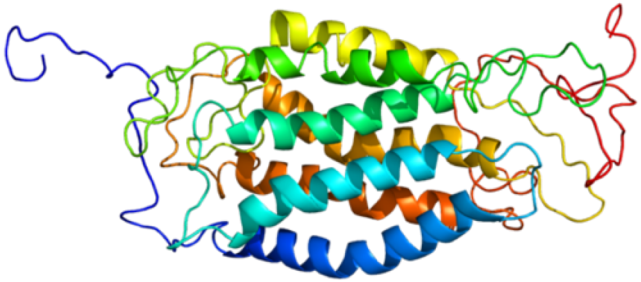


- ✓ **Shortly after emerged the idea that resistant individuals had mutated co-receptors**

(Liu et al., Cell 86:367-377, 1996; Samson et al., *Nature* 272:722-725, 1998)

The co-receptor story: the Δ32 mutation

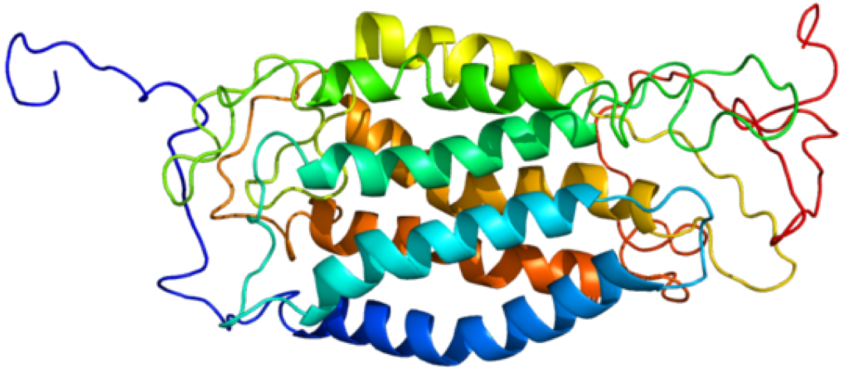
- ✓ To verify this hypothesis, Samson et al., sequenced CCR5 genes of three long term survivors and in one, they found a deletion defined to as **Δ32**



- ✓ This mutation has not a negative effect on the functions of macrophages, but appears to play a protective role against HIV. Despite the large genetic variability of CCR5, the Δ32 is associated to a CCR5 protein that does not sustain entry of R5-tropic HIV strains. Thus, **those individuals who are homozygous for this CCR5 mutation are resistant to HIV and rarely progress to AIDS.**

- ✓ Numerous studies of people infected by HIV have shown that the presence of one copy of CCR5-Δ32 delays the onset of AIDS for at least two years. It is also possible that a person with this mutation can not be infected with R5-tropic strains.

The co-receptor story: the Δ32 mutation



✓ This mutation is found at relatively high frequencies in Northern Europeans (4-16%). However, it absent in Asians and Africans.

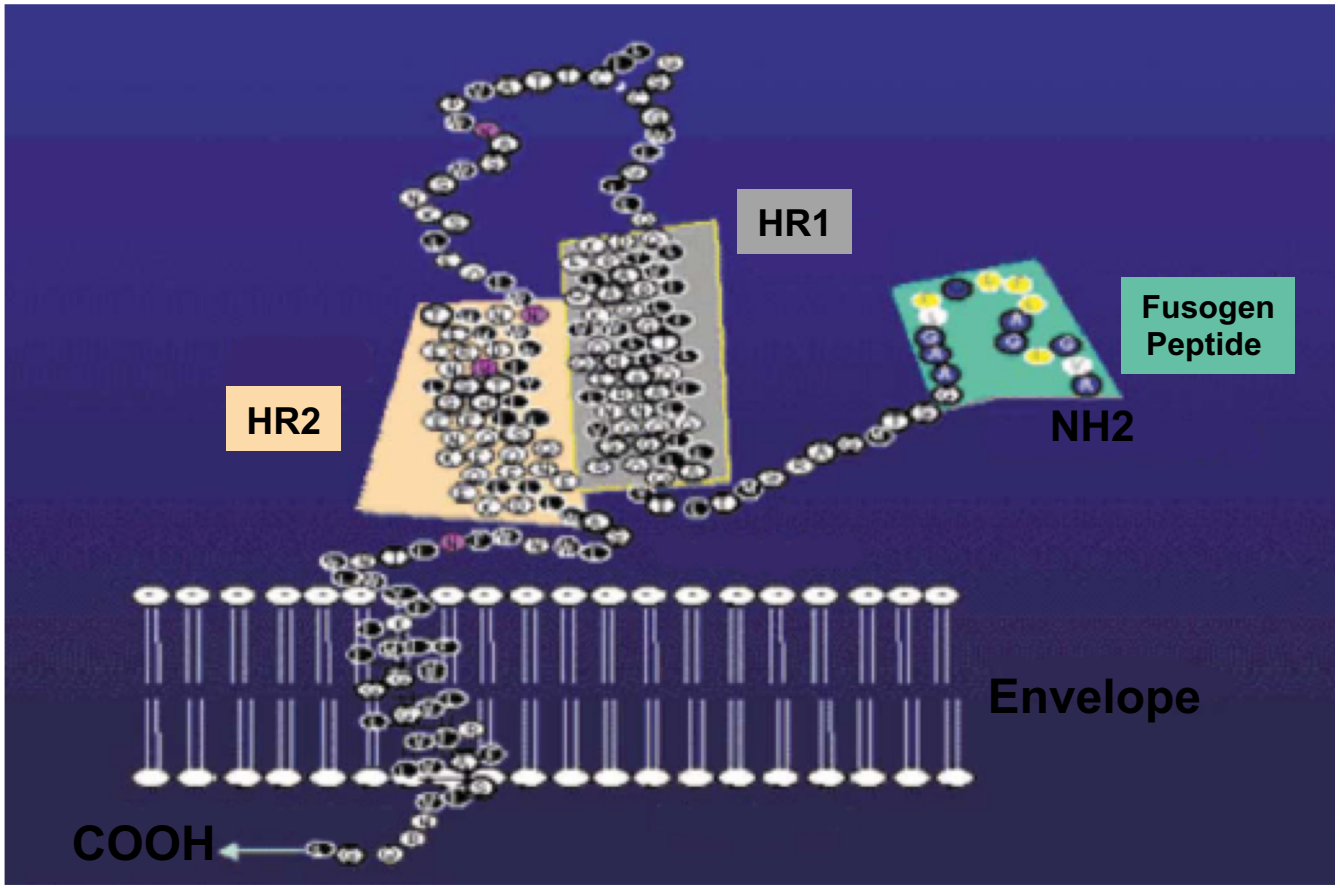
✓ Two hypotheses: genetic drift and natural selection

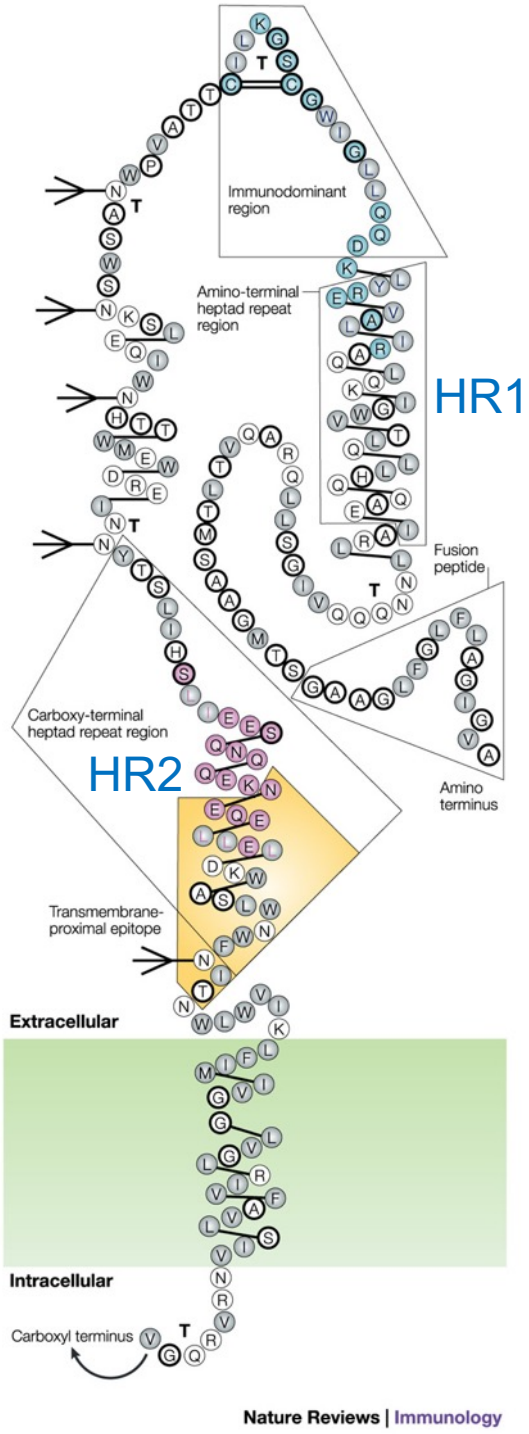
- During the plague of the 14th century in Europe, the CCR5 Δ32 allele would be selected because it conferred resistance to this infection. However, more recent studies have ruled out that possibility, revealing the absence of a protective role in this context.
- **It is believed now that Δ32 mutation may have given some kind of protection from smallpox during epidemics in Europe.**

The co-receptor story: the Berlin patient

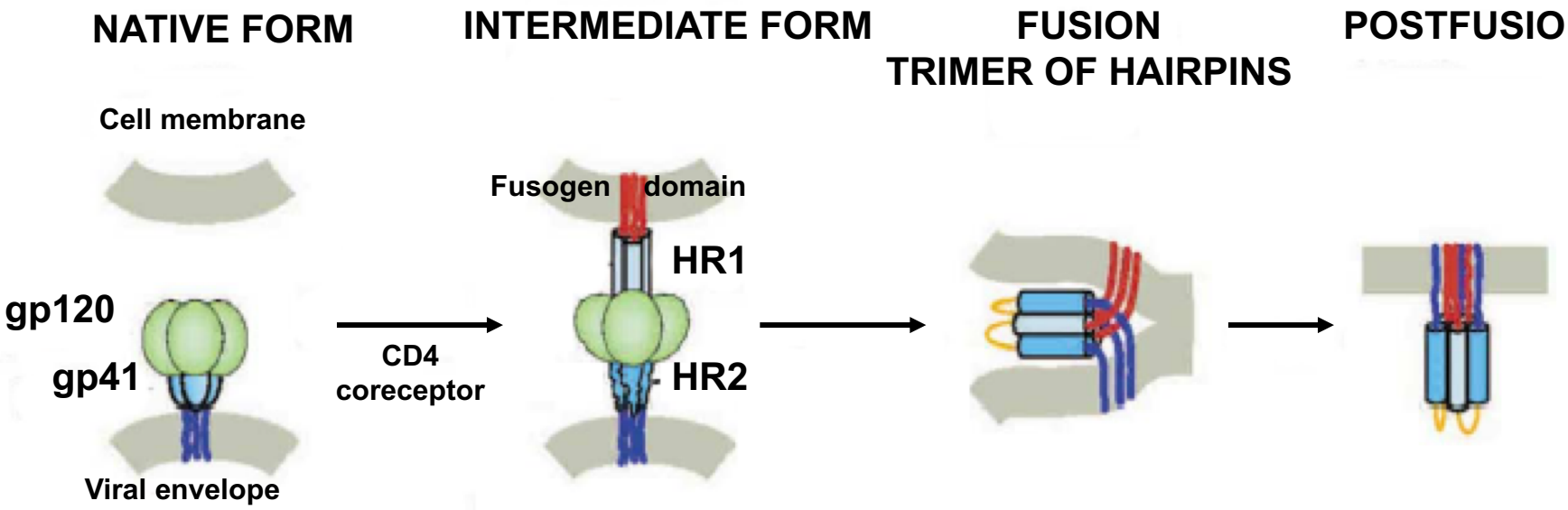
- ✓ The most famous Berlin patient is **Timothy Ray Brown**.
- ✓ He was diagnosed with HIV in 1995 and began antiretroviral therapy.
- ✓ In 2007, he was diagnosed with acute myeloid leukemia (AML). At the Charité Hospital in Berlin, he received a hematopoietic stem cell transplant (SCT) from a donor with the "Δ32" mutation on the CCR5.
- ✓ He received two SCT transplants from a donor homozygous for the "Δ32" mutation: one in 2007 and one in 2008. Then, he stopped taking antiretrovirals and three months after his first stem cell transplant, levels of HIV rapidly plummeted to undetectable levels, while his CD4 T cell count increased.
- ✓ Today, he still remains off antiretroviral therapy and is considered cured. He is the first, and as yet sole, individual to be cured of HIV.
- ✓ He received what is called a **sterilizing cure** (complete eradication) as opposed to a **functional cure** (to control infection with drugs).
- ✓ Limited chance of finding a matching donor homozygous for the "Δ32" mutation.

HIV entry: structure of gp 41



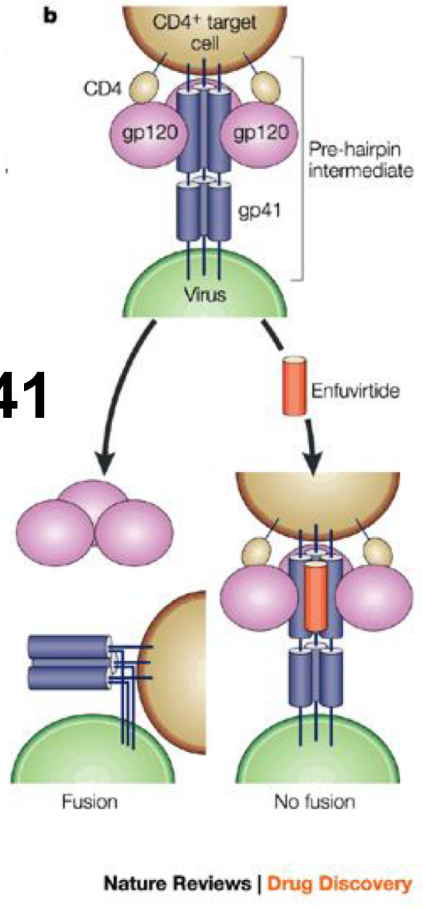


HIV entry: the gp41 role



Fusion inhibitors

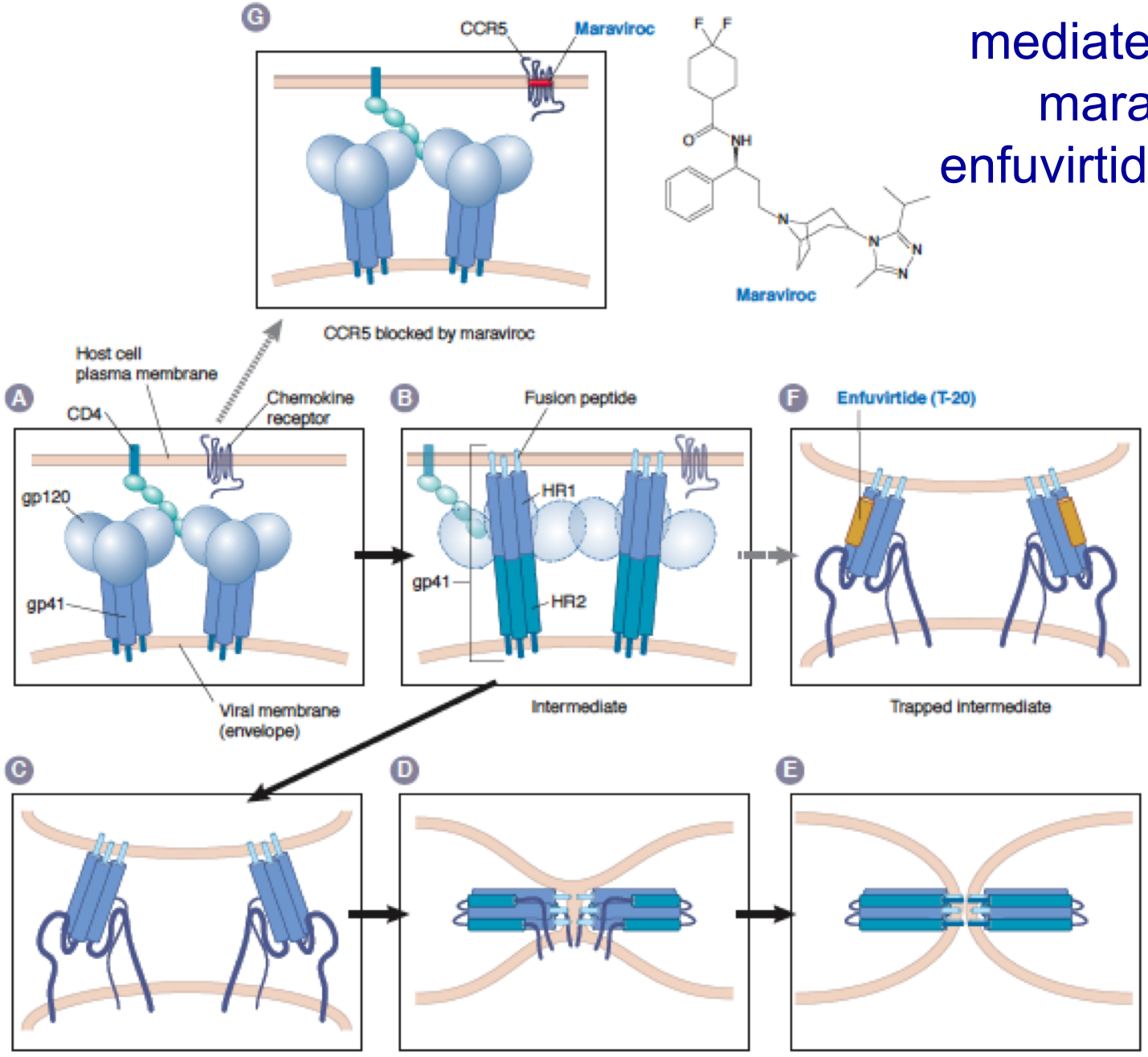
- ✓ They interact with the intermediate form of **gp41**



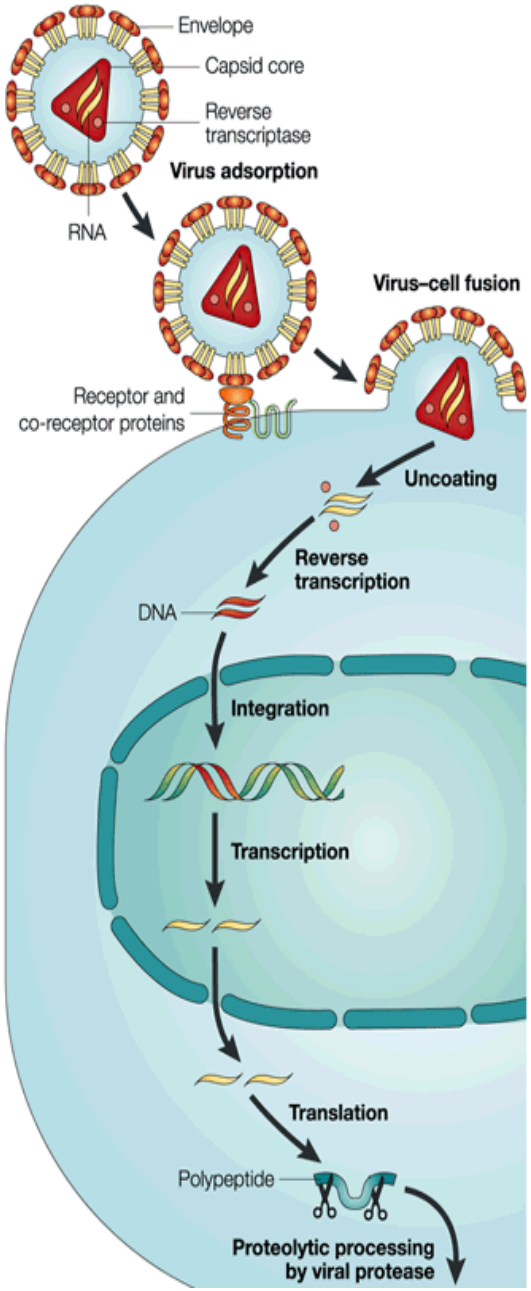
- ✓ **Enfuvirtide (T20)**

A synthetic peptide homologs to a conserved 36 aa sequence within the HR2 of gp41.
It hampers the formation of HR1/HR2 complex, thus preventing membranes fusion.
FDA Approved in 2003.
It should not develop cross-resistance with other available drugs.

Model for HIV gp41-mediated fusion and maraviroc and enfuvirtide (t20) action



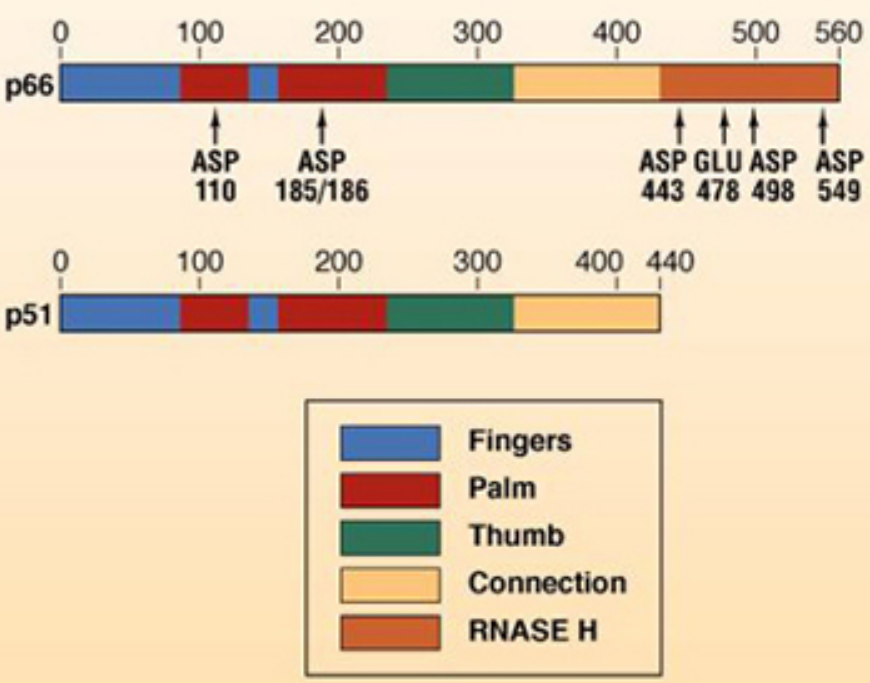
Druggable targets in HIV replication cycle

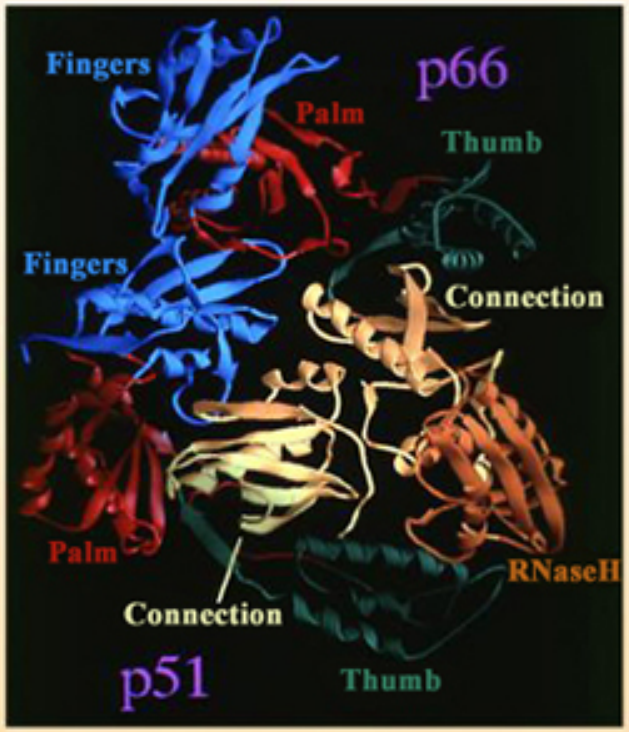


Reverse Transcription

Structure of HIV reverse transcriptase (RT)

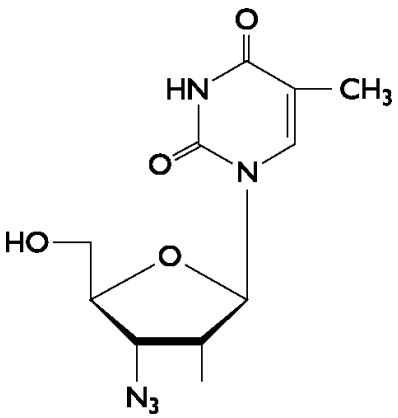
Heterodimer of **p51** and **p66** subunits



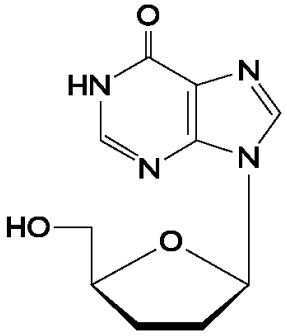


HIV RT inhibitors

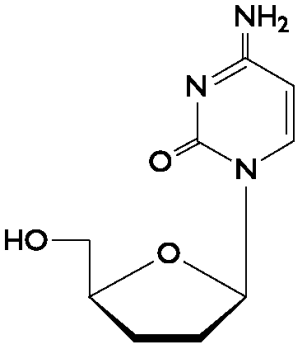
Nucleoside analogs (NRTI):



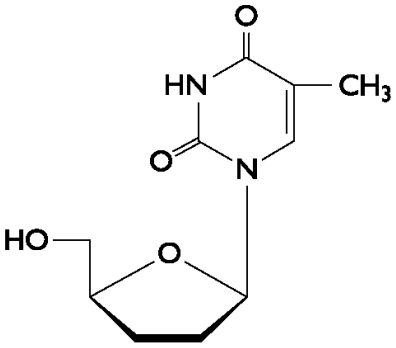
Zidovudine
(AZT)



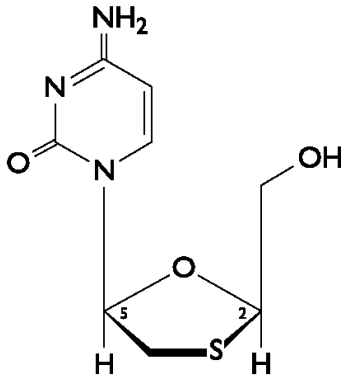
Didanosine
(ddI)



Zalcitabine
(ddC)



Stavudine
(d4T)



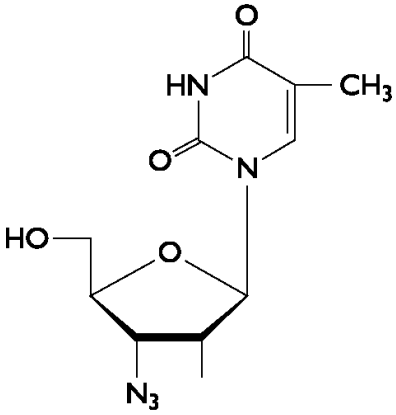
Lamivudine
(3TC)

✓ Competitive inhibitors acting as chain terminators in the synthesis of proviral DNA

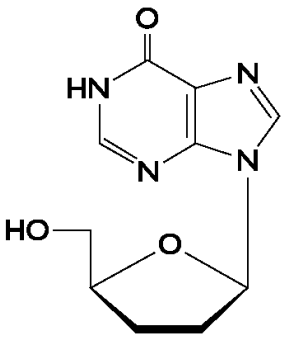
✓ Their selectivity of action stems from their affinity for RT that is 100-folds higher than that for human DNA polymerases

HIV RT inhibitors

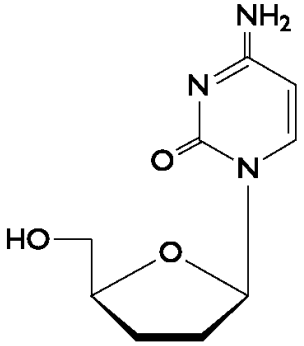
Nucleoside analogs (NRTI):



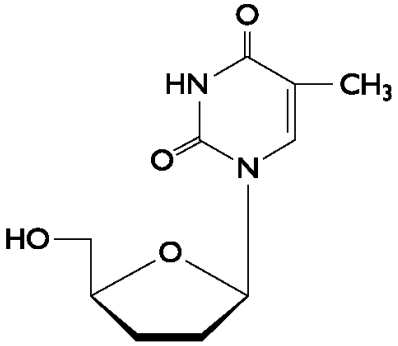
Zidovudine
(AZT)



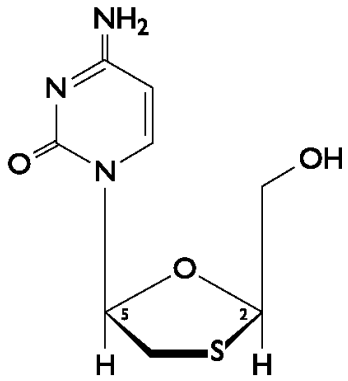
Didanosine
(ddI)



Zalcitabine
(ddC)



Stavudine
(d4T)



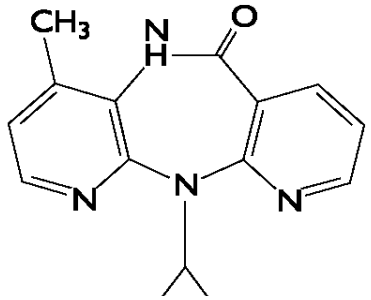
Lamivudine
(3TC)

- ✓ **RANGE OF ACTION:** HIV-1 HIV-2, HTLV-1
  Inhibit viral replication in peripheral blood lymphocytes and macrophages of the central nervous system, but are less active on monocyte-macrophages and resting cells.

- ✓ **RESISTANCES:** Frequent and sometimes crossresistance for RT mutations

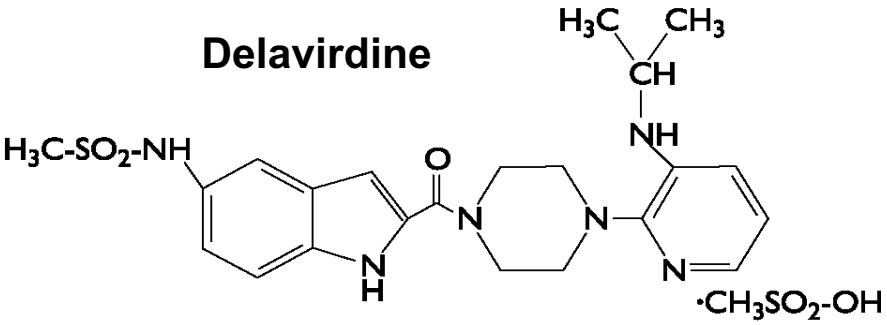
HIV RT inhibitors

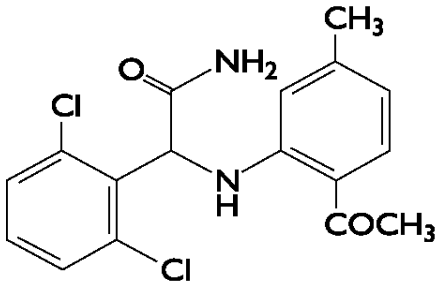
Non-nucleoside inhibitors (NNRTI):



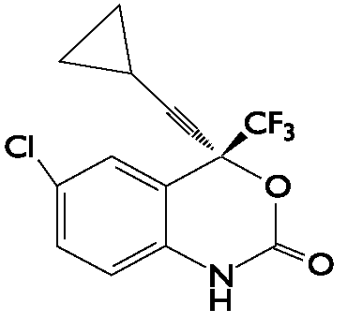
Nevirapine

Delavirdine





Loviride

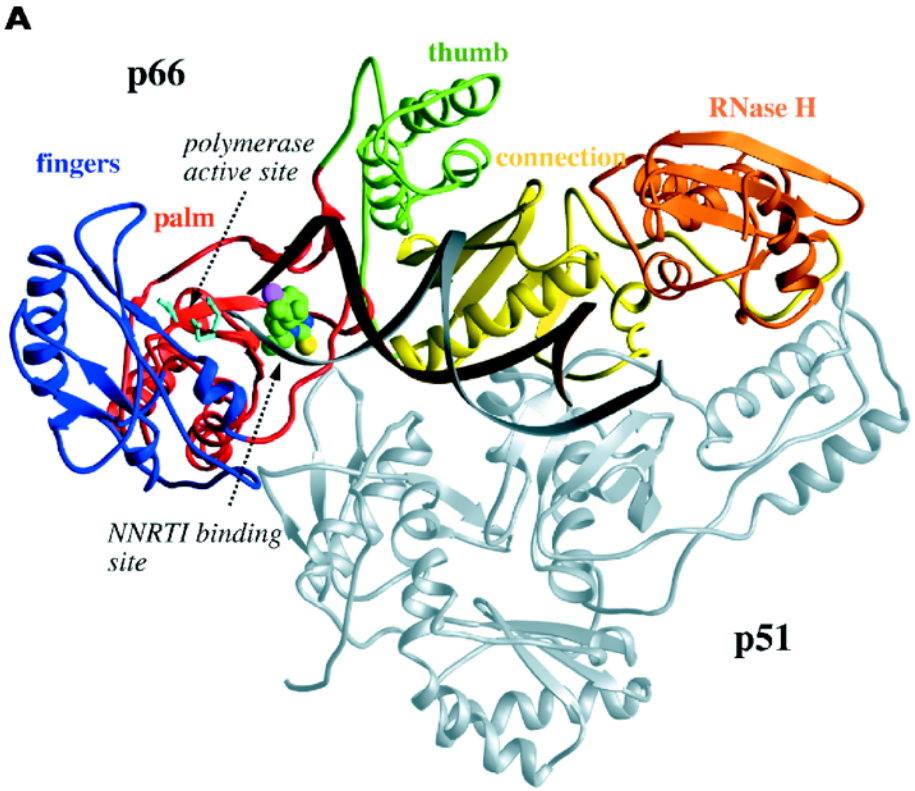


Efavirenz

- ✓ Non-competitive RT inhibitors that are not incorporated in the growing proviral DNA chain

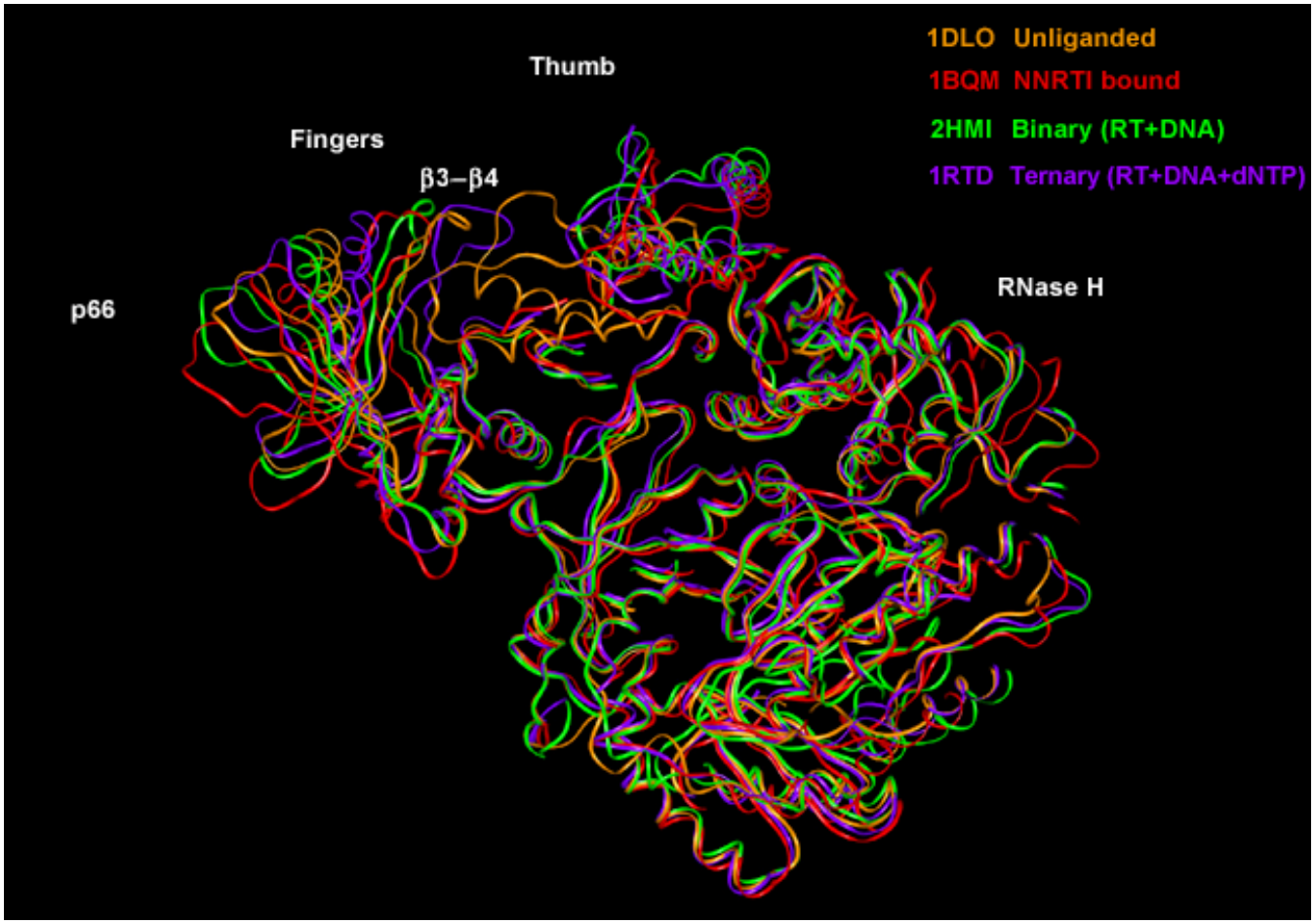
HIV RT inhibitors

Non-nucleoside inhibitors (NNRTI):



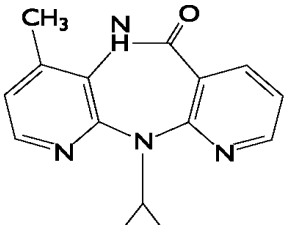
- ✓ They bind to a hydrophobic pocket close to the active site of RT (NNRTI pocket), thus blocking proviral DNA synthesis

Nonnucleoside inhibitors of HIV RT (NNRTI)

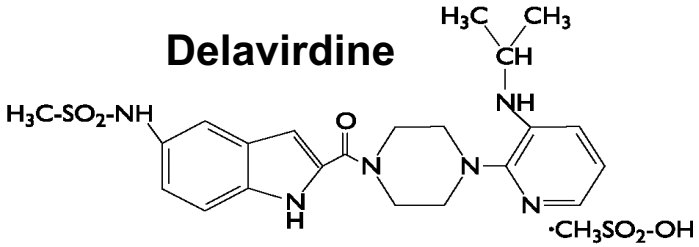


- ✓ The mechanism of action is unclear, however it is thought they alter the structure of the enzyme at the catalytic site, the binding site of template nucleic acid and of the binding site for dNTP

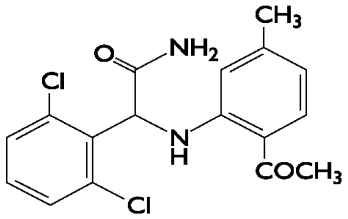
Nonnucleoside inhibitors of HIV RT (NNRTI)



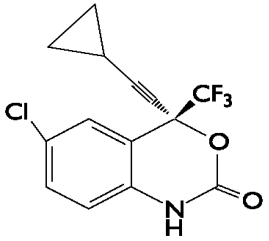
Nevirapine



Delavirdine



Loviride

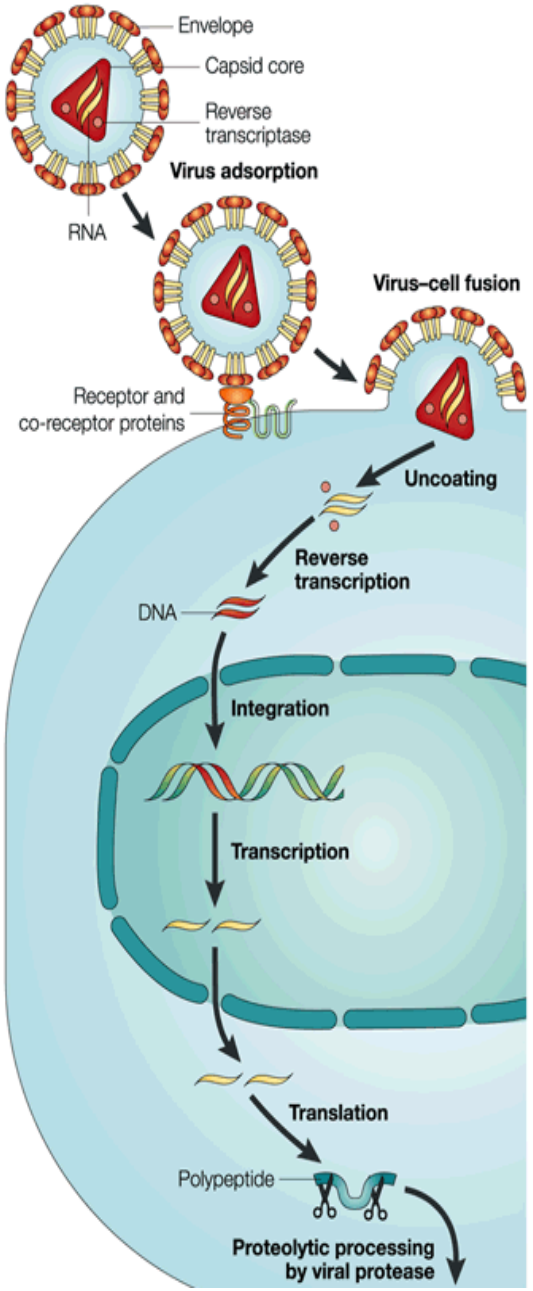


Efavirenz

- ✓ **RANGE OF ACTION:** Active against HIV-1 only.
  Always used in combination therapies.

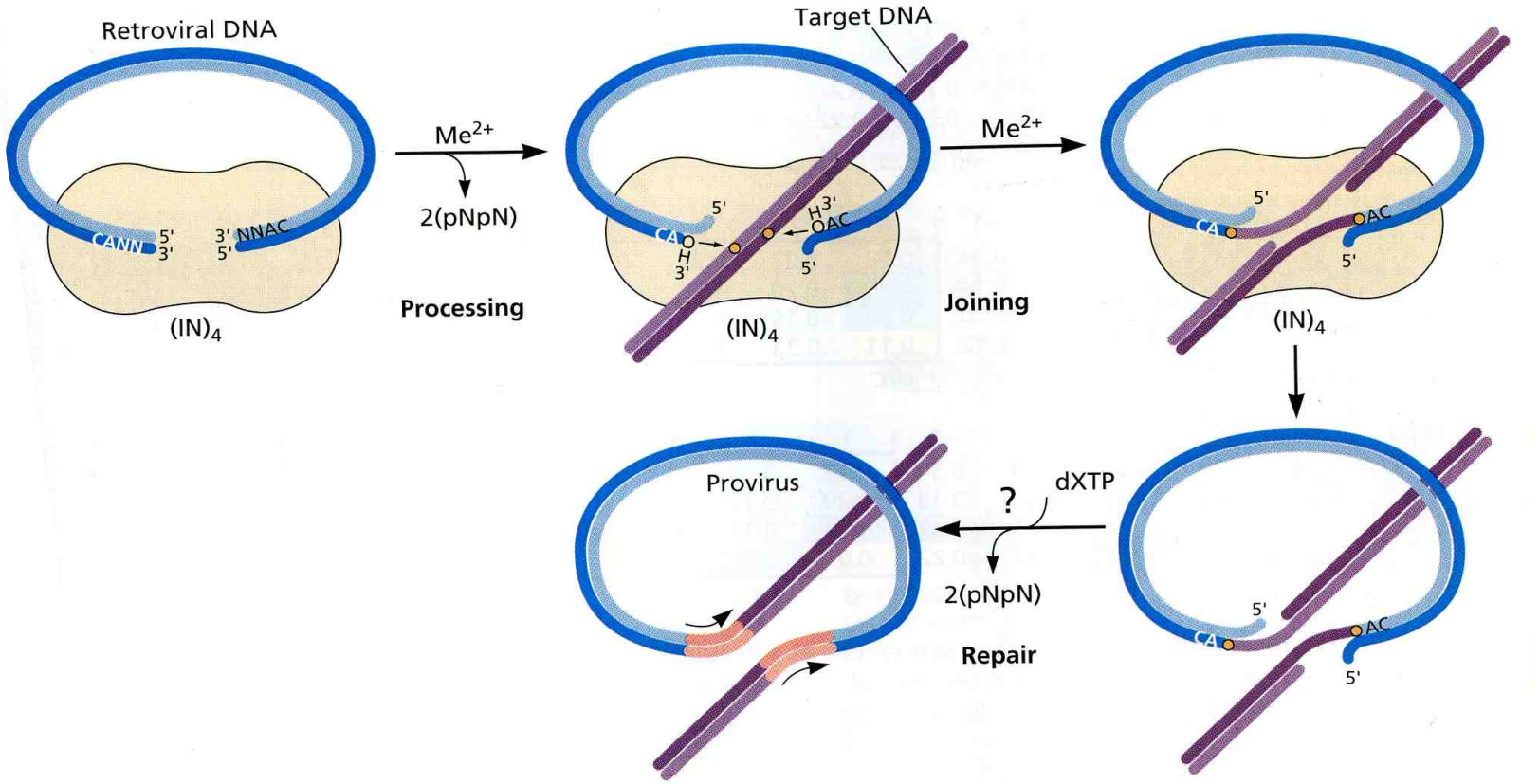
- ✓ **RESISTANCES:** Resistances and cross-resistances are frequent for point mutations in the RT. No reported cross-resistances with nucleoside analogues and protease inhibitors.

Druggable targets in HIV replication cycle



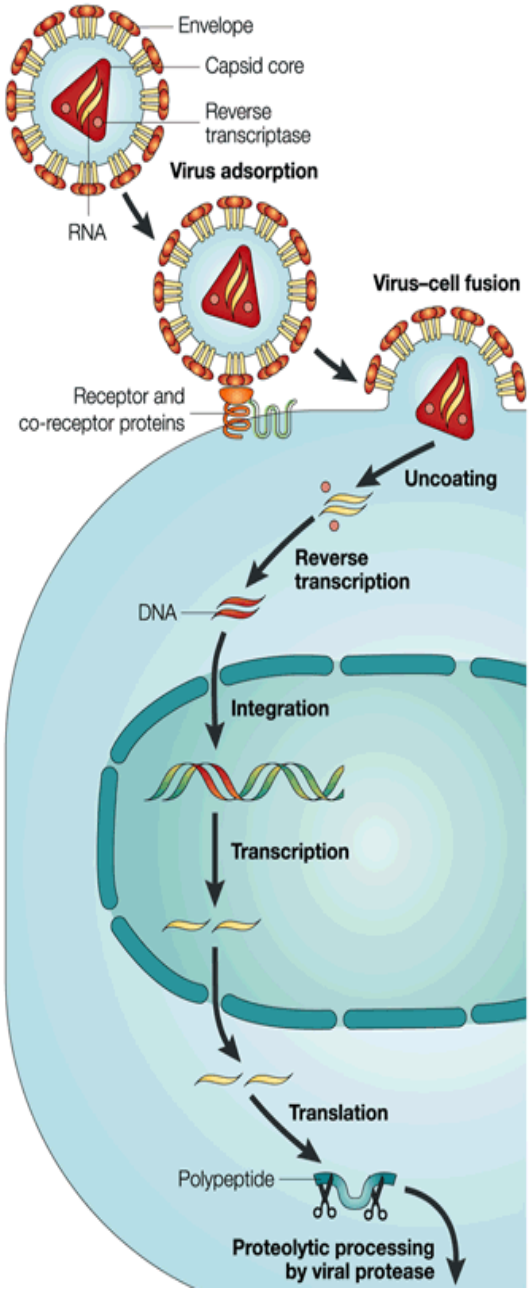
Integration

Development of HIV Integrase Inhibitors



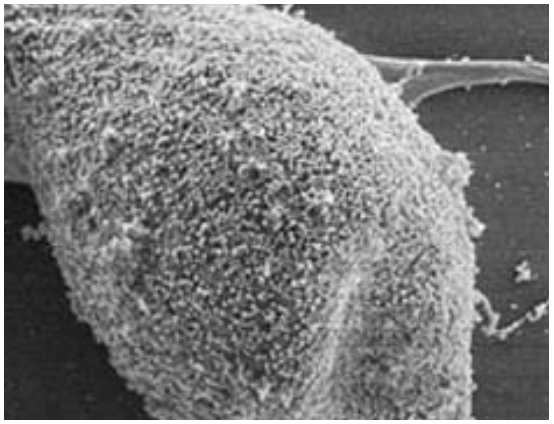
- ✓ The HIV integrase is an excellent drug target because it is a unique recombinase that mediates the insertion of retroviral DNA into host genome, where it is called a provirus
- ✓ HIV integrase is a tetrameric enzyme (crystal structure solved)
- ✓ These drugs act as strand transfer inhibitors that block the joining step

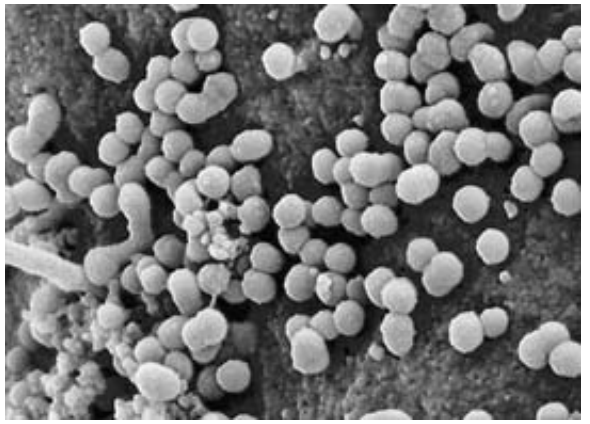
Druggable targets in HIV replication cycle

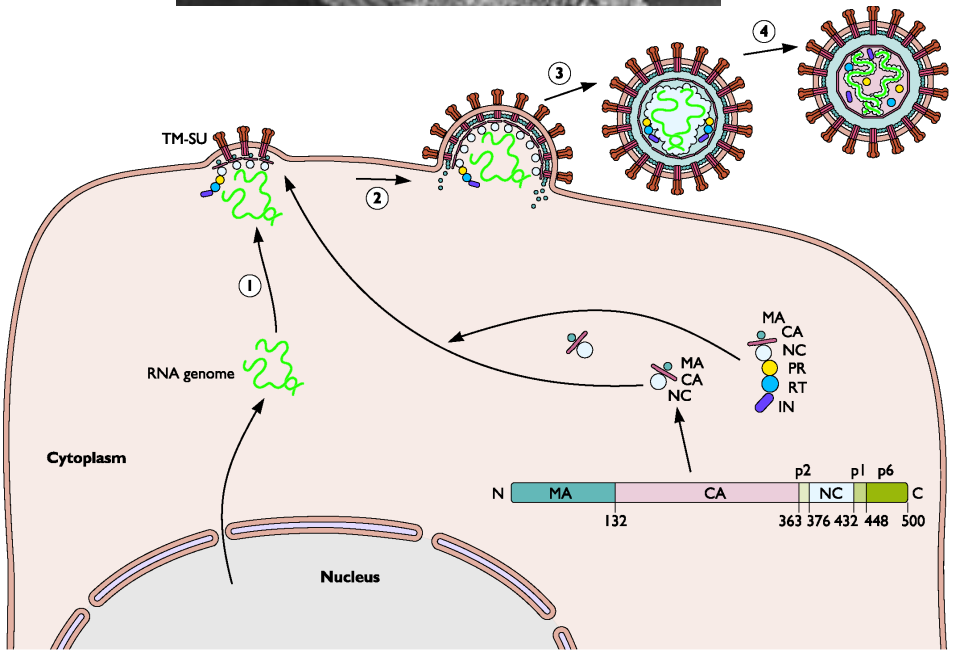


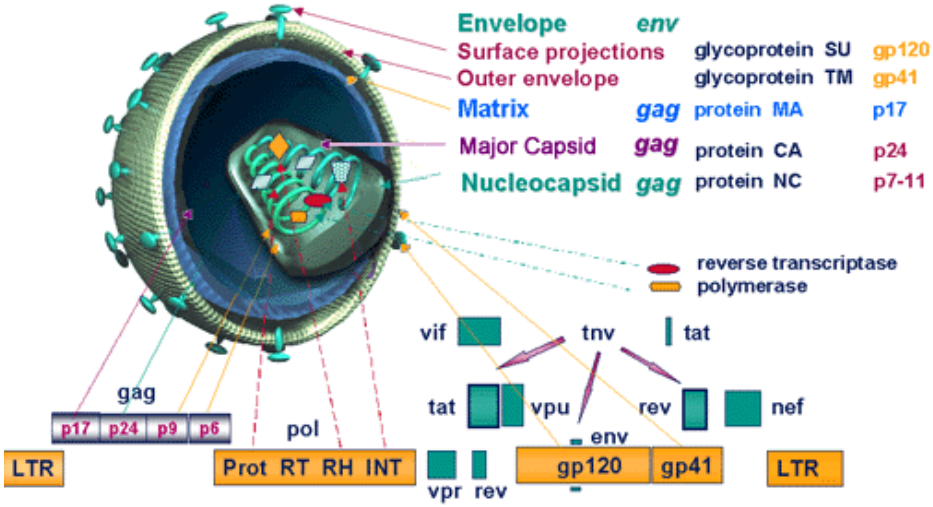
Maturation

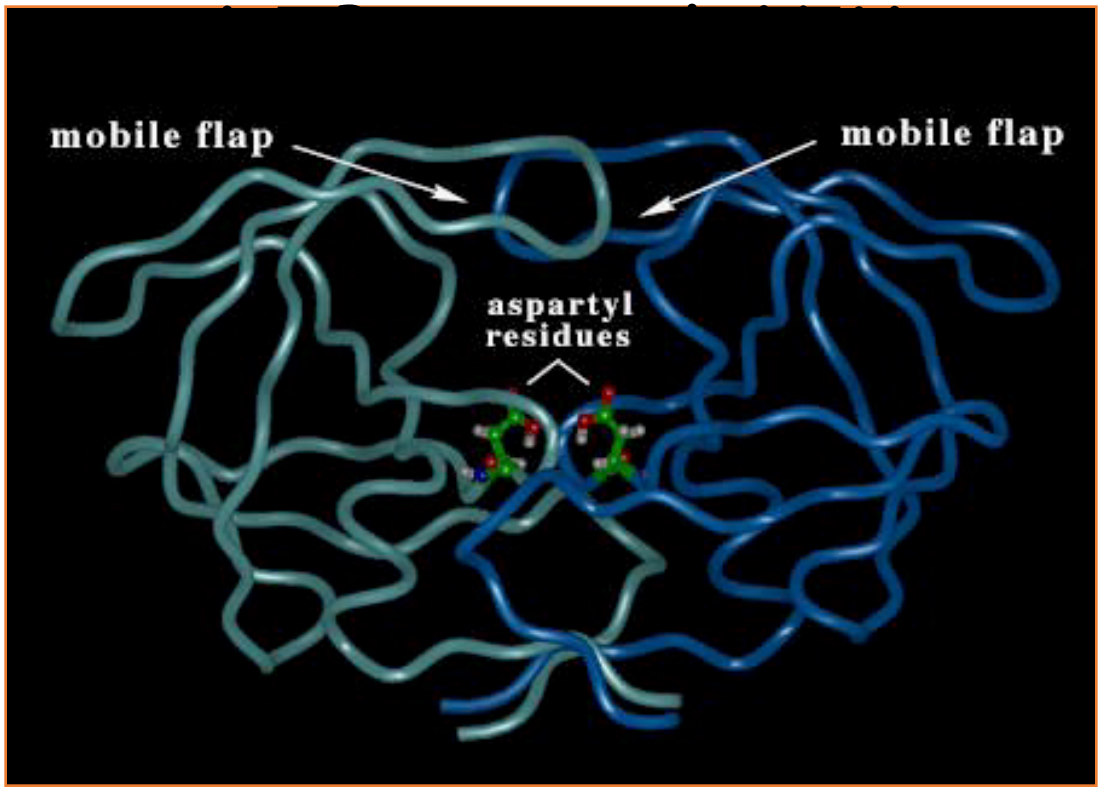
HIV egress by budding at the plasma membrane







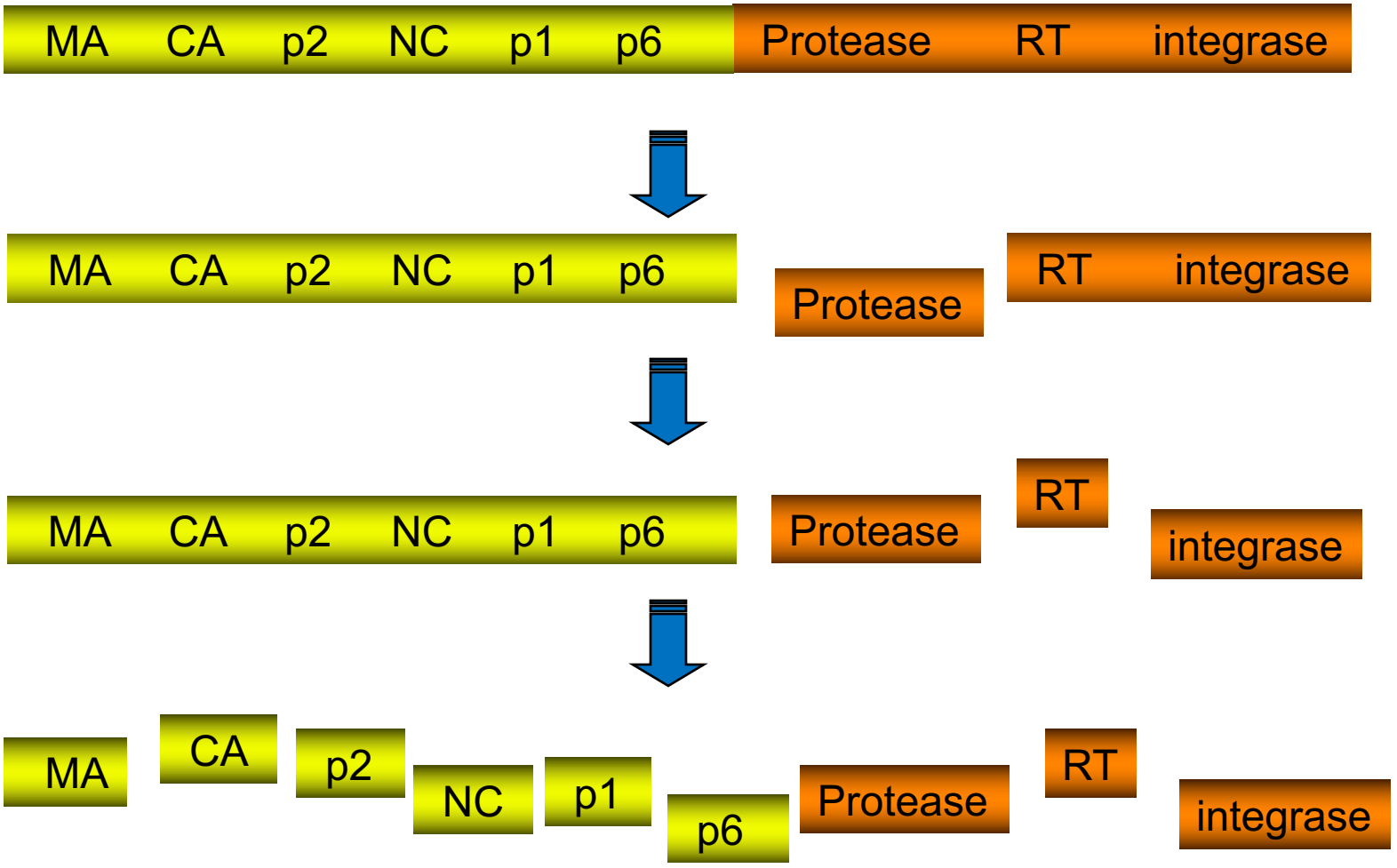




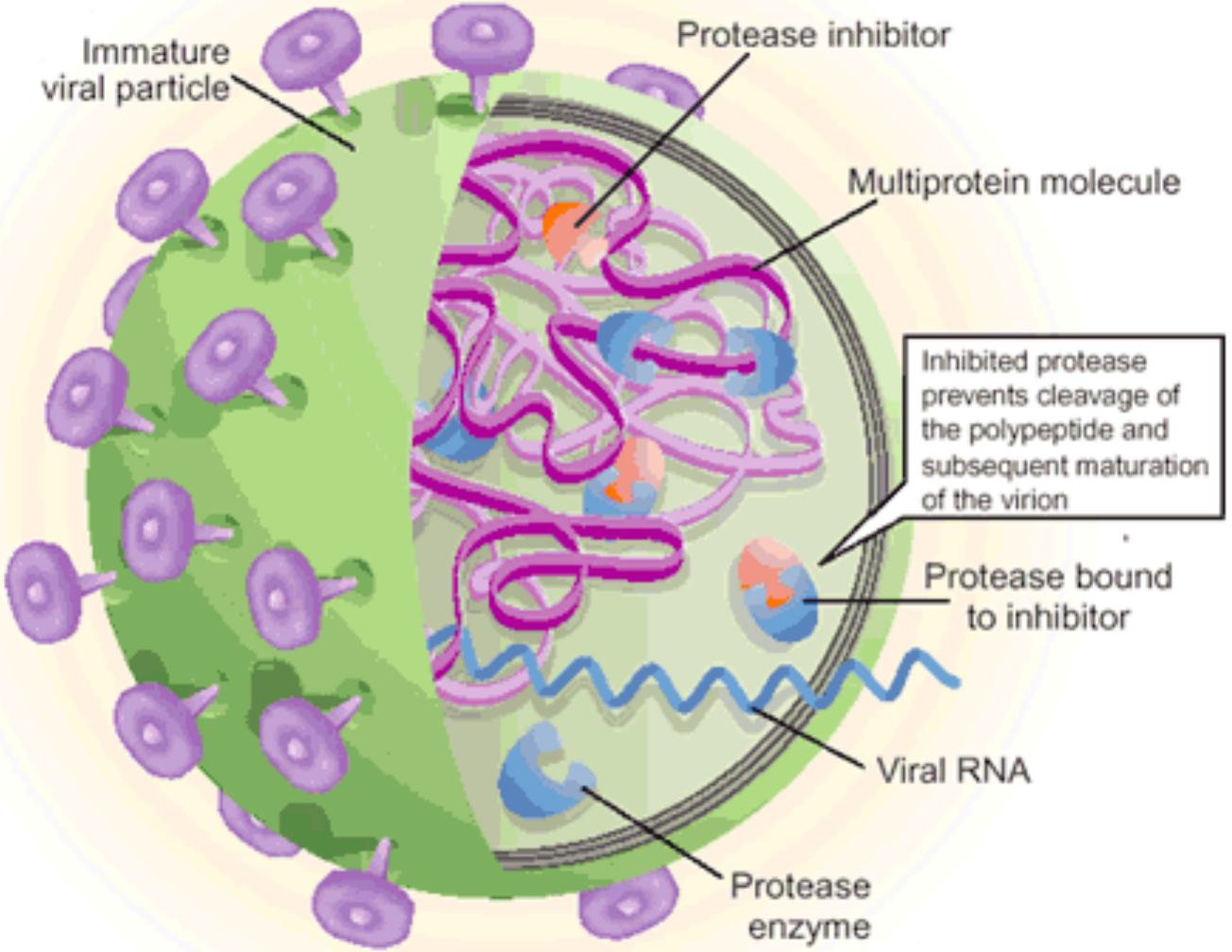
- ✓ The HIV-1 protease is a 99 aa protein encoded by ***pol***
- ✓ Dimeric
- ✓ Belongs to the aspartyl-proteases family (as rennin and pepsin)
- ✓ Asp 25 is crucial for enzyme activity
- ✓ HIV protease is essential for release of mature virions

Role of HIV protease

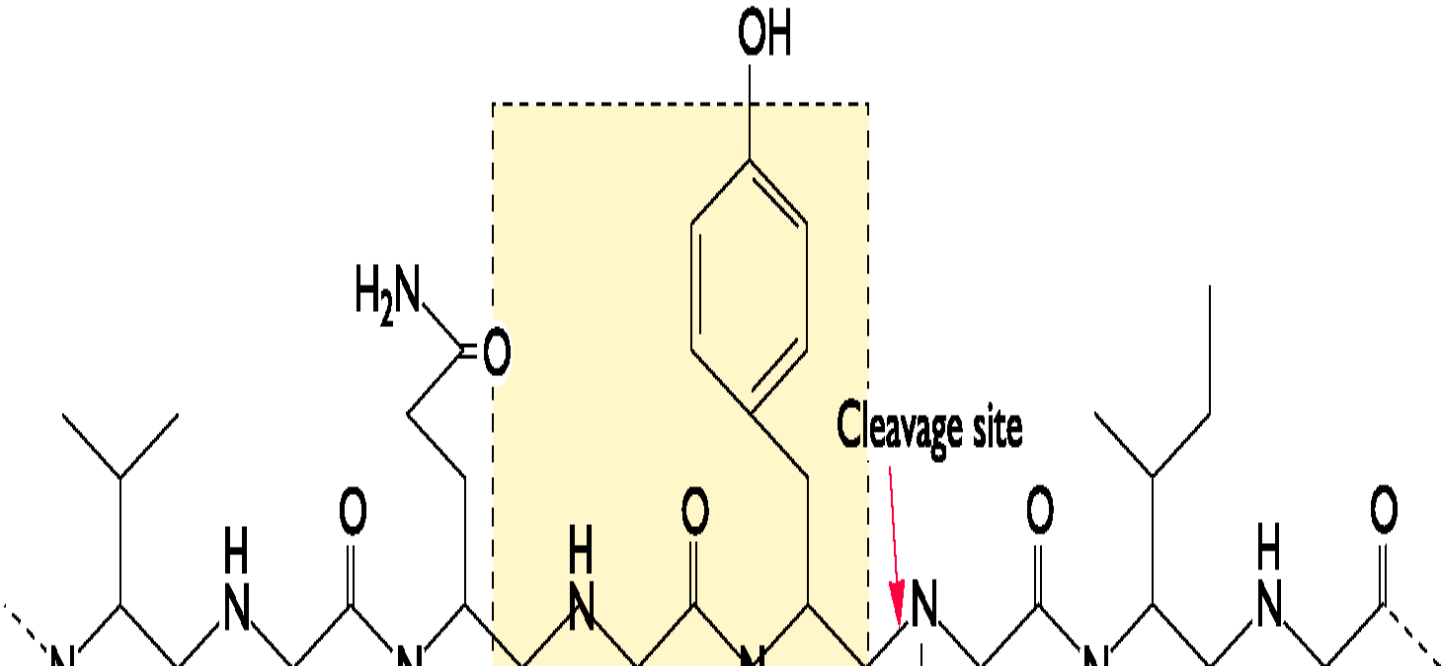
gag-pol polyprotein (9 cutting sites)



Role of HIV protease



Activity of HIV protease

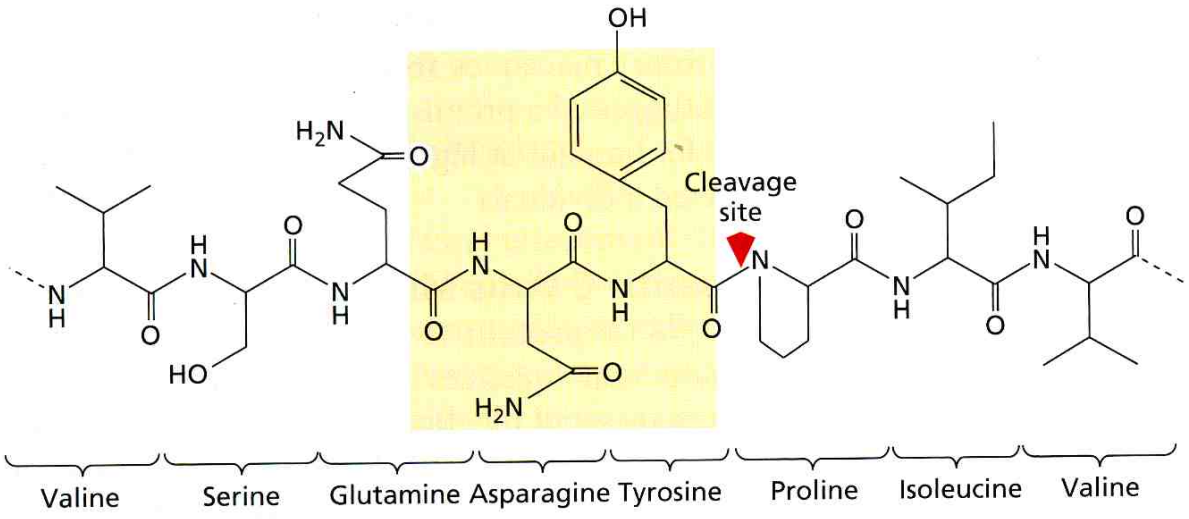


- ✓ HIV protease cuts between phenilalanine, or tyrosine, and a proline
- ✓ No human protease shows this specificity:

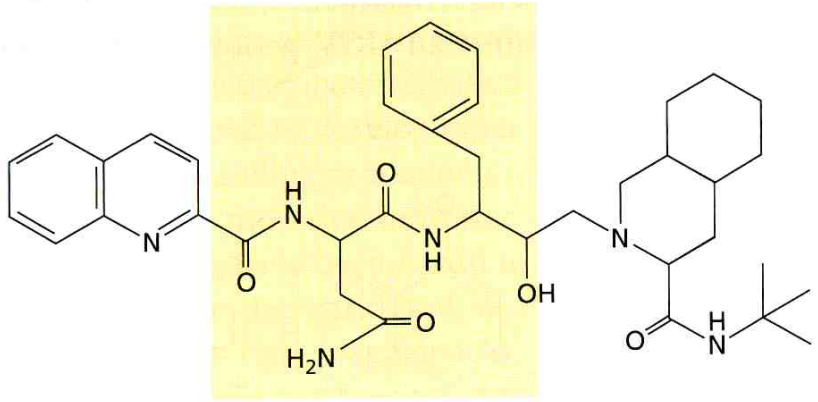
Thus, it possible to develop specific inhibitors!

Development of HIV PR inhibitors

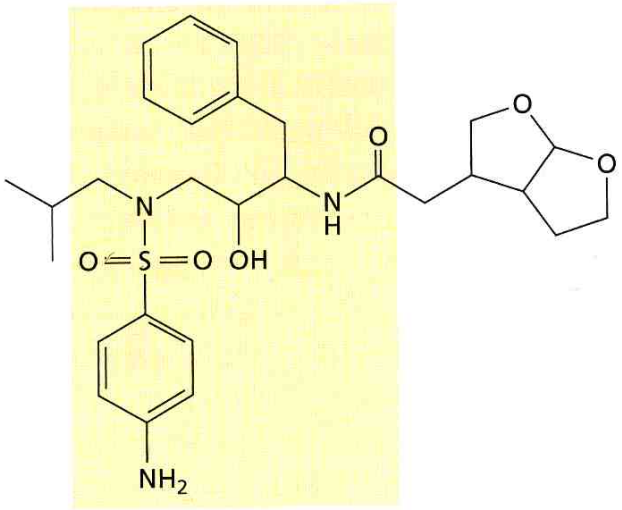
A Natural substrate of the HIV-1 protease



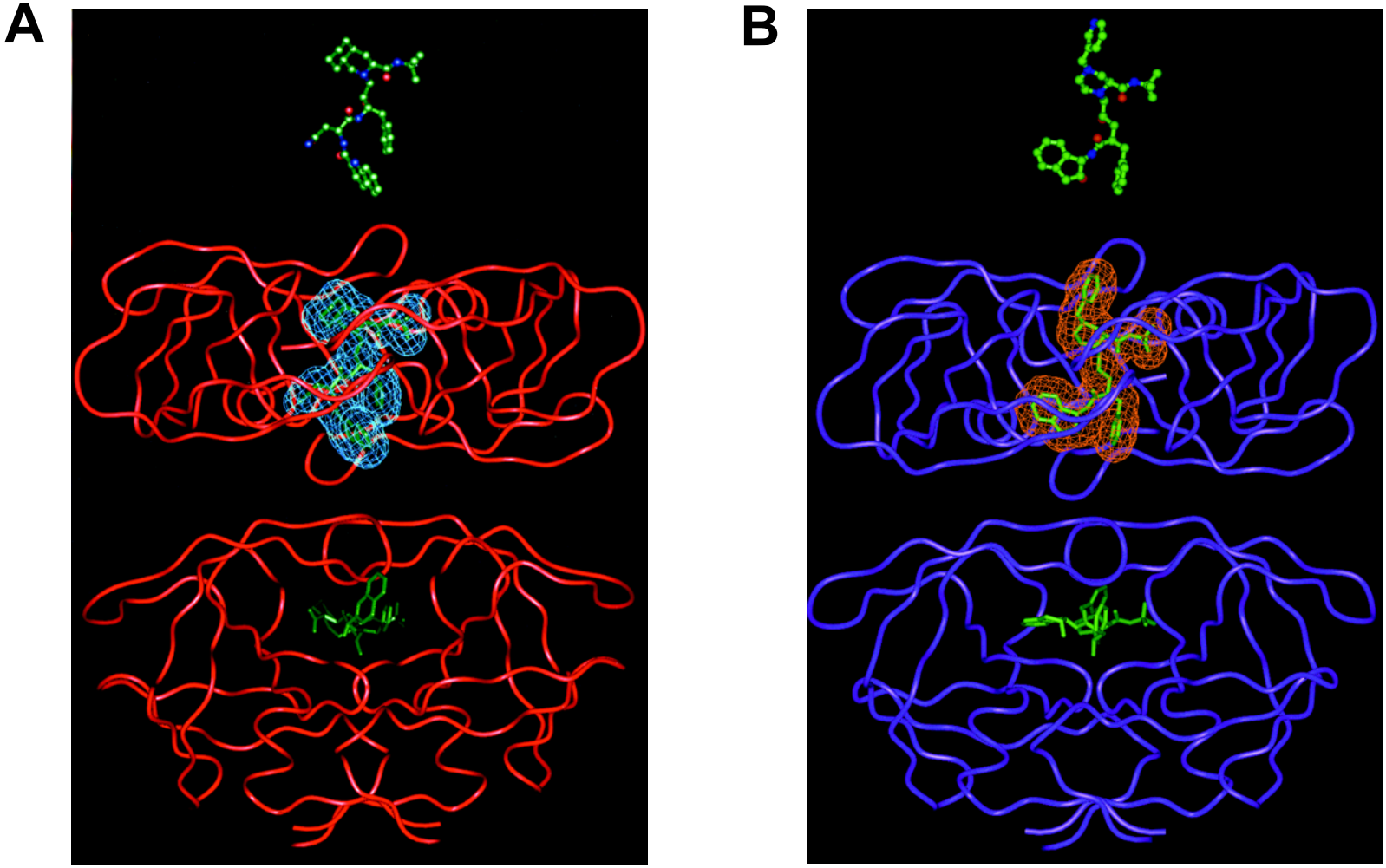
B Saquinavir



C Darunavir



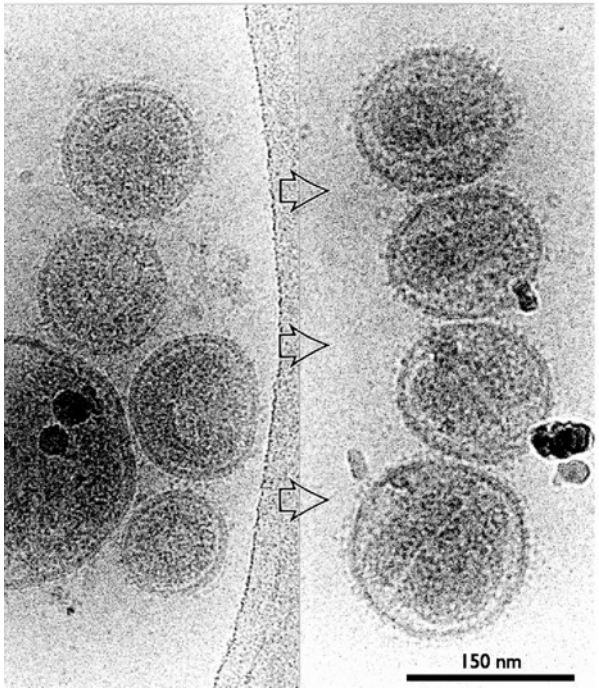
Development of HIV PR inhibitors



HIV PR structure bound to saquinavir (A) and indinavir (B) inhibitors

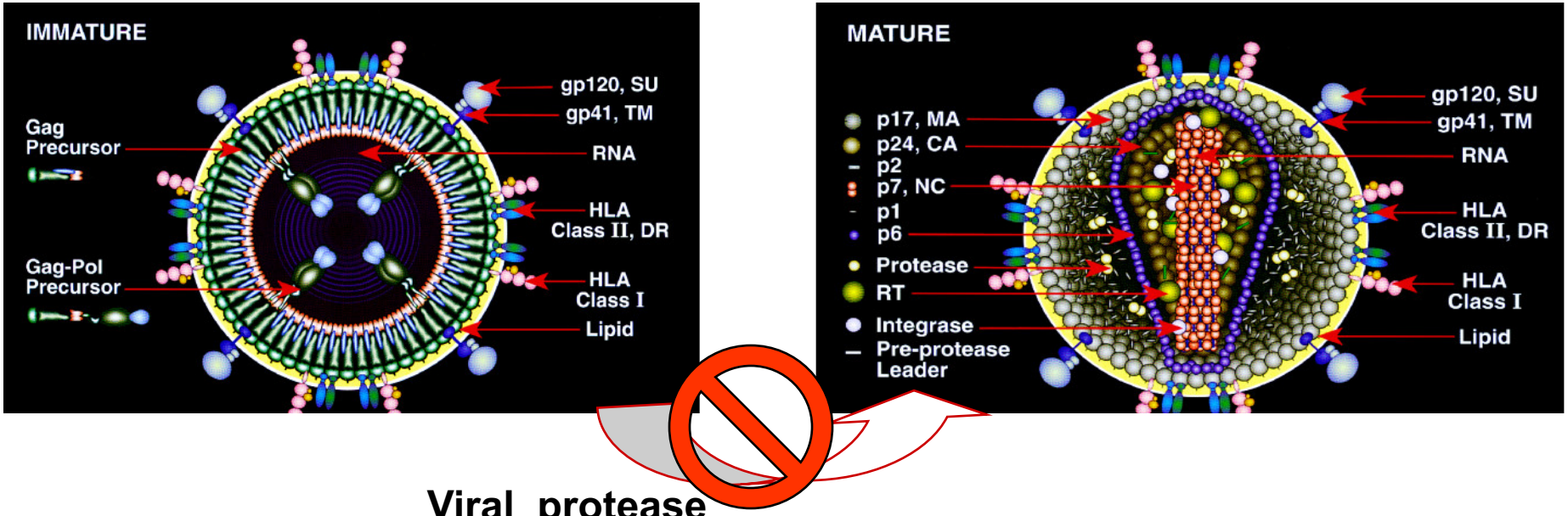
Vondrasek J. et al., *Nat. Struct. Biol.* 4:8, 1997

Effect of inhibition of HIV PR activity



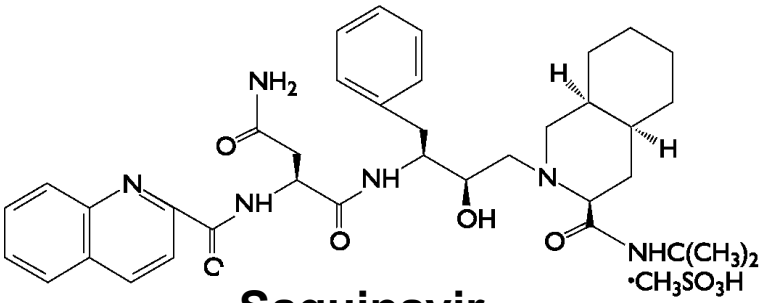
Immature virion

Mature virion

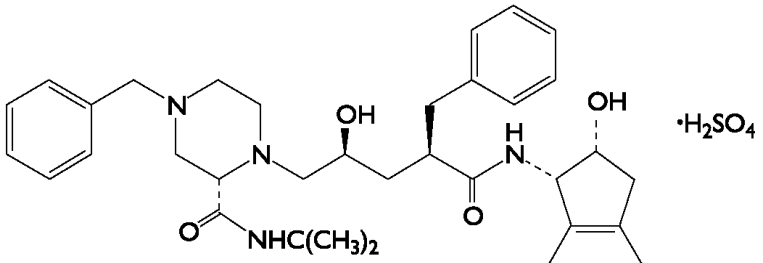


Viral protease

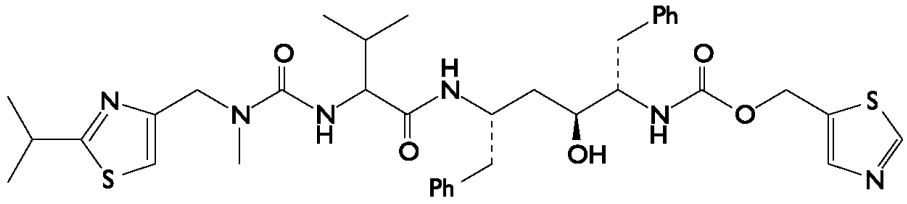
Inhibitors of HIV protease



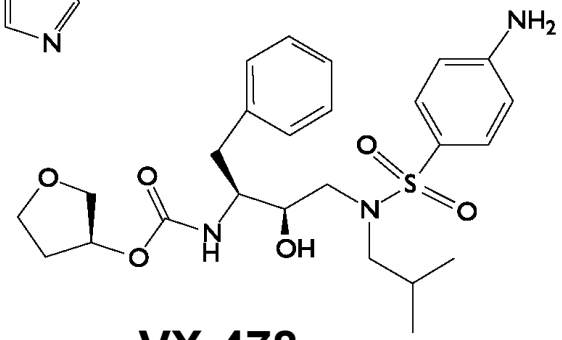
Saquinavir



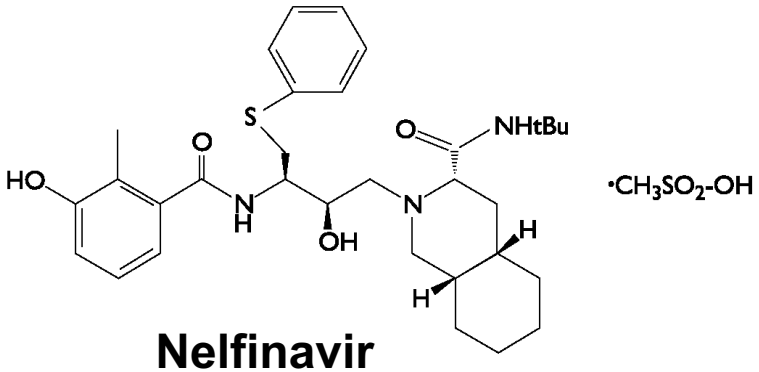
Indinavir



Ritonavir



VX-478



Nelfinavir

Inhibitors of HIV protease: summary

- ✓ **Peptidomimetics**
- ✓ They act as competitive inhibitors
- ✓ Active against HIV-1 and HIV-2
- ✓ Viral resistance frequent in monotherapy, sometimes cross-resistance

Summary of HIV pathogenesis

Human immunodeficiency virus types 1 and 2

Virus	Disease
Deltaretrovirus	
• Human T-lymphotropic virus type 1	Adult T-cell leukemia Tropical spastic paraparesis
• Human T-lymphotropic virus type 2	Hairy-cell leukemia
• Human T-lymphotropic virus type 5	Malignant cutaneous lymphoma
Lentivirus	
• **Human immunodeficiency virus types 1 and 2**	Acquired immune deficiency syndrome

Epidemiology

Transmission
- Virus in blood
  - Transfusions, needle sharing among drug users, needle sticks in health care workers, tattoo needles
- Virus in semen and vaginal secretions
  - Anal and vaginal intercourse
- Perinatal transmission
  - Intrauterine and peripartum transmission; breast milk

Distribution of virus
- Ubiquitous
- No seasonal incidence

At risk
- Intravenous drug users
- Homosexuals and heterosexuals with many partners
- Prostitutes
- Newborns of virus-positive mothers

Vaccines or antiviral drugs
- No vaccines
- Antiviral drugs
  - Nucleoside analog reverse transcriptase inhibitors (e.g., azidothymidine, dideoxycytidine)
  - Nonnucleoside reverse transcriptase inhibitors (e.g., nevirapine, delavirdine)
  - Protease inhibitors (e.g., saquinavir, ritonavir)

Disease mechanisms

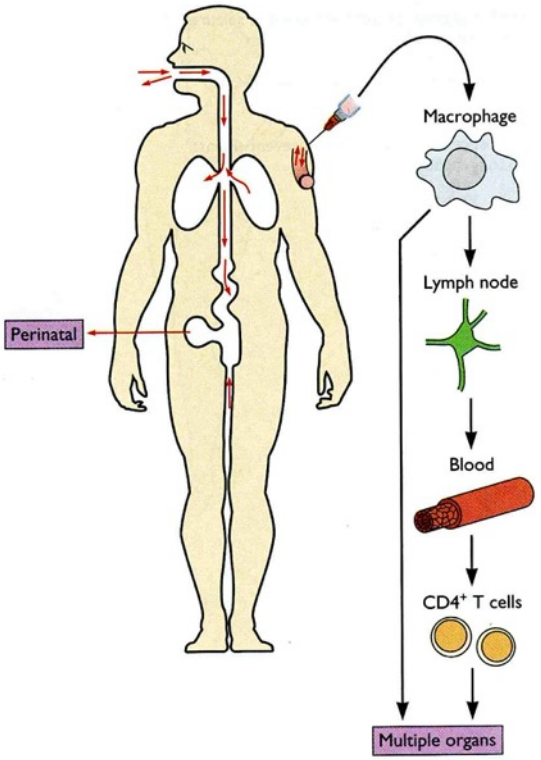
Infects mainly $CD4^+$ T cells and macrophages

Lyses $CD4^+$ T cells, persistently infects macrophages

Infection alters T-cell and macrophage function; immunosuppression leads to secondary infection and death

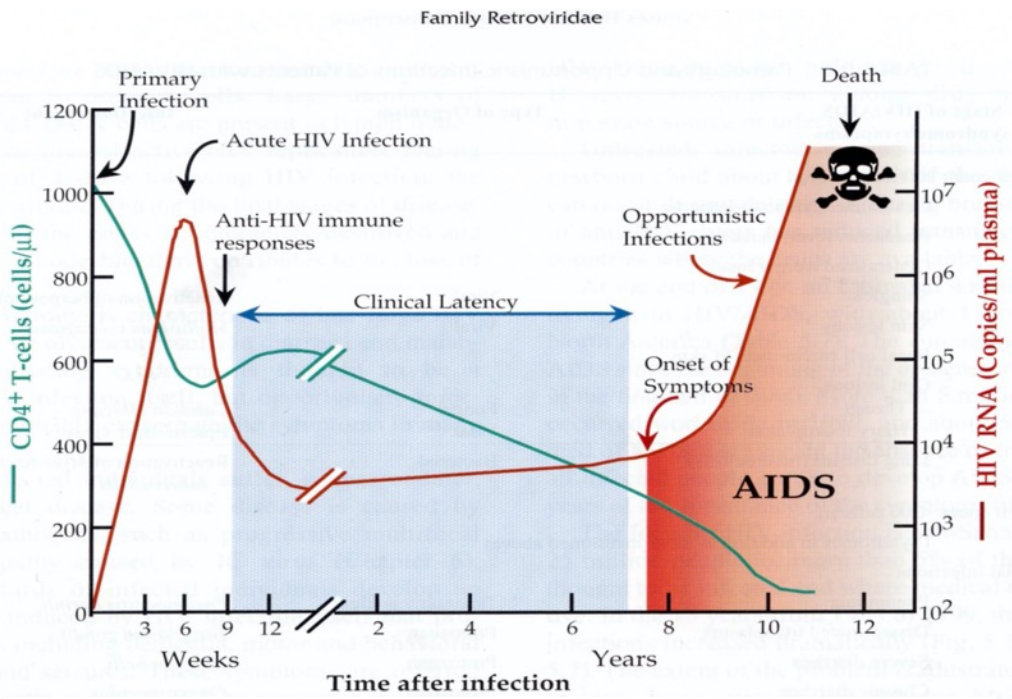
Infects long-lived cells, establishing reservoir for persistent infection

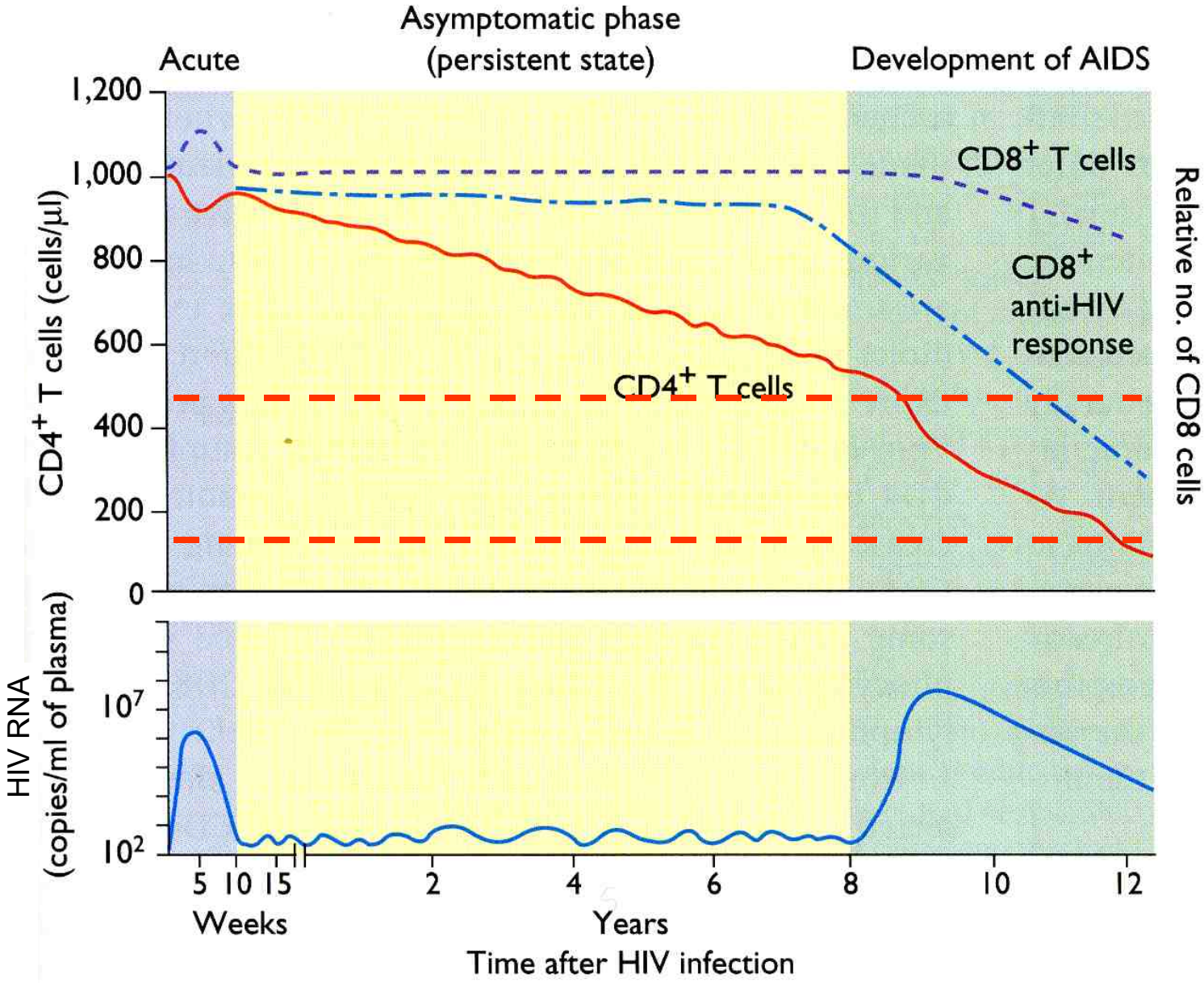
Infected monocytes spread to brain, causing dementia



The course of infection

- Infection
- The Acute Phase
- The Asymptomatic Phase
- The Symptomatic phase and AIDS




Asymptomatic phase
(persistent state)
Acute
Development of AIDS
1,200
1,000
800
600
400
200
0
CD4+ T cells (cells/μl)
CD8+ T cells
CD8+
anti-HIV
response
CD4+ T cells
Relative no. of CD8 cells
HIV RNA
(copies/ml of plasma)
10^7
10^2
5 10 15
2
4
6
8
10
12
Weeks
Years
Time after HIV infection

Monitoring the efficacy of anti-HIV therapy

Two indicators of disease state are used:

- ✓ **T lymphocytes CD4+ number**

  >500/ml normal value
  <200/ml AIDS

  Cytofluorimetry

- ✓ **HIV RNA copies number**

  50 copie/ml of plasma is the detection limit

  Real Time PCR

Monitoring the efficacy of anti-HIV therapy

When anti-HIV therapy works…..

The n° of T CD4+ lymphocytes: INCREASES

Immunoreconstitution

The n° of HIV RNA copies: DECREASES

Viral replication is blocked

The problem of viral resistances

REPLICATION=MUTATION

Mechanims of drug resistance

- RNA viruses: error prone RNA polymerase, no correction mechanism
- One misincorporation in 10^4-10^5 nucleotides polymerized (10^3-10^4 greater than host DNA genome)
- In a viral RNA genome of 10 kb, a mutation frequency of 1 in 10^4 per template copied corresponds to an average of 1 mutation in ***every*** replicated genome.

Math of HIV drug resistance

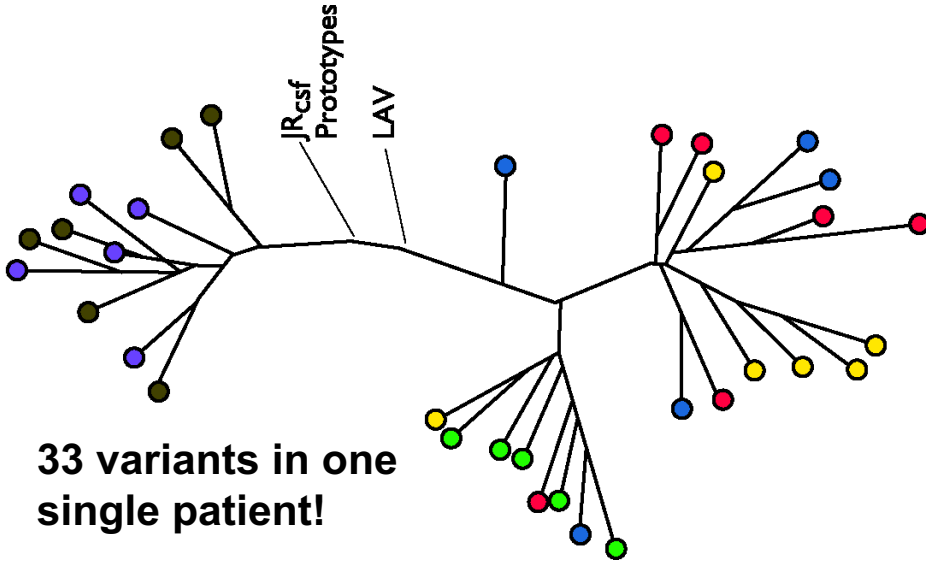
- Assume that one mutation is needed for drug resistance
- Mutation rate of 1 every 10^4 bases polymerized
- Each base in the genome is substituted in every 10^4 viruses
- Each HIV-infected individual makes 10^{10} new viruses/day
- $10^{10}/10^4 = 10^6$ viruses will be produced each day with resistance to one drug

The problem of viral resistances

REPLICATION=MUTATION

- ✓ **10^{10}** virons are produced every day…
- ✓ Assuming a mutation rate of about **10^{-4}** …
- ✓ Without proofreading activity of RT…

Each new viral genome (9200 nt) bears a mutation

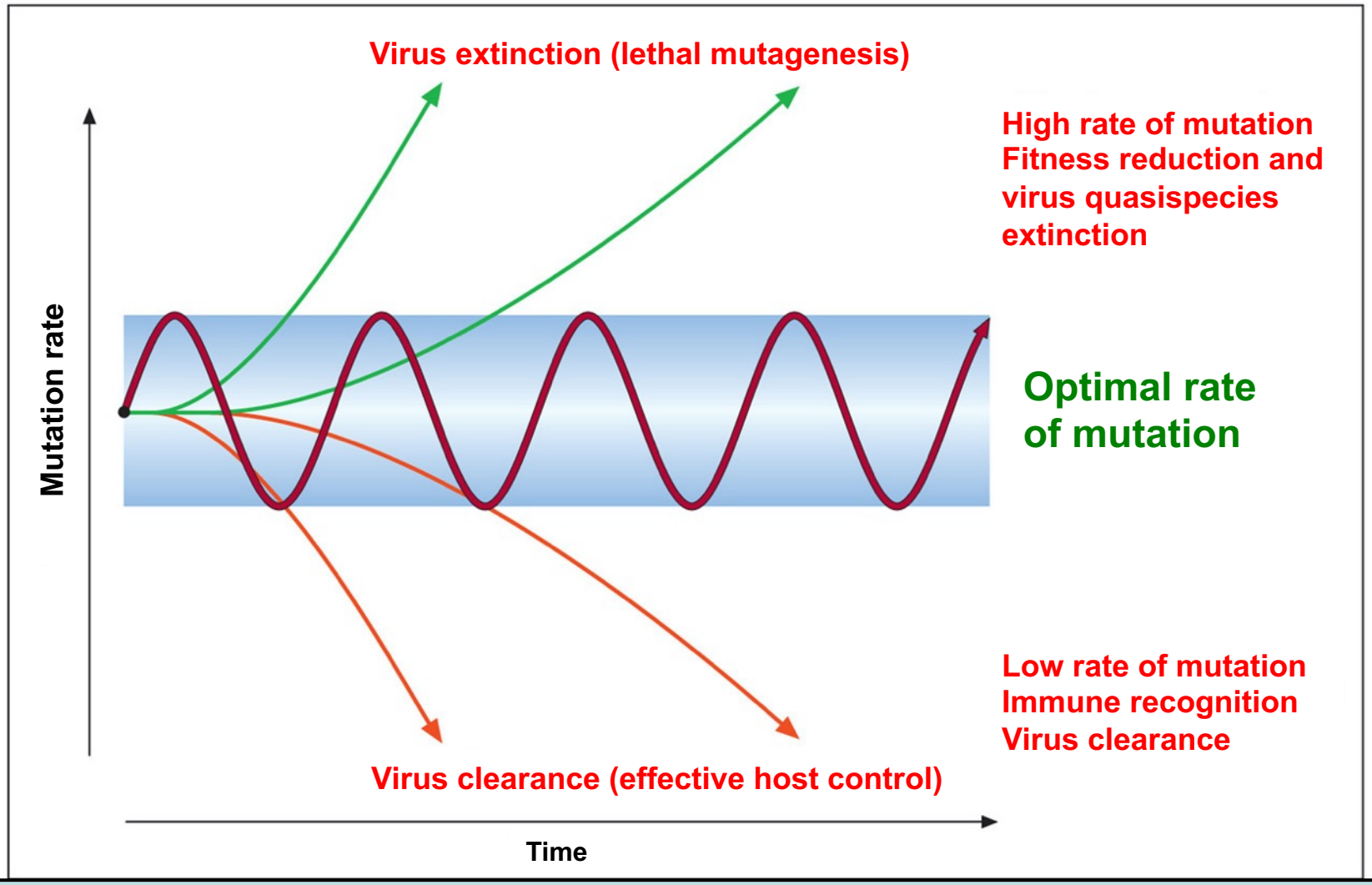


33 variants in one single patient!

HIV evolution in a single patient	
BLU	lymphonodes
RED	blood
YELLOW	spleen
GREEN	lungs
BLACK	dorsal ganglia

Ait-Khaled et al., AIDS 9:657-683, 1995

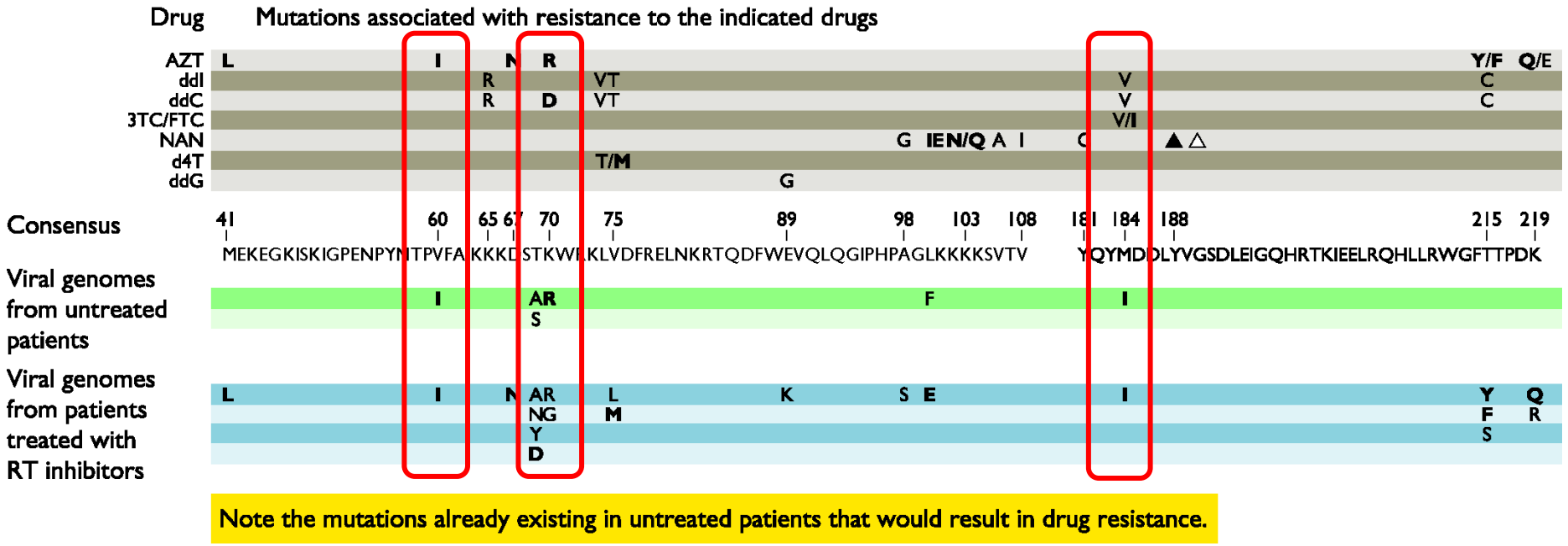
Genetic variability of RNA viruses (HIV, HCV), optimal rate of mutation and virus evolution



The quasispecies concept: The genome of virus population exists as a weigthed average of a large number of different individual sequences as a dynamic distributions of nonidentical but related replicons, called **quasispecies**.

The problem of viral resistances

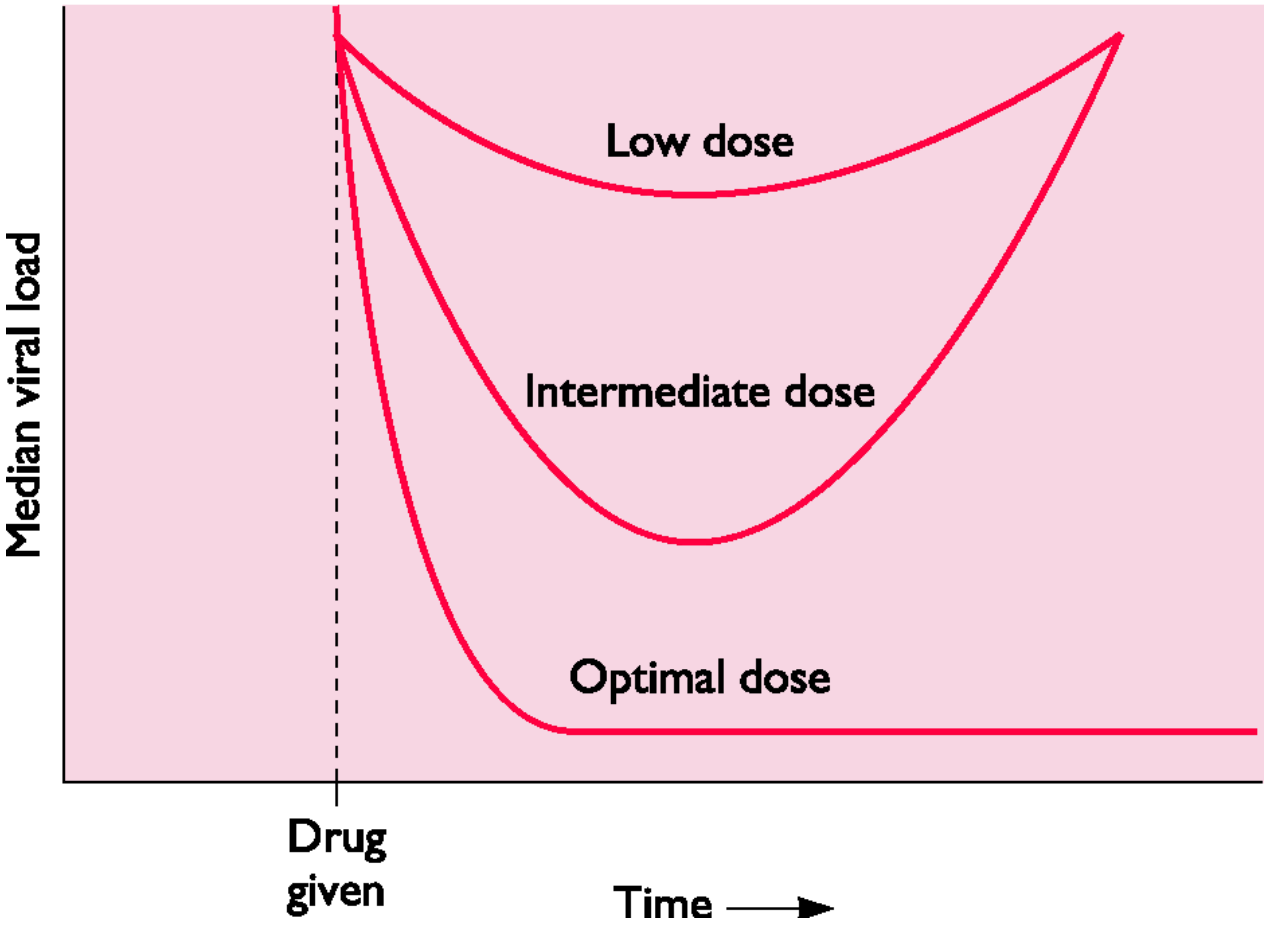
REPLICATION=MUTATION



MUTATIONS EMERGE SPONTANEOUSLY WITHOUT SELECTION

The problem of viral resistances

REPLICATION=MUTATION



If the administered drug dose does not completely suppresses viral replication, mutant viruses will be selected

HAART: highly active antiretroviral therapy

- ✓ It combines the use of of RT and protease inhibitors
- ✓ It allows to obtain a greater antiretroviral potency

- ✓ It reduces the occurrence of resistant strains.

  Eg. If the resistance to a drug occurs once every 10^4 virion and that of another drug occurs every 10^4 virions, the probability to generate a genome bearing the two mutations is given by the product of the two probabilities, so: 1 of 10^8 virions

  $10^{10}/10^8$= 100 viruses resistant to the two drugs per day

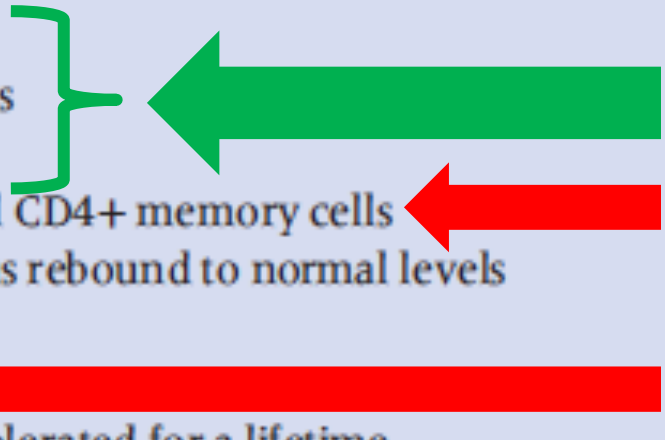
  Resistance to thre drugs: $10^4 \times 10^4 \times 10^4 = 10^{12}$ virus needed

- ✓ Remember that replication is suppressed by drugs

HAART: highly active antiretroviral therapy

Key points about HAART for HIV infection

- Uses three drugs simultaneously; these bind different parts of the viral reverse transcriptase or protease molecules or sometimes now other HIV molecular targets
- In use since 1994
- Reduces virus load in plasma
- Restores lost immune functions
- Halts progression to AIDS
- Does not clear latently infected CD4+ memory cells
- Lapses in therapy result in virus rebound to normal levels
- Is not tolerated by all people
- Has to be taken indefinitely
- Not known if therapy can be tolerated for a lifetime
- Uncertain if resistant virus will eventually break through



Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents

Initiating Antiretroviral Therapy in Treatment-Naive Patients

(Last updated:3/29/2012; last reviewed:3/27/2012)

Printer-Friendly Files

- Section Only PDF (213 KB)
- Entire Guideline PDF (3.1 MB)
- All Tables PDF (496 KB)
- All Recommendations PDF (131 KB)

Panel's Recommendations

- Antiretroviral therapy (ART) is recommended for all HIV-infected individuals. The strength of this recommendation varies on the basis of pretreatment CD4 cell count:
  - CD4 count <350 cells/mm^3 **(AI)**
  - CD4 count 350 to 500 cells/mm^3 **(AII)**
  - CD4 count >500 cells/mm^3 **(BIII)**

- Regardless of CD4 count, initiation of ART is strongly recommended for individuals with the following conditions:
  - Pregnancy **(AI)** (see perinatal guidelines for more detailed discussion)
  - History of an AIDS-defining illness **(AI)**
  - HIV-associated nephropathy (HIVAN) **(AII)**
  - HIV/hepatitis B virus (HBV) coinfection **(AII)**

- Effective ART also has been shown to prevent transmission of HIV from an infected individual to a sexual partner; therefore, ART should be offered to patients who are at risk of transmitting HIV to sexual partners (**AI** [heterosexuals] or **AIII** [other transmission risk groups]; see text for discussion).

- Patients starting ART should be willing and able to commit to treatment and should understand the benefits and risks of therapy and the importance of adherence **(AIII).** Patients may choose to postpone therapy, and providers, on a case-by-case basis, may elect to defer therapy on the basis of clinical and/or psychosocial factors.

Rating of Recommendations: *A = Strong; B = Moderate; C = Optional*

Rating of Evidence: *I = data from randomized controlled trials; II = data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = expert opinion*

http://aidsinfo.nih.gov/

AIDSinfo

FDA-Approved Anti-HIV Medications

Antiretroviral therapy (ART) is the recommended treatment for HIV infection. ART involves taking a combination of anti-HIV medications (a regimen) daily. A regimen contains three or more anti-HIV medications from at least two different drug classes. Anti-HIV medications prevent HIV from multiplying in the body, which helps people infected with HIV live longer, healthier lives. ART may reduce the risk of transmission of HIV but anti-HIV medications can't cure HIV/AIDS.

The following table lists anti-HIV medications approved by the U.S. Food and Drug Administration (FDA) for treatment of HIV in the United States. The medications are presented by drug class and identified by generic name/acronym and brand name.

Drug Class	Generic Name (Acronym)	Brand Name	Manufacturer	FDA Approval Date
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)				
NNRTIs bind to and alter reverse transcriptase, an enzyme HIV needs to make copies of itself.	Delavirdine (DLV)	Rescriptor	Pfizer	April 4, 1997
	Efavirenz (EFV)	Sustiva	Bristol-Myers Squibb	Sept. 17, 1998
	Etravirine (ETR)	Intelence	Tibotec	Jan. 18, 2008
	Nevirapine (NVP)	Viramune	Boehringer Ingelheim	June 21, 1996
	Rilpivirine (RPV)	Edurant	Janssen Pharmaceuticals, Inc.	May 20, 2011
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)				
NRTIs block reverse transcriptase, an enzyme HIV needs to make copies of itself.	Abacavir (ABC)	Ziagen	GlaxoSmithKline	Dec. 17, 1998
	Didanosine (ddI)	Videx Videx EC (enteric-coated)	Bristol-Myers Squibb Bristol-Myers Squibb	Oct. 9, 1991 Oct. 31, 2000
	Emtricitabine (FTC)	Emtriva	Gilead Sciences	July 2, 2003
	Lamivudine (3TC)	Epivir	GlaxoSmithKline	Nov. 17, 1995
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	June 24, 1994
	Tenofovir DF (TDF)	Viread	Gilead Sciences	Oct. 26, 2001
	Zidovudine (ZDV, AZT)	Retrovir	GlaxoSmithKline	March 19, 1987
Protease Inhibitors (PIs)				
PIs block HIV protease, an enzyme HIV needs to make copies of itself.	Atazanavir (ATV)	Reyataz	Bristol-Myers Squibb	June 20, 2003
	Darunavir (DRV)	Prezista	Janssen Pharmaceuticals, Inc.	June 23, 2006
	Fosamprenavir (FPV)	Lexiva	GlaxoSmithKline	Oct. 20, 2003
	Indinavir (IDV)	Crixivan	Merck	March 13, 1996
	Nelfinavir (NFV)	Viracept	Agouron Pharmaceuticals	March 14, 1997
Protease Inhibitors (PIs), continued				
PIs block HIV protease, an enzyme HIV needs to make copies of itself.	Ritonavir (RTV)	Norvir	Abbott Laboratories	March 1, 1996
	Saquinavir (SQV)	Invirase	Hoffmann-La Roche	Dec. 6, 1995
	Tipranavir (TPV)	Aptivus	Boehringer Ingelheim	June 20, 2005
Fusion Inhibitors				
Fusion inhibitors block HIV from entering the CD4 cells of the immune system.	Enfuvirtide (T-20)	Fuzeon	Hoffmann-La Roche, Trimeris	March 13, 2003
CCR5 Antagonists				
CCR5 entry inhibitors block CCR5, a protein on the CD4 cells that HIV needs to enter the cells.	Maraviroc (MVC)	Selzentry	Pfizer	Aug. 6, 2007
Integrase Inhibitors				
Integrase inhibitors block HIV integrase, an enzyme HIV needs to make copies of itself.	Raltegravir (RAL)	Isentress	Merck	Oct. 12, 2007
Fixed-Dose Combination				
Fixed-dose combination tablets contain two or more anti-HIV medications from one or more drug classes.	Abacavir, Lamivudine	Epzicom	GlaxoSmithKline	Aug. 2, 2004
	Abacavir, Lamivudine, Zidovudine	Trizivir	GlaxoSmithKline	Nov. 14, 2000
	Efavirenz, Emtricitabine, Tenofovir DF	Atripla	Bristol-Myers Squibb, Gilead Sciences	July 12, 2006
	Elvitegravir*, Cobicistat†, Emtricitabine, Tenofovir DF	Stribild	Gilead Sciences	Aug. 27, 2012
	Emtricitabine, Rilpivirine, Tenofovir DF	Complera	Gilead Sciences	Aug. 10, 2011
	Emtricitabine, Tenofovir DF	Truvada	Gilead Sciences	Aug. 2, 2004
	Lamivudine, Zidovudine	Combivir	GlaxoSmithKline	Sept. 27, 1997
	Lopinavir, Ritonavir	Kaletra	Abbott Laboratories	Sept. 15, 2000

* Elvitegravir is currently approved only for use as a component of Stribild.

† Cobicistat, a pharmacokinetic enhancer, inhibits an enzyme that metabolizes certain HIV drugs and is used to prolong the effect of elvitegravir.

AIDSinfo | OFFERING INFORMATION ON HIV/AIDS TREATMENT, PREVENTION, AND RESEARCH

FDA-Approved HIV Medicines

Last Reviewed: June 24, 2019

Drug Class	Generic Name (Other names and acronyms)	Brand Name	FDA Approval Date
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)			
NRTIs block reverse transcriptase, an enzyme HIV needs to make copies of itself.	abacavir (abacavir sulfate, ABC)	Ziagen	December 17, 1998
	emtricitabine (FTC)	Emtriva	July 2, 2003
	lamivudine (3TC)	Epivir	November 17, 1995
	tenofovir disoproxil fumarate (tenofovir DF, TDF)	Viread	October 26, 2001
	zidovudine (azidothymidine, AZT, ZDV)	Retrovir	March 19, 1987
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
NNRTIs bind to and later alter reverse transcriptase, an enzyme HIV needs to make copies of itself.	doravirine (DOR)	Pifeltro	August 30, 2018
	efavirenz (EFV)	Sustiva	September 17, 1998
	etravirine (ETR)	Intelence	January 18, 2008
	nevirapine (extended-release nevirapine, NVP)	Viramune	June 21, 1996
		Viramune XR (extended release)	March 25, 2011
	rilpivirine (rilpivirine hydrochloride, RPV)	Edurant	May 20, 2011
Protease Inhibitors (PIs)			
PIs block HIV protease, an enzyme HIV needs to make copies of itself.	atazanavir (atazanavir sulfate, ATV)	Reyataz	June 20, 2003
	darunavir (darunavir ethanolate, DRV)	Prezista	June 23, 2006
	fosamprenavir (fosamprenavir calcium, FOS-APV, FPV)	Lexiva	October 20, 2003
	ritonavir (RTV) *Although ritonavir is a PI, it is generally used as a pharmacokinetic enhancer as recommended in the *Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV* and the *Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection*.	Norvir	March 1, 1996
	saquinavir (saquinavir mesylate, SQV)	Invirase	December 6, 1995
	tipranavir (TPV)	Aptivus	June 22, 2005
Fusion Inhibitors			
Fusion inhibitors block HIV from entering the CD4 cells of the immune system.	enfuvirtide (T-20)	Fuzeon	March 13, 2003
CCR5 Antagonists			
CCR5 antagonists block CCR5 coreceptors on the surface of certain immune cells that HIV needs to enter the cells.	maraviroc (MVC)	Selzentry	August 6, 2007
Integrase Inhibitors			
Integrase inhibitors block HIV integrase, an enzyme HIV needs to make copies of itself.	dolutegravir (DTG, dolutegravir sodium)	Tivicay	August 13, 2013
	raltegravir (raltegravir potassium, RAL)	Isentress	October 12, 2007
		Isentress	May 26,

AIDSinfo

Recommended HIV Treatment Regimens

What is the treatment for HIV?

Antiretroviral therapy (ART) is the recommended treatment for HIV. ART involves taking a combination of anti-HIV medications (a **regimen**) every day. Anti-HIV medications (also called **antiretrovirals**) are grouped into six **drug classes** according to how they fight HIV. The six classes are non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5 antagonists, and integrase inhibitors.

Recommended HIV treatment regimens include three or more anti-HIV medications from at least two different drug classes. Taking a combination of anti-HIV medications from different classes is the most effective way to control the virus. Some anti-HIV medications are available in combination (two or more medications in one pill).

Anti-HIV medications are approved by the U.S. Food and Drug Administration (FDA). See the FDA-Approved Anti-HIV Medications fact sheet for a complete list of medications used in HIV treatment regimens in the United States.

How will I know which anti-HIV medications to take?

The best combination of anti-HIV medications for you depends on your individual needs. Factors that you and your health care provider will consider when selecting your HIV regimen include:

- Other diseases or conditions you may have
- Possible side effects of anti-HIV medications
- The risk of interactions between anti-HIV medications and other medications you take
- Results of **drug-resistance testing** and other tests
- Convenience of the regimen. (For example, a regimen that involves taking only one pill a day is convenient to follow.)
- Any personal issues that can make following a regimen difficult (such as depression or alcohol or drug abuse)

What are the recommended regimens for people taking anti-HIV medications for the first time?

After considering your individual needs, you and your health care provider may select one of the following regimens recommended for people taking anti-HIV medications for the first time:

- **Atripla** (a combination of three anti-HIV medications in one pill)
- **Reyataz** + **Norvir** + **Truvada** (Truvada is a combination of two anti-HIV medications in one pill.)
- **Prezista** + **Norvir** + **Truvada**
- **Isentress** + **Truvada**

Reyatax = Atazanavir (PI)
Norvir = Ritonavir (PI)
Truvada = Emtricitabine+Tenofovir (NRTI)

Terms Used in This Fact Sheet:

Antiretroviral: A medication that prevents a retrovirus, such as HIV, from making copies of itself. Anti-HIV medications are also called antiretrovirals.

Antiretroviral therapy (ART): The recommended treatment for HIV. ART involves taking a combination of three or more anti-HIV medications from at least two different drug classes every day to control the virus.

Atripla: A combination of three anti-HIV medications in one pill—Sustiva (also called efavirenz or EFV), Emtriva (also called emtricitabine or FTC), and Viread (also called tenofovir or TDF).

Drug class: A group of medications that work in the same way.

Drug-resistance testing: Testing to identify which anti-HIV medications will or will not be effective against a person's specific strain of HIV. Drug-resistance testing is done using a sample of blood.

Isentress: An anti-HIV medication in the integrase inhibitor class. Isentress is also called raltegravir or RAL.

Norvir: An anti-HIV medication in the protease inhibitor (PI) class. Norvir is also called ritonavir or RTV.

Prezista: An anti-HIV medication in the protease inhibitor (PI) class. Prezista is also called darunavir or DRV.

Regimen: A combination of three or more anti-HIV medications from at least two different drug classes.

Reyataz: An anti-HIV medication in the protease inhibitor (PI) class. Reyataz is also called atazanavir or ATV.

Sustiva: An anti-HIV medication in the non-nucleoside reverse transcriptase inhibitor (NNRTI) class. Sustiva is also called efavirenz or EFV.

Truvada: Two anti-HIV medications from the nucleoside reverse transcriptase (NRTI) class—Emtriva and Viread—combined in a single pill. Emtriva is also called emtricitabine or FTC. Viread is also called tenofovir or TDF.

This information is based on the U.S. Department of Health and Human Services' *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents* (available at http://aidsinfo.nih.gov/guidelines).

Reviewed August 2012

Table 16. Monthly Average Wholesale Price[b] of Commonly Used[c] Antiretroviral Drugs (Last updated July 14, 2016; last reviewed July 14, 2016) (page 2 of 4)

ARV Drug (Generic and Brand Names)	Strength Formulation	Dosing	Tablets, Capsules, or mLs per Month[a]	AWP[b] (Monthly)
Co-Formulated Combination Products as Single Tablet Regimens				
Dolutegravir/Abacavir/Lamivudine • Triumeq	50/600/300 mg tablet	1 tablet daily	30 tablets	$2,889.22
Efavirenz/Tenofovir Disoproxil Fumarate/Emtricitabine • Atripla	600/300/200 mg tablet	1 tablet daily	30 tablets	$2,869.86
Elvitegravir/Cobicistat/Tenofovir Alafenamide/Emtricitabine • Genvoya	150/150/10/200 mg tablet	1 tablet daily	30 tablets	$3,093.19
Elvitegravir/Cobicistat/Tenofovir Disoproxil Fumarate/ Emtricitabine • Stribild	150/150/300/200 mg tablet	1 tablet daily	30 tablets	$3,244.76
Rilpivirine/Tenofovir Alafenamide/Emtricitabine • Odefsey	25/25/200 mg tablet	1 tablet daily	30 tablets	$2,815.04
Rilpivirine/Tenofovir Disoproxil Fumarate/Emtricitabine • Complera	25/300/200 mg tablet	1 tablet daily	30 tablets	$2,815.04
Pharmacokinetic Enhancers (Boosters)				
Cobicistat • Tybost	150 mg tablet	1 tablet daily	30 tablets	$230.90
Ritonavir: Total daily dose depends on the dose of the concomitant PI (100 mg once or twice daily, or 200 mg twice daily) • Norvir	100 mg tablet	1 tablet once daily	30 tablets	$308.60
	80 mg/mL solution	100 mg daily	37.5 mL (of a 240 mL bottle)	$270.04

AIDSinfo

Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents

Appendix B: Drug Characteristics Tables

Coformulated Single-Tablet Regimens

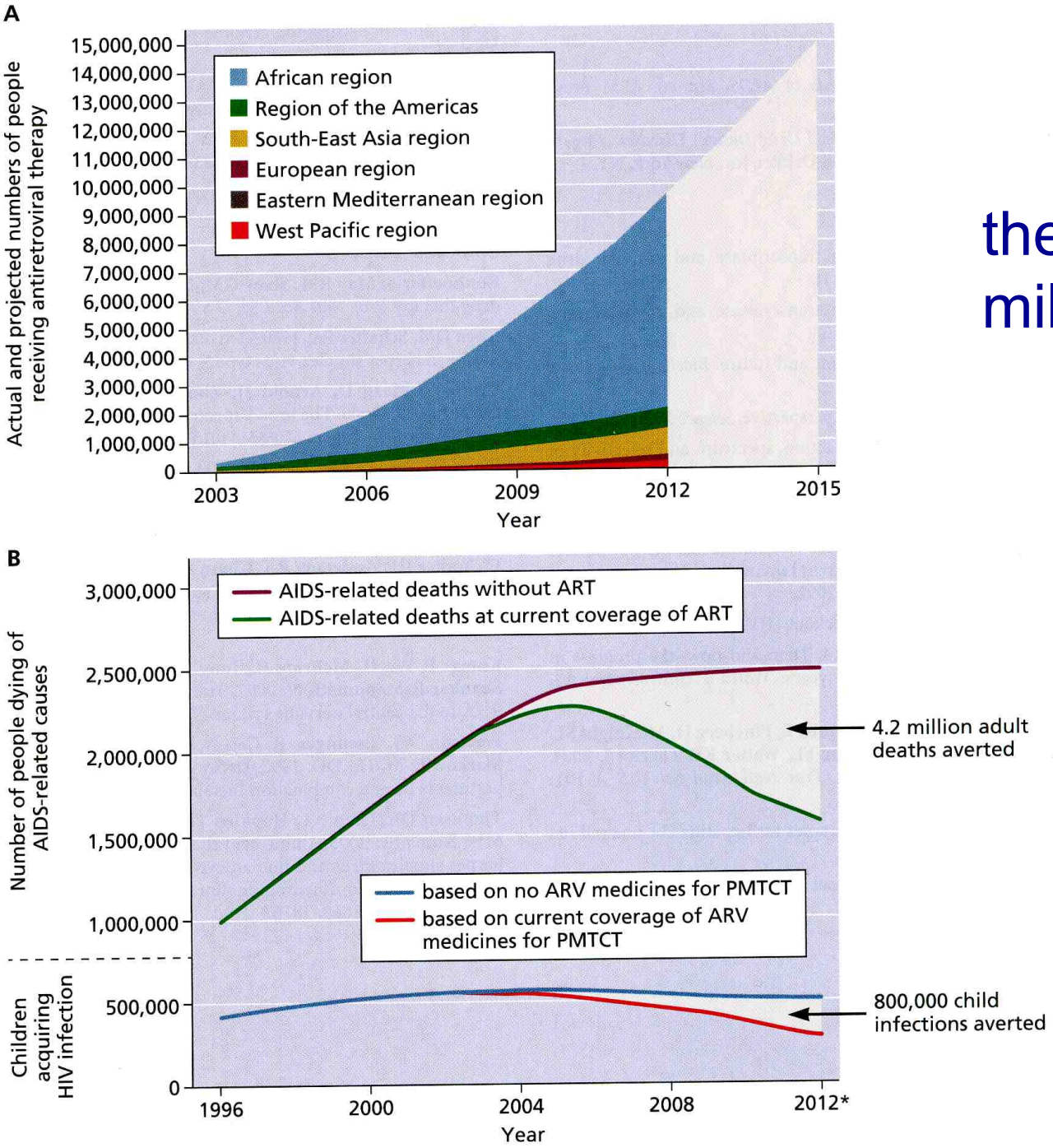
Last Updated: July 10, 2019; Last Reviewed: July 10, 2019

Trade Name (Abbreviations)	ARV Drugs Included in the STR	Dosing Recommendation[a]
INSTI plus Two NRTIs		
Biktarvy (BIC/TAF/FTC)	Bictegravir 50 mg/tenofovir alafenamide 25 mg/emtricitabine 200 mg	One tablet once daily
Genvoya (EVG/c/TAF/FTC)	Elvitegravir 150 mg/cobicistat 150 mg/tenofovir alafenamide 10 mg/emtricitabine 200 mg	One tablet once daily with food
Stribild (EVG/c/TDF/FTC)	Elvitegravir 150 mg/cobicistat 150 mg/tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg	One tablet once daily with food
Triumeq (DTG/ABC/3TC)	Dolutegravir 50 mg/abacavir 600 mg/lamivudine 300 mg	One tablet once daily
INSTI plus One NRTI		
Dovato (DTG/3TC)	Dolutegravir 50 mg/lamivudine 300 mg	One tablet once daily
PI plus Two NRTIs		
Symtuza (DRV/c/TAF/FTC)	Darunavir 800 mg/cobicistat 150 mg/tenofovir alafenamide 10 mg/emtricitabine 200 mg	One tablet once daily with food
NNRTI plus Two NRTIs		
Atripla (EFV/TDF/FTC)	Efavirenz 600 mg/tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg	One tablet once daily on an empty stomach, preferably at bedtime
Complera (RPV/TDF/FTC)	Rilpivirine 25 mg/tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg	One tablet once daily with a meal
Delstrigo (DOR/TDF/3TC)	Doravirine 100 mg/tenofovir disoproxil fumarate 300 mg/lamivudine 300 mg	One tablet once daily
Odefsey (RPV/TAF/FTC)	Rilpivirine 25 mg/tenofovir alafenamide 25 mg/emtricitabine 200 mg	One tablet once daily with a meal
Symfi (EFV/TDF/3TC)	Efavirenz 600 mg/tenofovir disoproxil fumarate 300 mg/lamivudine 300 mg	One tablet once daily on an empty stomach, preferably at bedtime
Symfi Lo (EFV/TDF/3TC)	Efavirenz 400 mg/tenofovir disoproxil fumarate 300 mg/lamivudine 300 mg	One tablet once daily on an empty stomach, preferably at bedtime
INSTI plus One NNRTI		
Juluca (DTG/RPV)	Dolutegravir 50 mg/rilpivirine 25 mg	One tablet once daily with a meal

[a] For dose adjustments in patients with renal or hepatic insufficiency, see Appendix B, Table 10. When no food restriction is listed, the STR can be taken with or without food.

Key: 3TC = lamivudine; ABC = abacavir; ARV = antiretroviral; BIC = bictegravir; c = cobicistat; DOR = doravirine; DRV = darunavir; DTG = dolutegravir; EFV = efavirenz; EVG = elvitegravir; FDA = Food and Drug Administration; FTC = emtricitabine; INSTI = integrase strand transfer inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; RPV = rilpivirine; STR = single-tablet regimen; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate

Anti-HIV therapy saves million of lives



HAART: highly active antiretroviral therapy

There are 10^{16} HIV genomes on the Earth today

With this number of genomes, it is highly probable that HIV genomes exist that are resistant to every one of the antiviral drugs that we have now, or EVER WILL HAVE