VIROLOGY

Research, Development and Applications of Antiviral Agents

EPIDEMIOLOGY OF VIRAL DISEASES

Prevention and control

Vaccines:
the proven best defense
against viruses

Vaccines have been very successful in preventing some viral diseases; however, they provide modest to no therapeutic effect in individuals that are already infected

Prevention and control

Consequently, the second arm of antiviral defense has been the development and use of molecules capable of preventing an infection or stopping it once started.

Antiviral drugs:
small molecules that block virus replication

EPIDEMIOLOGY OF VIRAL DISEASES

Prevention and control

Antiviral drugs:
small molecules that block virus replication

However, despite 50 years of research, our arsenal of antiviral drugs remains dangerously small.

Only less than 100 antiviral drugs are available on the market

Most against HIV, HCV, Herpesviruses (Persistent infections)

THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

• Safety is the overriding concern in anviral drug discovery! Compounds interfering with virus growth often have adverse severe effects on the host:

- Side effects are common (unacceptable)
- Every step in viral replicative cycle engages host functions

• Many medically important viruses are difficult or impossible to grow in laboratory (eg. HBV, HPV), or are dangerous (Ebola).

• Some viruses have no available animal model of human disease (eg. Measle, HCV).

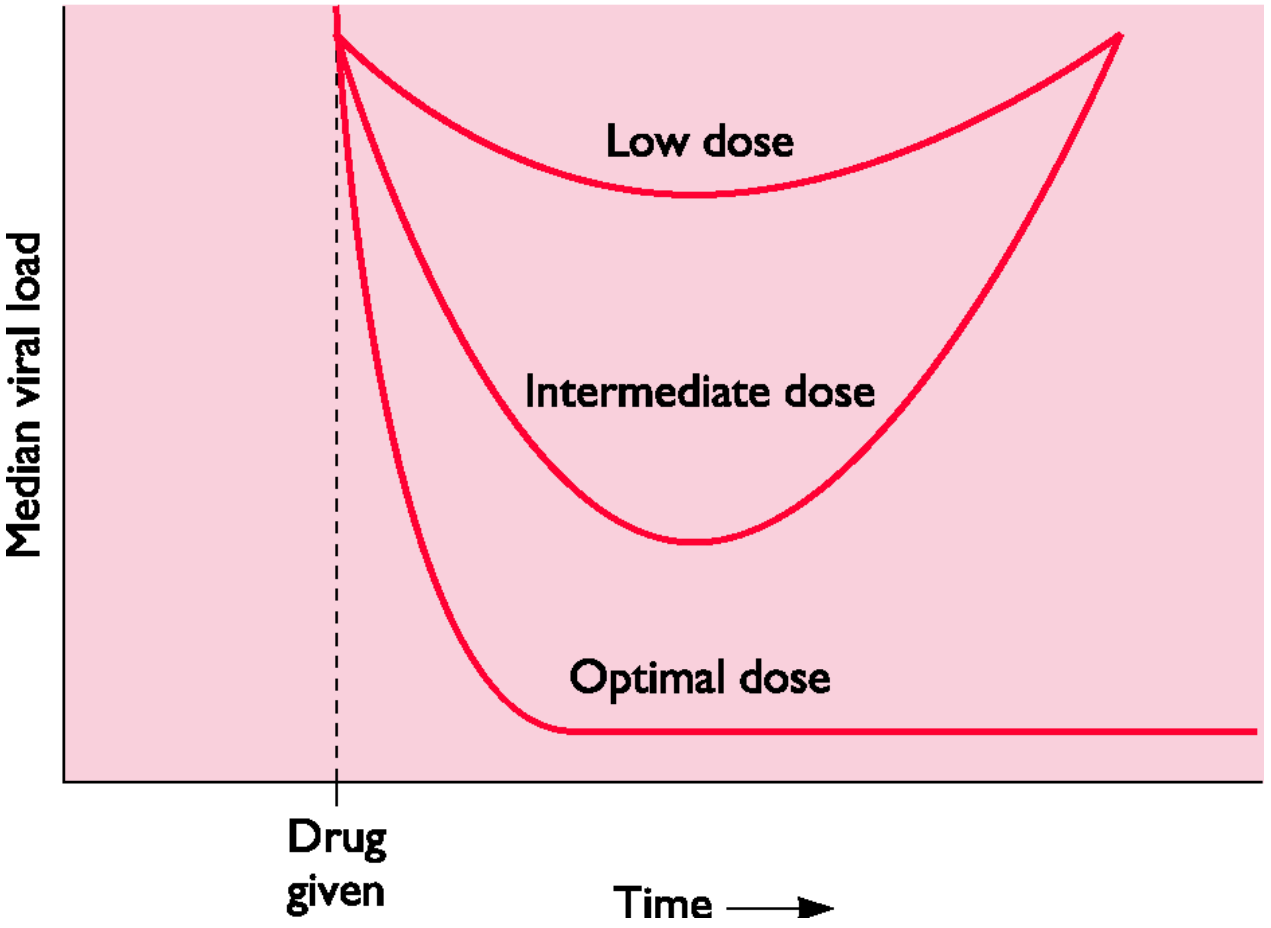
THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

An unappreciated third reason may be the most important

- Antiviral compounds must be virtually 100% efficient in blocking virus growth.
- A compound must block virus replication completely!
- So, it must be extremely potent!!!
- Partially inhibition is not acceptable for an antivirals: even a modest viral replication in the presence of a drug provides the opportunity that drug–resistant mutants arise.
- Makes drug discovery expensive.

The problem of viral resistances

REPLICATION=MUTATION



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

THE CONUNDRUM OF ANTIVIRAL CHEMOTHERAPY:
So Much Knowledge, So Few Antivirals

- **Another serious problem for antiviral discovery:**
  - Many acute infections are of short duration and by the time the indiduals feel ill the virus is no longer replicating and indeed may have already been cleared from the host. Thus, too late to impact clinical disease.
  - Antivirals drugs for these viruses must be given early in infection or prophylactically to populations at risk.
  - Safety issues: giving drugs to healthy people not wise

- The lack of rapid diagnostic tests has hampered development of antiviral drugs

- No broad-spectrum of antiviral agents are currently available

ANTIVIRAL HISTORY

- The first modest search for antiviral drugs occurred in 1950s.
  - *Chemists looked at derivatives of the sulfonamide antibiotics.*
  - *Synthesis of thiosemicarbazones actve against poxviruses.*
  - *Smallpox was still a major threat after WWII.*
- 1960s and 1970s: “blind screening” programs to find chemicals with antiviral activity spurred on by successes in the treatment of bacterial infections with antibiotics.
- No attempt to focus discovery on a virus or a virus-specific mechanism
- Random chemicals and natural product mixtures tested for ability to block replication of a variety of viruses in cell culture systems.
- Considerable effort, very little success: one exception!
- Symmetrel (Amantadine) approved late 1960s for Influenza A virus infections. Mechanism of action discovered only in 1990s.

Antiviral discovery today

Modern antiviral discovery

The advent of modern molecular virology, recombinant DNA technology and sophisticated chemistry make targeted discovery possible

- Essential viral genes cloned, expressed in genetically tractable organisms, purified, and analyzed in atomic detail.
- Life cycles of most viruses known, targets for intervention can be generalized.
- Modern technology allows inhibitors to be found even for viruses that cannot be propagated in cell culture

Blind screening  Is no longer attractive

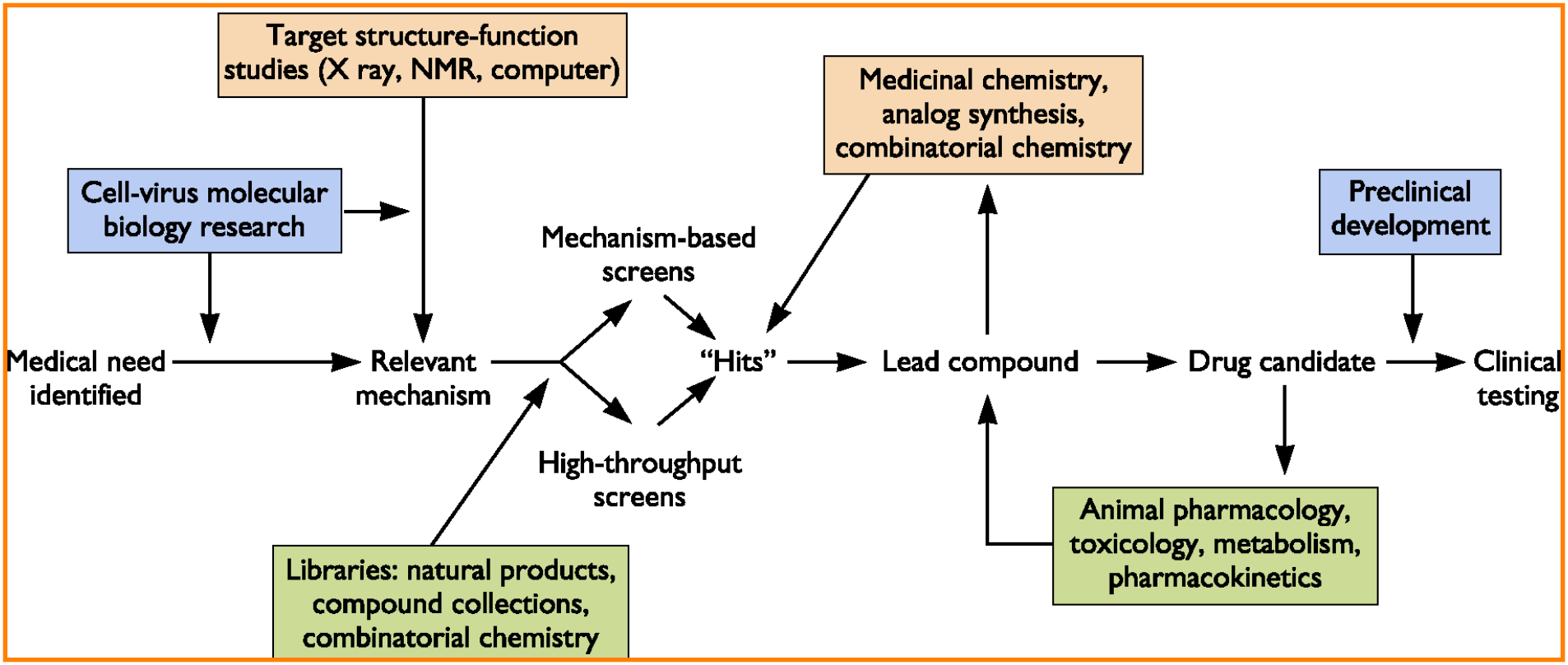
Antiviral discovery today

Blind screening  Is no longer attractive

Modern antiviral discovery

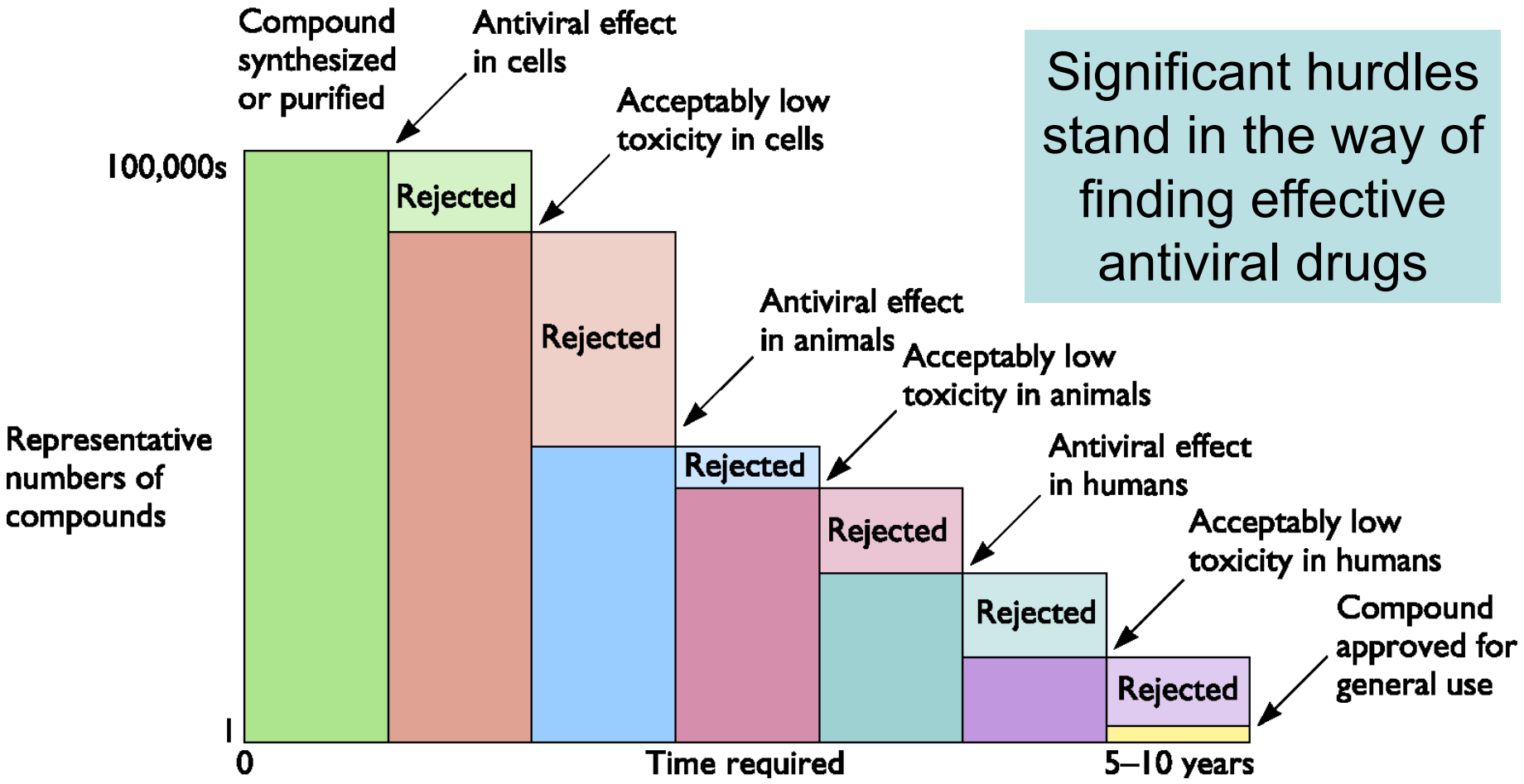
- Mechanism-based screens
- Cell-based assays
- Combinatorial chemistry
- High-throughput screens
- Computational Approaches:
  - Structure-assisted drug design
  - Genome sequencing, proteomic analyses
  - *In silico* discovery via Virtual Screening

Search and development of antiviral drugs



The path of antiviral drug discovery

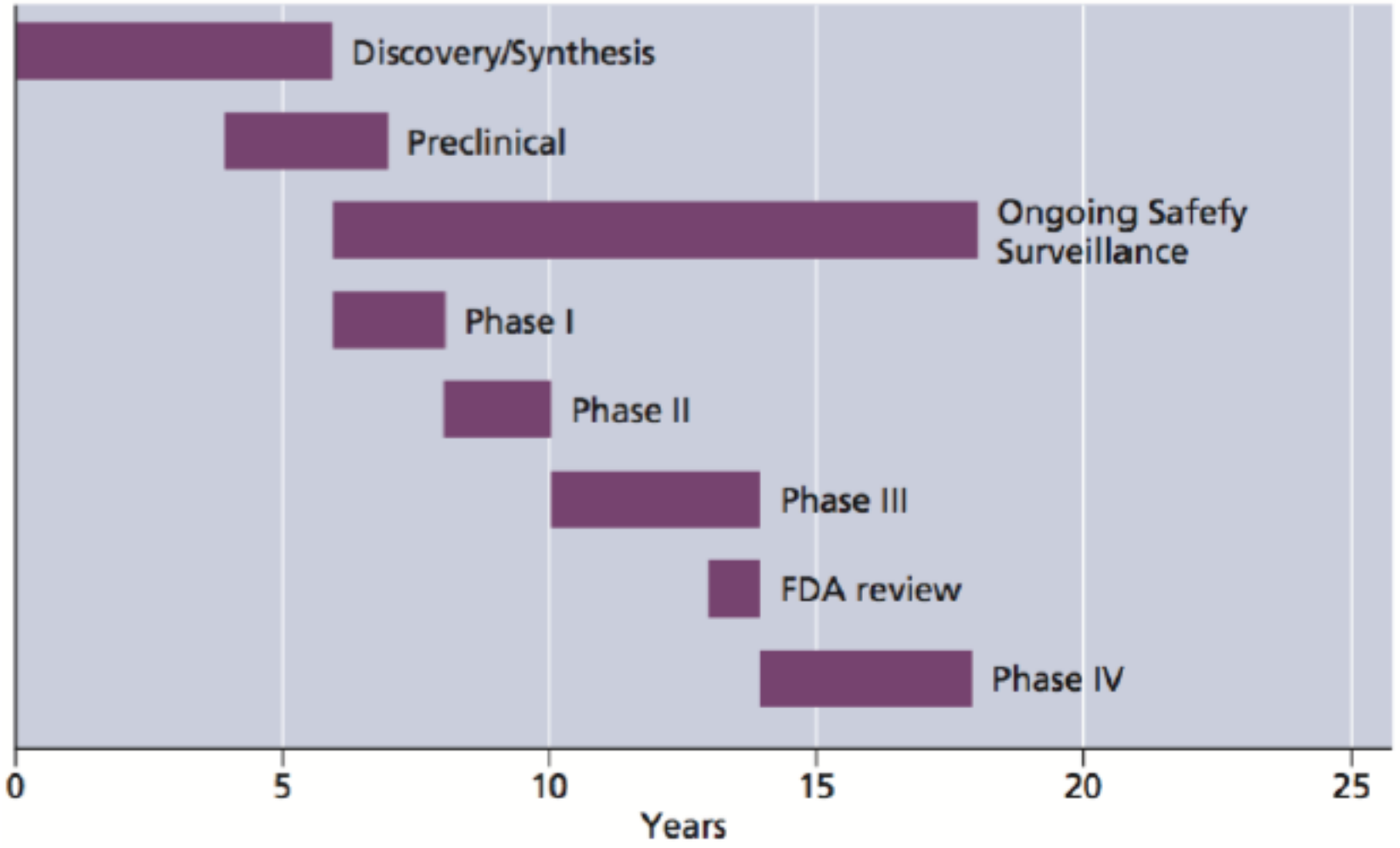
R&D of antiviral discovery: antiviral drugs are expensive to discovery, develop, and bring to market



Significant hurdles stand in the way of finding effective antiviral drugs

Staircase of drug discovery: it can takes 5 to years after the initial lead is found to get a drug to the market and it may cost 100M$ to 500M$

R&D of antiviral discovery: antiviral drugs are expensive to discovery, develop, and bring to market



From drug discovery to the hospital

Search and development of antiviral drugs

Table 19.8 Key points for drug hunters seeking commercially viable antiviral drugs

Virology issues

Must obtain proof of principle as soon as possible.

- What is the molecular mechanism? Does the drug work in tissue culture and in animal models as predicted?

Compound should block viral spread early to limit cytopathology and host cytokine/inflammatory response.

- Drug must block replication completely and must not make an infection "persistent" by slow replication and spread.

Resistance to the antiviral drug must be manageable.

- Resistance mutations arise when any virus is permitted to replicate.
- Viruses with resistance to other drugs must not be resistant to the new drug.
- The virulence of resistant mutants must be understood.
- Noncompliance by patient may select for drug resistance or may encourage persistent infection; multiple dosing, stringent dietary requirements, or unpleasant taste or side effects affect compliance.

Search and development of antiviral drugs

Business issues

Compound should be safe with no side effects.

Compound should be inexpensive to manufacture.

Compound should be easy to formulate and deliver.

- A pill to be swallowed is much preferred over injection.

Compound must satisfy an unmet medical need.

- That is, it must be better than any competitive drug or, better yet, have no competition.

Ultimately, a profit should be possible.

- The market should be large enough to allow a profit to be made.

Search and development of antiviral drugs

Blind screening  Is no longer attractive

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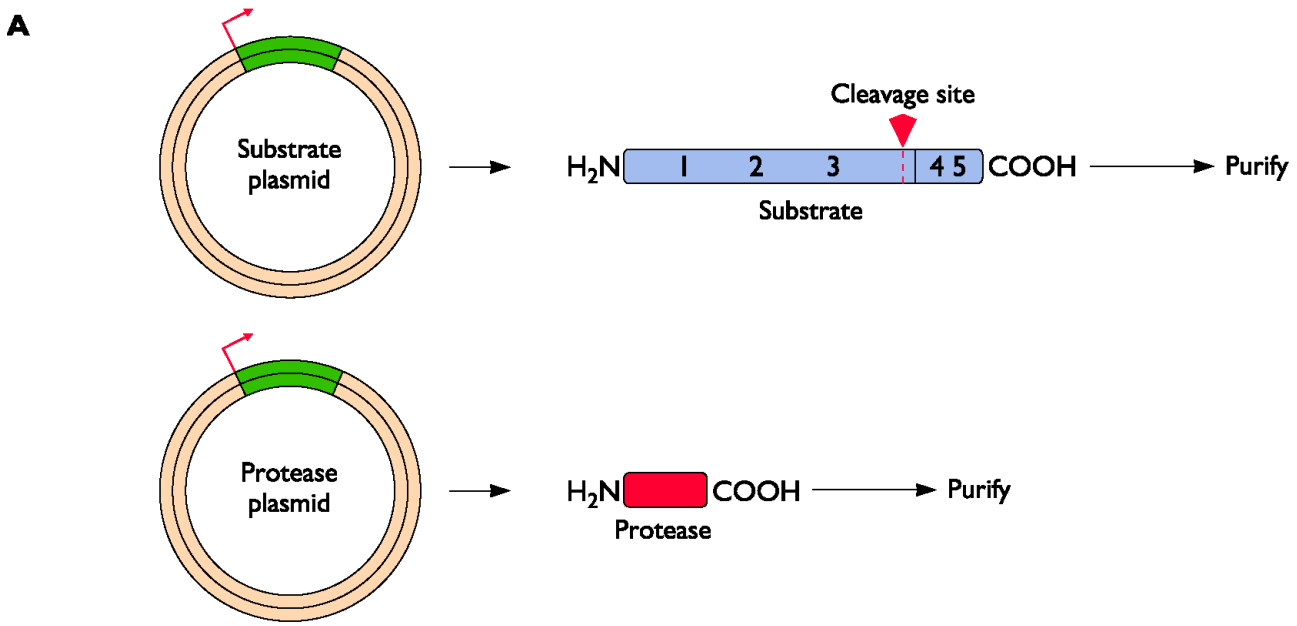
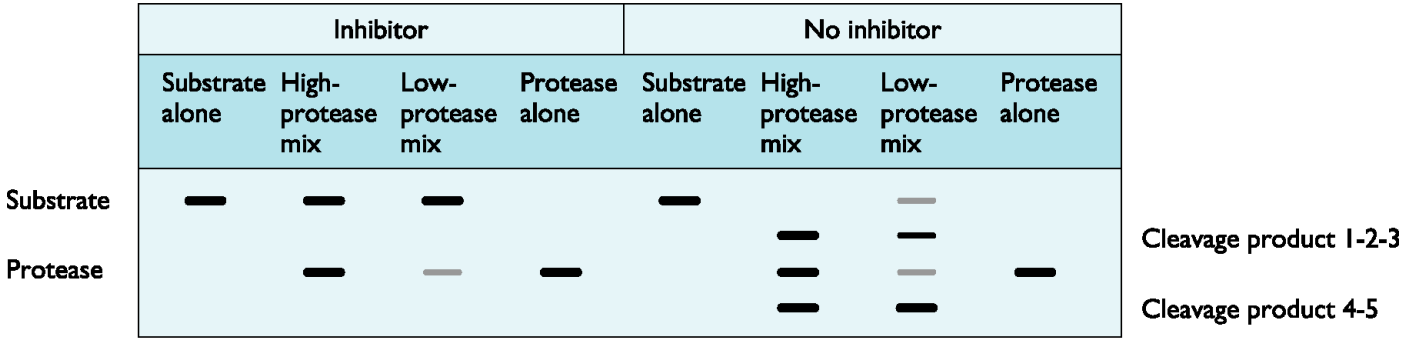
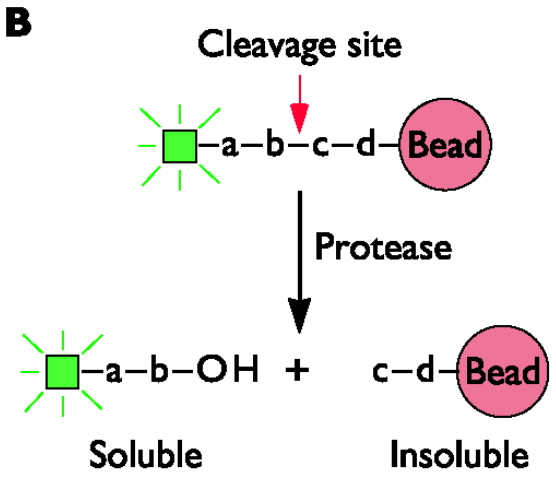
Mechanism-based assay to select inhibitors of a viral protease

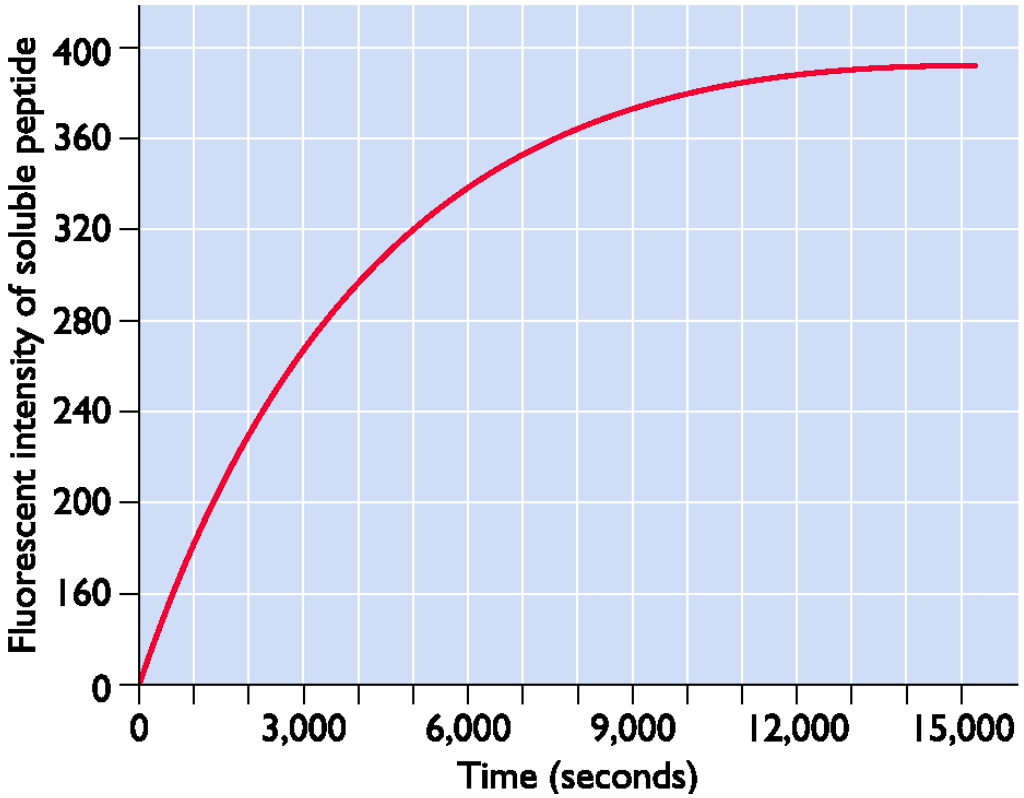


Assay:
1) Mix a constant amount of substrate with a "high" and "low" concentration of protease.
2) Controls: protease and substrate alone.
3) Incubate for set periods under appropriate conditions with and without inhibitor.
4) Sample; separate proteins by electrophoretic mobility.



Mechanism-based assay to select inhibitors of a viral protease



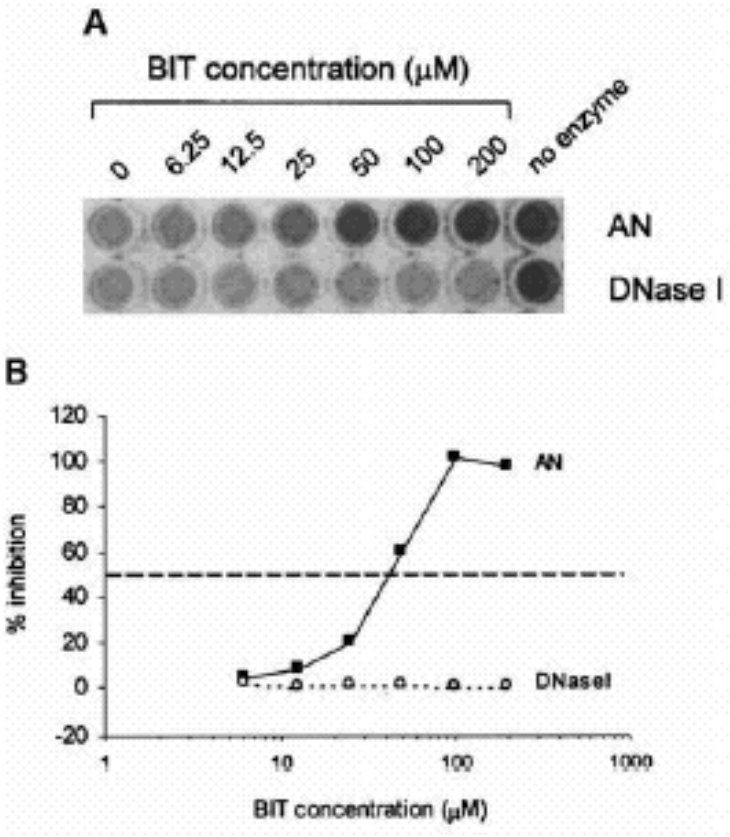


An example of a mechanism-based assay to select inhibitors of a viral nuclease

Analytical Biochemistry 293, 239–245 (2001)
doi:10.1006/abio.2001.5144, available online at http://www.idealibrary.com on IDEAL®

A Colorimetric Assay for High-Throughput Screening of Inhibitors of Herpes Simplex Virus Type 1 Alkaline Nuclease

Joel C. Bronstein and Peter C. Weber[1]
Infectious Diseases Section, Pfizer Global Research and Development, Ann Arbor, Michigan 48105



Nuclease assays. The DNA–methyl green degradation assay utilized a DNA substrate complexed with the intercalating dye methyl green and was obtained commercially (Sigma Chemical Co.). Degradation of the DNA substrate resulted in release of the dye and a corresponding loss of solution color. Assays were carried out as 150-μl reactions in 96-well plates and included 75 μl water, 15 μl 10× assay buffer (0.5 M Tris–HCl, pH 7.5, 30 mM $MgSO_4$), 5 μl compound stock prepared in methanol, 30 μl AN (2 μg) or bovine pancreatic DNaseI (0.15 units) diluted in DE buffer just prior to use, and 25 μl DNA–methyl green substrate (25 μg from a 1 mg/ml stock). Typically all of the components of the reaction were added with the exception of the substrate, and the plate was mixed and incubated at 37°C for 5 min. The DNA–methyl green substrate was then added to start the reaction, and the plate was then mixed again and incubated at 37°C for 3 h. The reaction was terminated by the addition of 50 μL sodium citrate (0.2 M, pH 7.5), and the plate was then mixed, sealed, and stored in the dark at room temperature for at least 12 h to allow color equilibration before reading on a spectrophotometer to determine A_{650}. AN inhibitor screens typically included the

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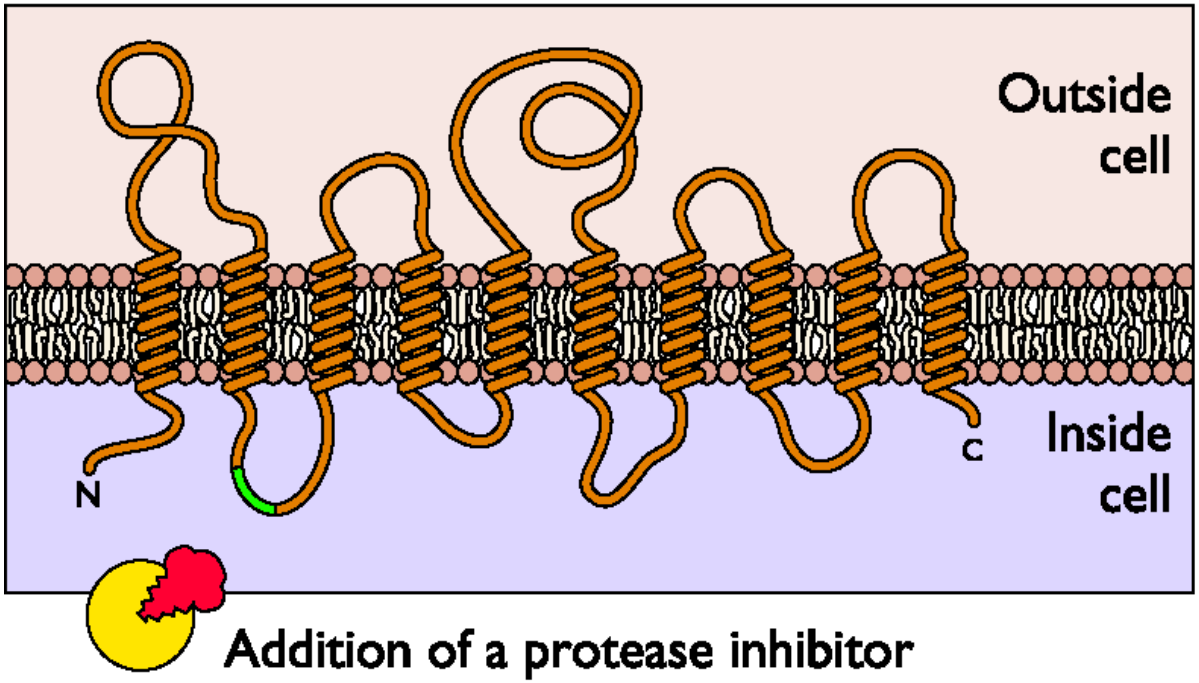
A cell-based assay to select inhibitors of HIV protease

Grafsrom et al., *Adv. Exp. Med. Biol.* 312:25-40, 1992

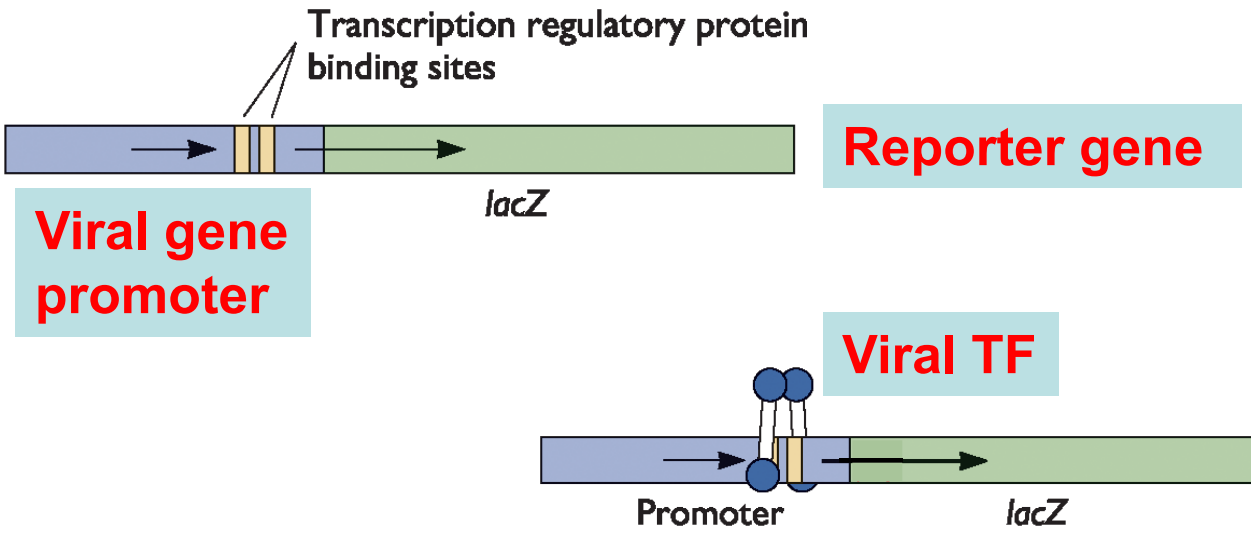
Active tetracycline efflux protein



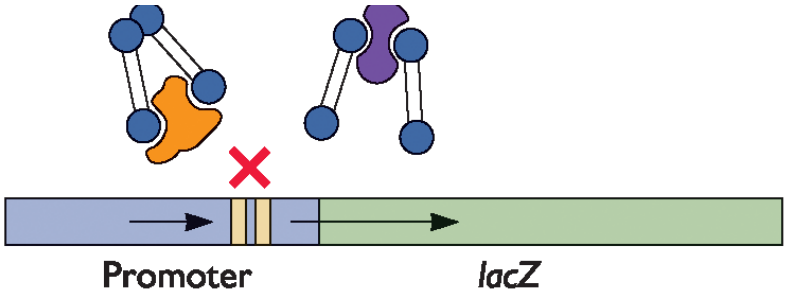
Tetracycline-resistant bacteria

Addition of a protease inhibitor blocks cleavage, leaving an active tetracycline efflux protein

A cell-based assay to select inhibitors of viral transcription factor



A small molecule that directly blocks DNA binding of the protein or blocks a protein-protein interaction required for the cooperative binding can be identified



ORIGINAL ARTICLE

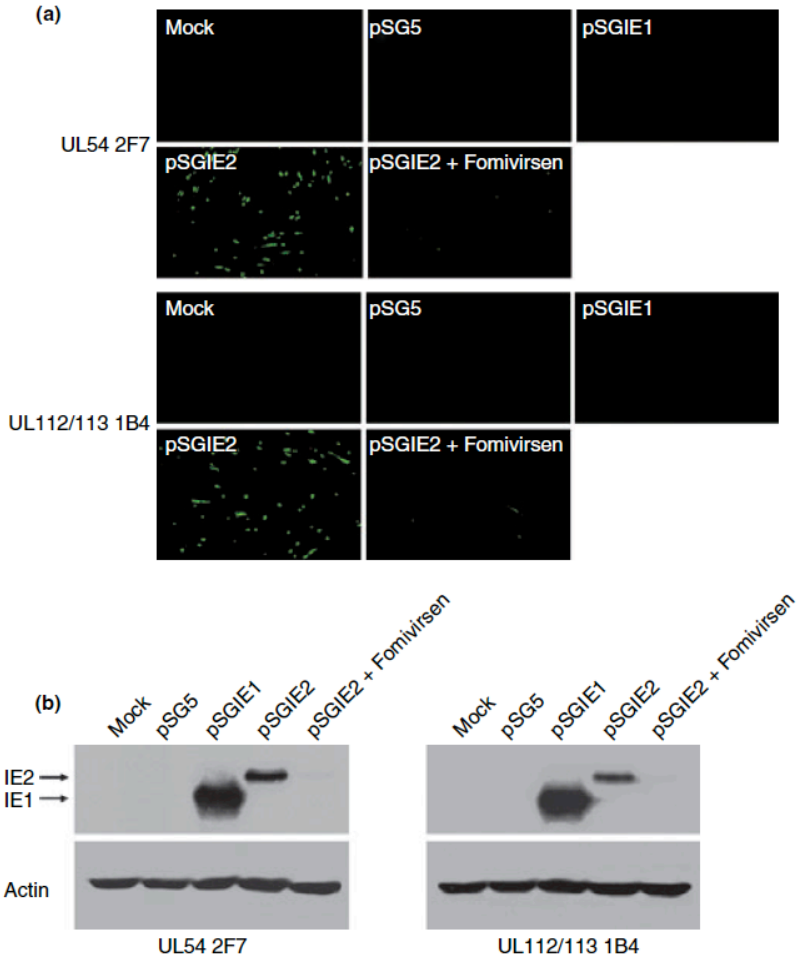
Journal of Applied Microbiology **105** (2008) 1791–1801

New cell-based indicator assays for the detection of human cytomegalovirus infection and screening of inhibitors of viral immediate-early 2 protein activity

A. Luganini[1], P. Caposio[1], M. Mondini[2], S. Landolfo[1] and G. Gribaudo[1]

1 Department of Public Health and Microbiology, University of Torino, Torino, Italy
2 Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy

HCMV IE2-dependent cell-based assay



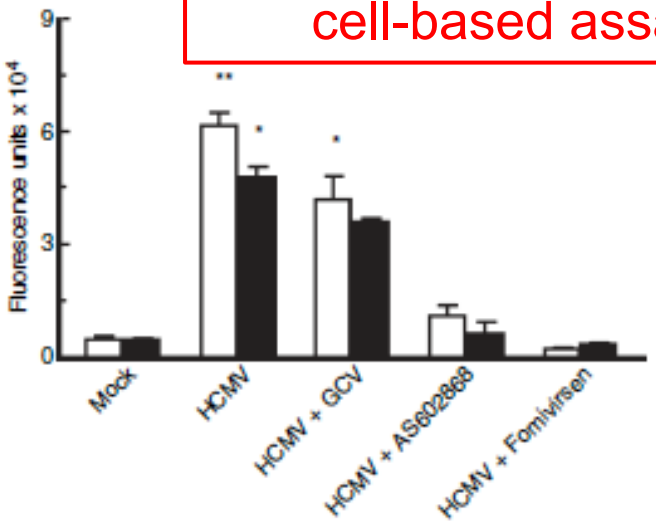
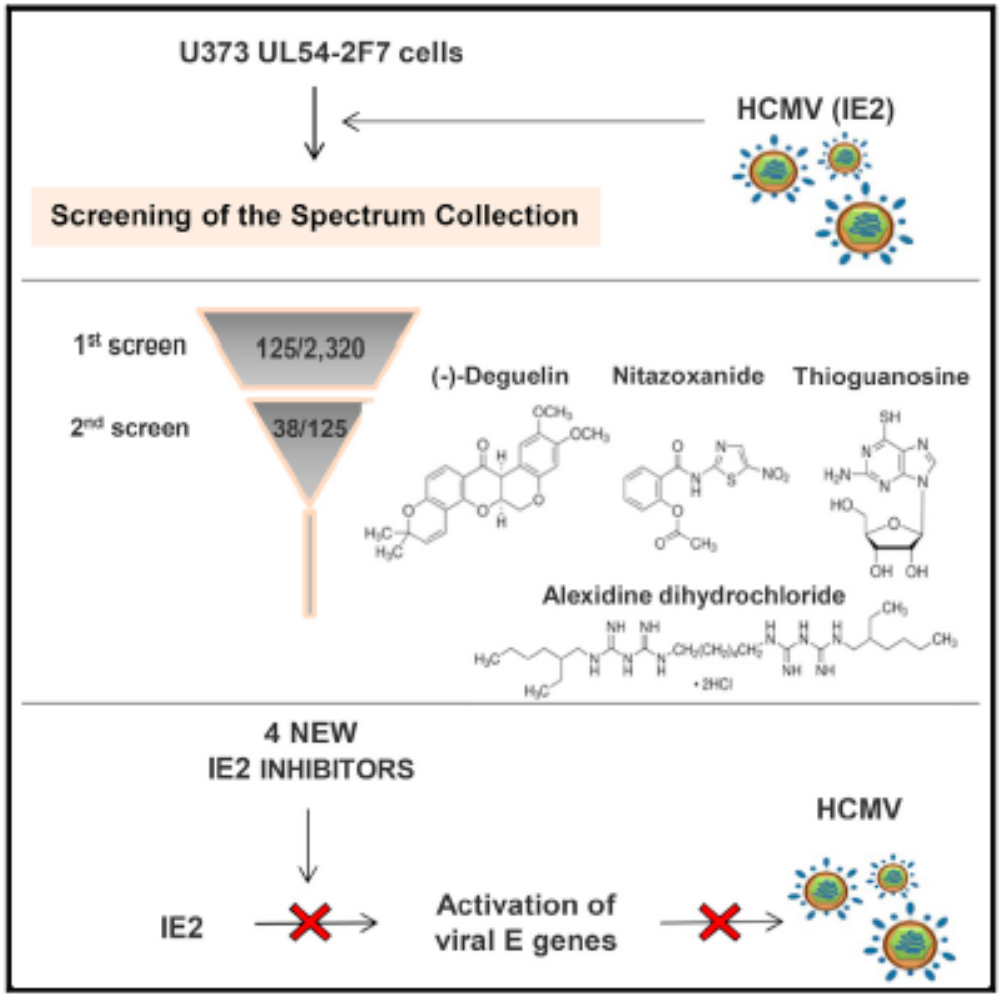


Figure 4 Effect of anti-cytomegaloviral drugs on EGFP expression in UL54 2F7 and UL112/113 1B4 cells. UL54 2F7 and UL112/113 1B4 cells were infected HCMV AD169 (moi 5) or mock-infected. Where indicated, the cells were pretreated and treated with 5 μmol l^{-1} fomivirsen (ISIS 2922) or 20 μmol l^{-1} AS602868 1 h prior to and during infection, or 100 μmol l^{-1} GCV after virus adsorption. At 72 h p.i., the cells were lysed and assayed for quantitative EGFP expression by automated fluorometry. The data shown in each column are the mean ± SD (error bars) of three independent experiments. $*P < 0.05$ compared with control, $**P < 0.01$ compared with control. (□), UL54 2F7; (■), UL112/113 1B4.

CellPress

Drug Repurposing Approach Identifies Inhibitors of the Prototypic Viral Transcription Factor IE2 that Block Human Cytomegalovirus Replication

Beatrice Mercorelli,[1,4] Anna Luganini,[2,4] Giulio Nannetti,[1] Oriana Tabarrini,[3] Giorgio Palù,[1] Giorgio Gribaudo,[2,5] and Arianna Loregian[1,5,*]
[1]Department of Molecular Medicine, University of Padua, 35121 Padua, Italy
[2]Department of Life Sciences and Systems Biology, University of Turin, 10123 Turin, Italy
[3]Department of Pharmaceutical Sciences, University of Perugia, 06123 Perugia, Italy
[4]Co-first author
[5]Co-senior author



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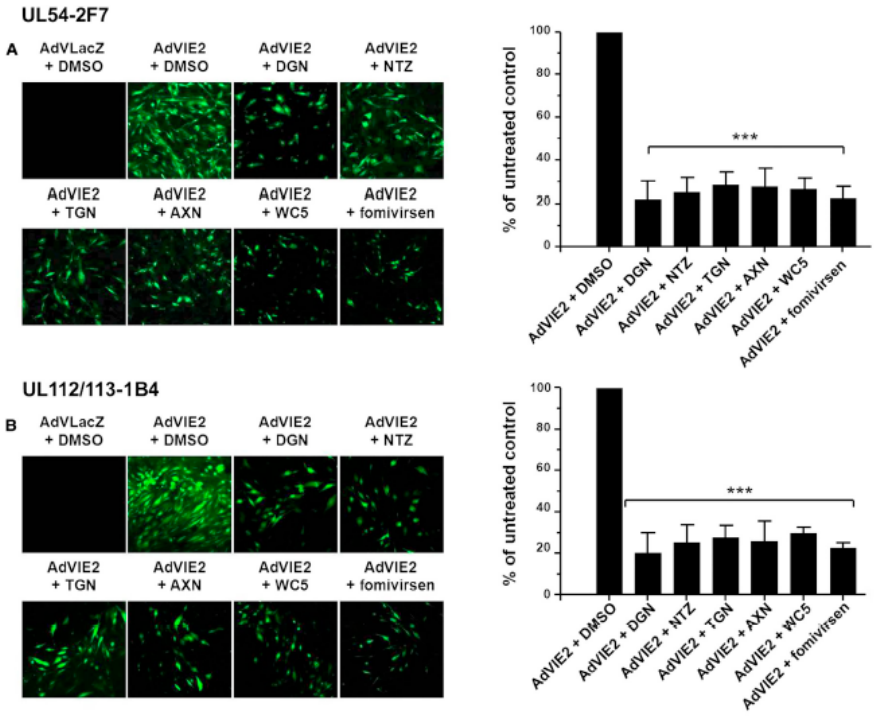
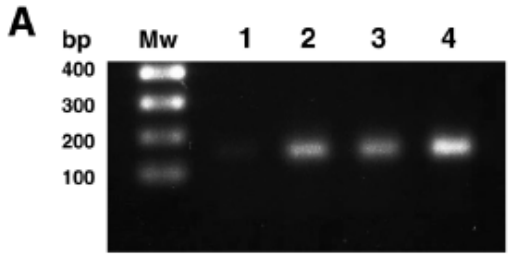
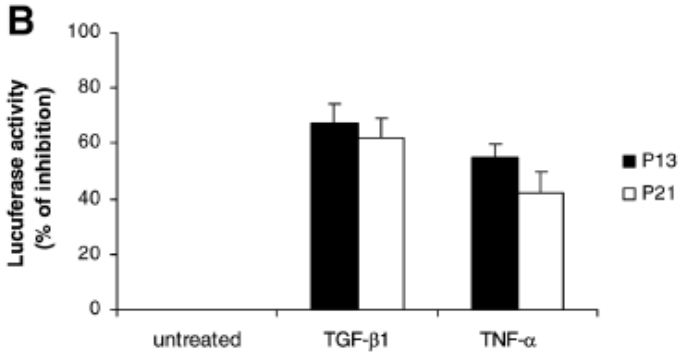


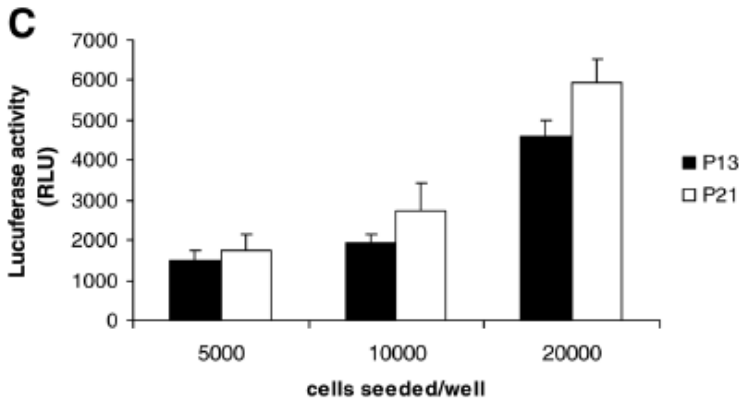
Mercorelli et al., 2016, Cell Chemical Biology 23, 1–12
March 17, 2016
http://dx.doi.org/10.1016/j.chembiol.2015.12.012

The FASEB Journal express article 10.1096/fj.05-3904fje. Published online October 27, 2005.

A cell-based high-throughput assay for screening inhibitors of human papillomavirus-16 long control region activity







Effect of cytokines on HPV-16 LCR activity

Cytokines	% of LCR inhibition (mean values ± SD)	Group[a]
Anti-inflammatory		
IL-4	56.6 ± 7.2	III
IL-10	0	I
IL-13	64.3 ± 5.8	III
TGF-β1	61.4 ± 8.4	III
TGF-β2	60.1 ± 6.5	III
TGF-β3	58.9 ± 5.9	III
Activin	32.4 ± 4.3	II
GDF-15	16.3 ± 2.1	I
Osteonectin	0.2 ± 0.03	I
Pro-inflammatory		
IL-1β	33.3 ± 4.6	II
IL-15	12.6 ± 1.8	I
IL-17	29.6 ± 3.6	I
IL-18	3 ± 0.5	I
IL-19	9 ± 1.2	I
IL-20	10.2 ± 0.8	I
IL-22	10.3 ± 1.7	I
TNF-α	53.7 ± 4.3	III
GM-CSF	12 ± 0.7	I
Growth factors		
IL-3	2.1 ± 0.09	I
IL-6	5.2 ± 0.3	I
IL-7	2.5 ± 0.5	I
IL-21	2.4 ± 0.4	I
Chemotactic factors		
IL-8	0	I
IP-10	5.2 ± 0.9	I
LEC	16.1 ± 2.3	I
MIP-1α	0	I
MIP-1β	27.3 ± 3.8	I
NAP-2	0	I
I-309	0.9 ± 0.2	I
Interferons		
IFN-α	58.2 ± 7.6	III
IFN-β	63.1 ± 4.8	III
IFN-γ	35.5 ± 4.3	II

[a]Groups are formed according to the % of LCR inhibition: I, 0–29%; II, 30–49%; III, 50–70%.

Search and development of antiviral drugs

Blind screening  Is no longer attractive

Modern antiviral discovery

- Mechanism-based screens
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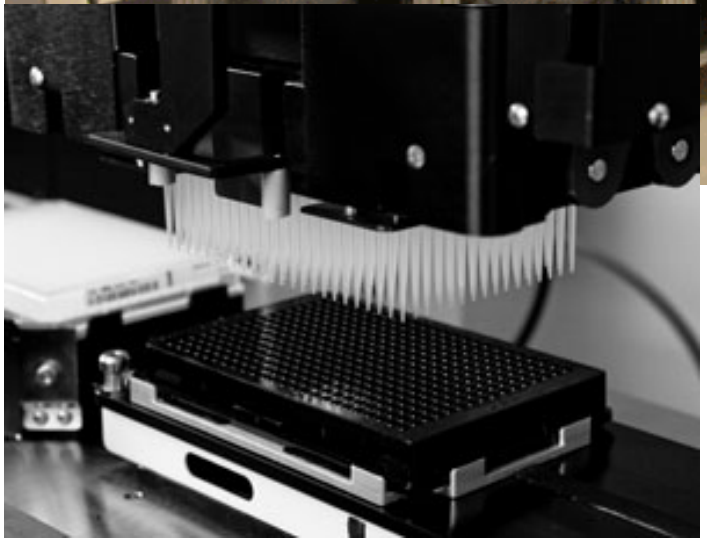
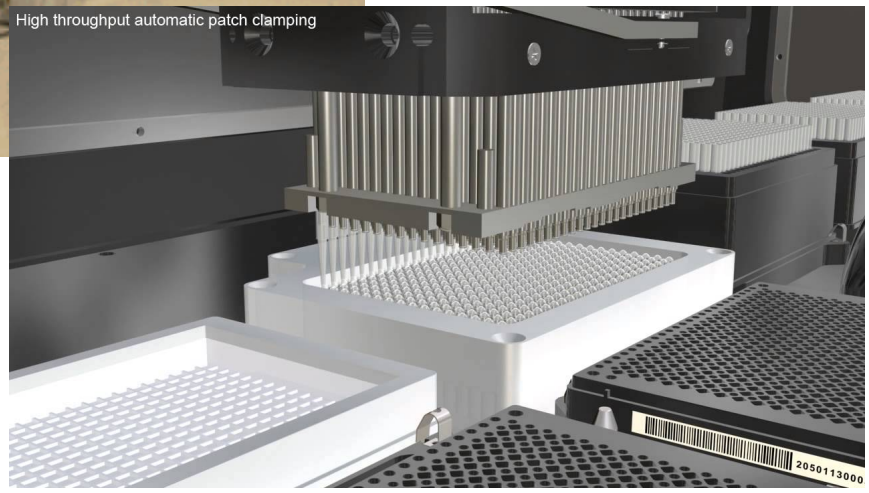
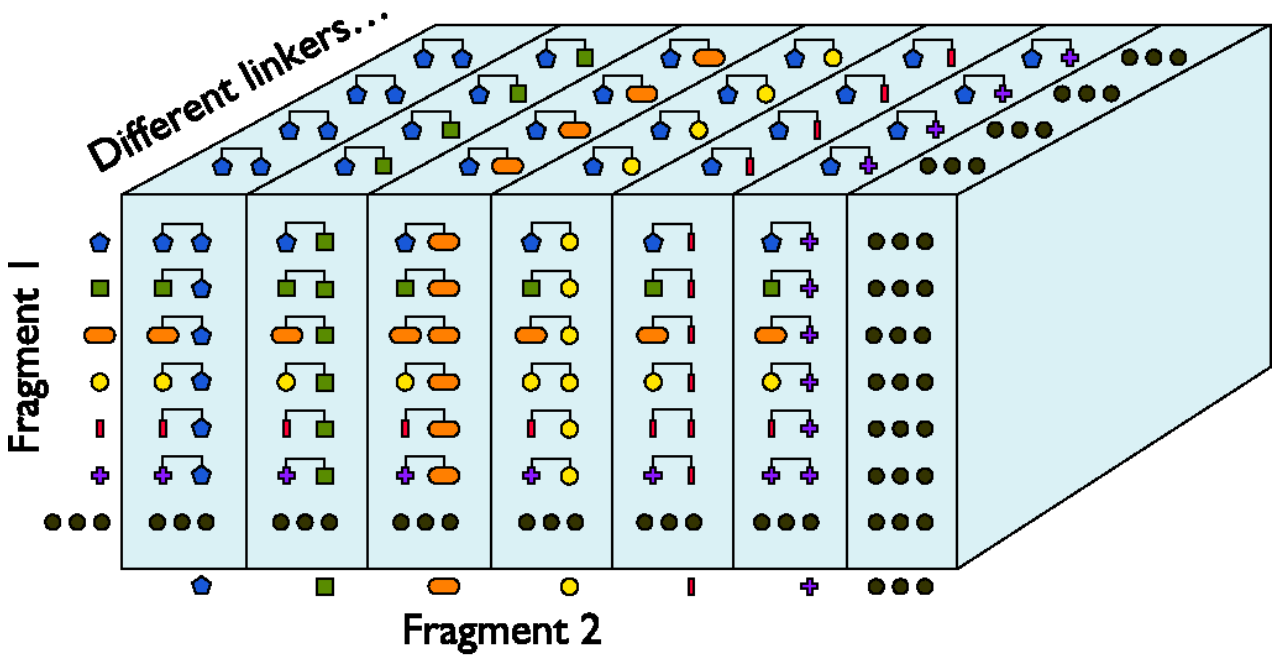
Automated high-throughput screens

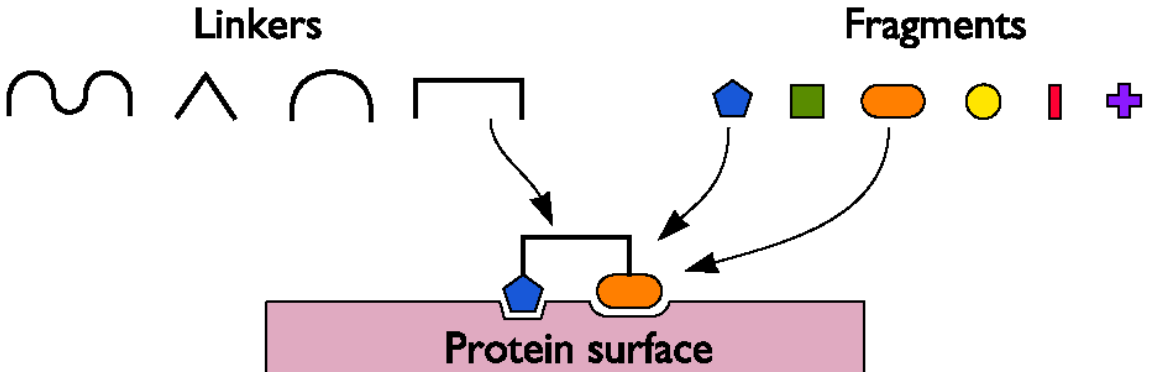




Combinatorial chemistry



Eg. If assembled pairwise with 10 linkers, a collection of 10,000 small molecules yields a library of 10^9 new combinations.



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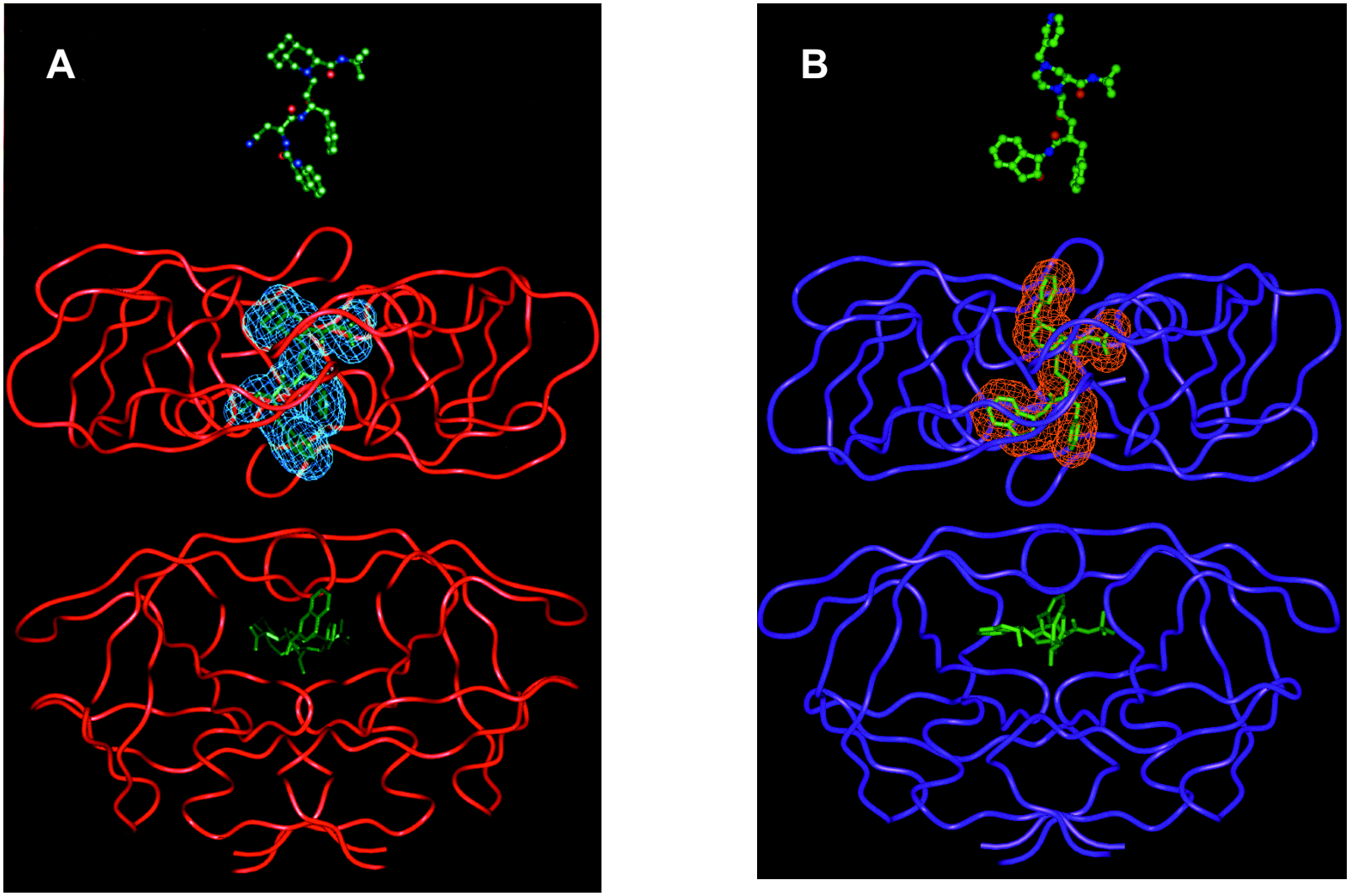
Stucture-assisted drug design as an example of computational approach to drug discovery



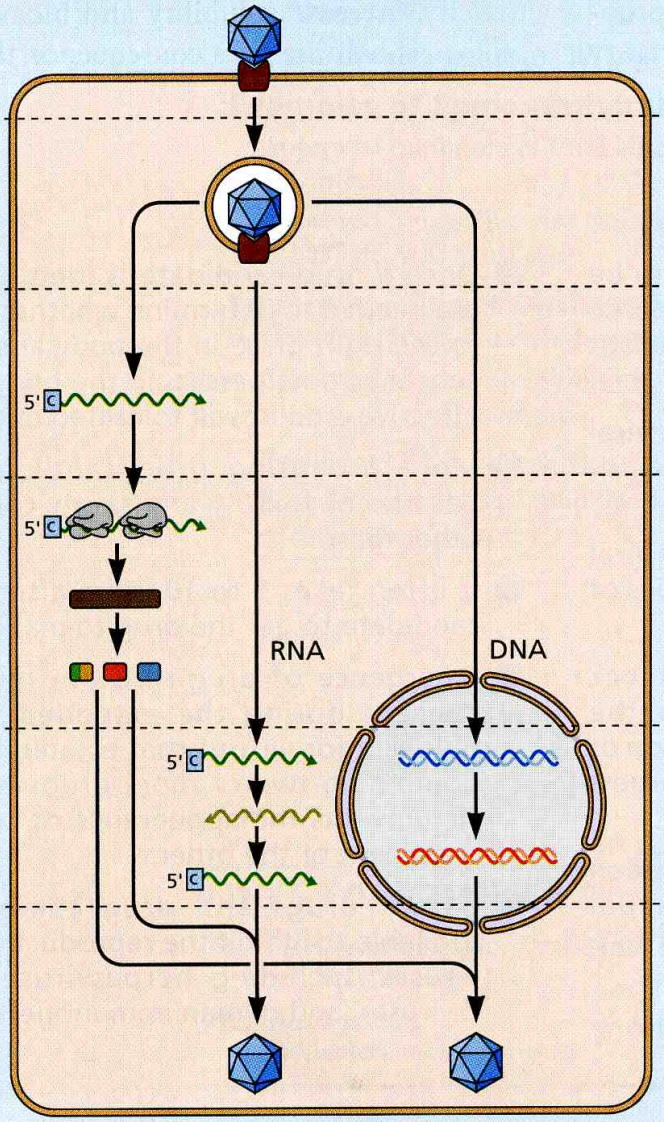
Structure of HIV protease with the inhibitors saquinavir (A) e indinavir (B)

Druggable Human Viruses

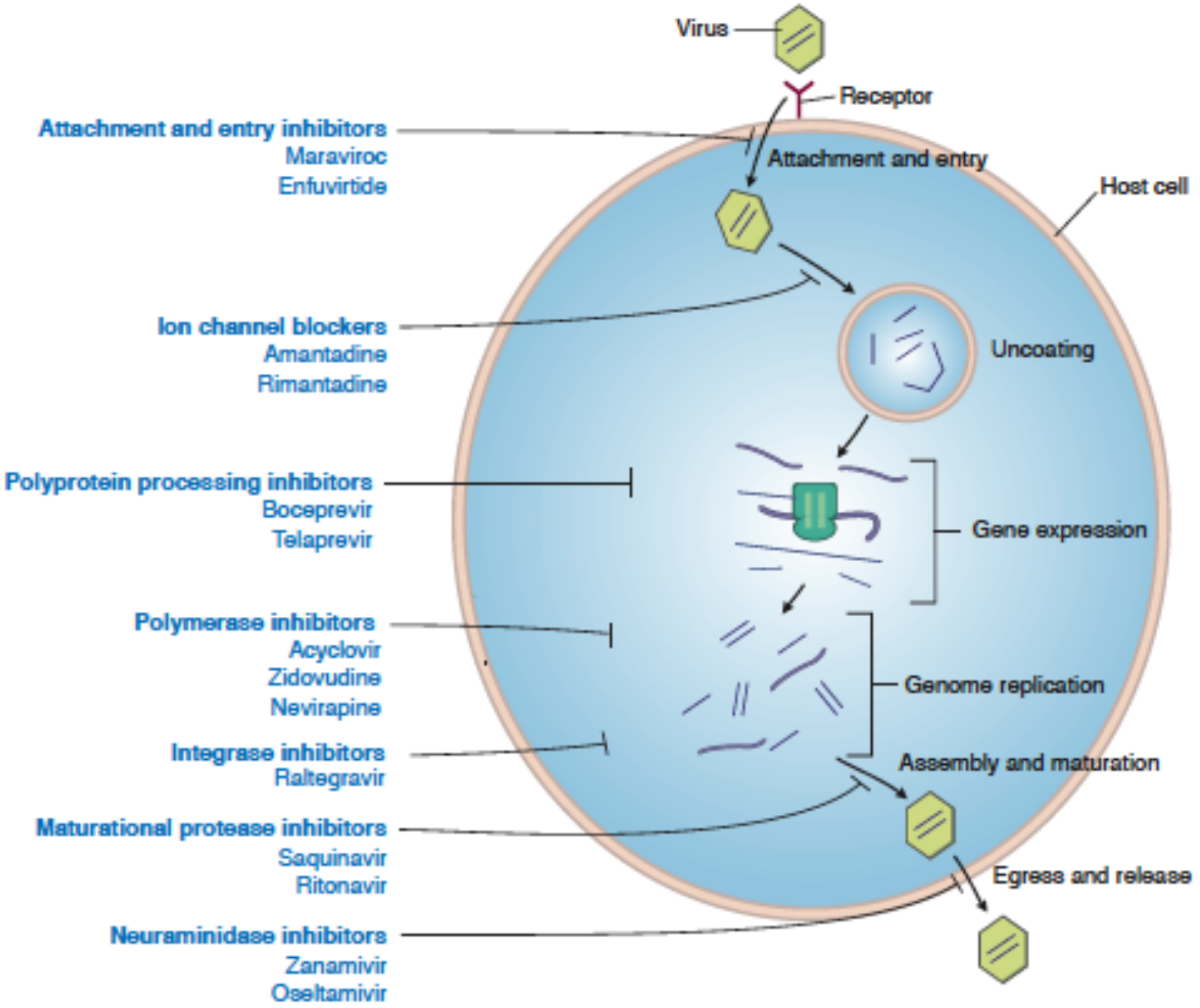
Herpes simplex virus (HSV)
Varicella-zoster virus (VZV)
Human Citomegalovirus (CMV)
Human Immunodeficiency virus (HIV)
Influenza virus
Respiratory Syncitial virus (RSV)
Hepatitis viruses A, B, C (HAV, HBV, HBC)
Human Papillomavirus (HPV)
Picornavirus

Knowledge of viral cycles identifies general targets for antiviral drug discovery

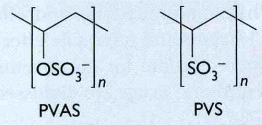
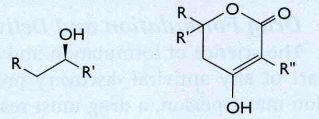
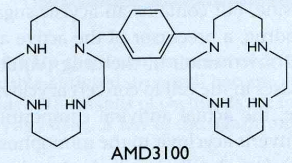
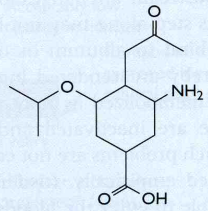
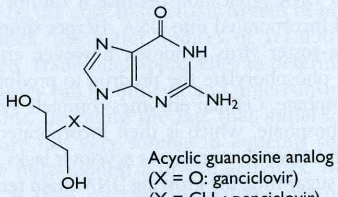
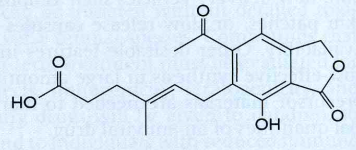
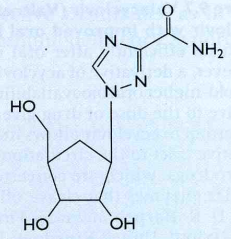
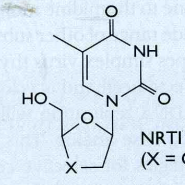
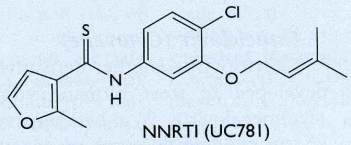
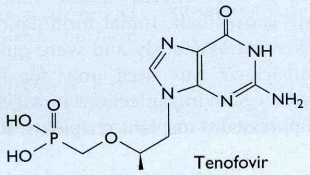
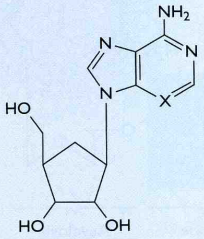
Function	Lead compound or example	Virus
Attachment	Peptide analogs of attachment protein	HIV
Penetration and uncoating	Dextran sulfate, heparin	HIV, herpes simplex virus
mRNA synthesis	Interferon	Hepatitis A, B, and C viruses; papillomavirus
	Antisense oligonucleotides	Papillomavirus, human cytomegalovirus
Protein synthesis/ Initiation	Interferon	Hepatitis A, B, and C viruses; papillomavirus
DNA/RNA replication	Nucleoside, nonnucleoside analogs	Herpesviruses, HIV, hepatitis B and C virus
Assembly	Peptidomimetics	HIV, herpes simplex virus



Some viral targets for antiviral drug discovery



The prototypic compounds (pharmacophores) of important classes of antiviral agents

Important classes	Structures	Important classes	Structures
Viral adsorption inhibitors	 PVAS PVS	**Protease inhibitors**	 Peptidomimetic Nonpeptidomimetic
Viral-cell fusion inhibitors	 TAK779  AMD3100	**Neuraminidase inhibitor**	 Oseltamivir
Viral DNA polymerase inhibitor	 Acyclic guanosine analog (X = O: ganciclovir) ($X = CH_2$: penciclovir)	**IMP dehydrogenase inhibitors**	 Mycophenolic acid  Ribavirin
Reverse transcriptase inhibitors	 NRTI (X = CHN_3, AZT)  NNRTI (UC781)		
Acyclic nucleoside phosphonate	 Tenofovir	**SAH hydrolase inhibitor**	 (X = N or CH)

Examples of viral targets for antiviral drugs

- ✓Attachment
- ✓Entry and uncoa[illegible]g
- ✓Viral gene expressio[illegible]
- ✓Replication of viral geno[illegible]
- ✓Assembly
- ✓Maturation and release

Attachment

Agent	Virus
Receptor antagonists - soluble receptors - peptidomimetics - shRNAs	**HIV**
Neutralizing antibodies	**Many**
Destran sulphate, heparin, peptides	**Herpesvirus, HPV**

BMC Biotechnology 2008, 8:85 doi:10.1186/1472-6750-8-85

BMC Biotechnology

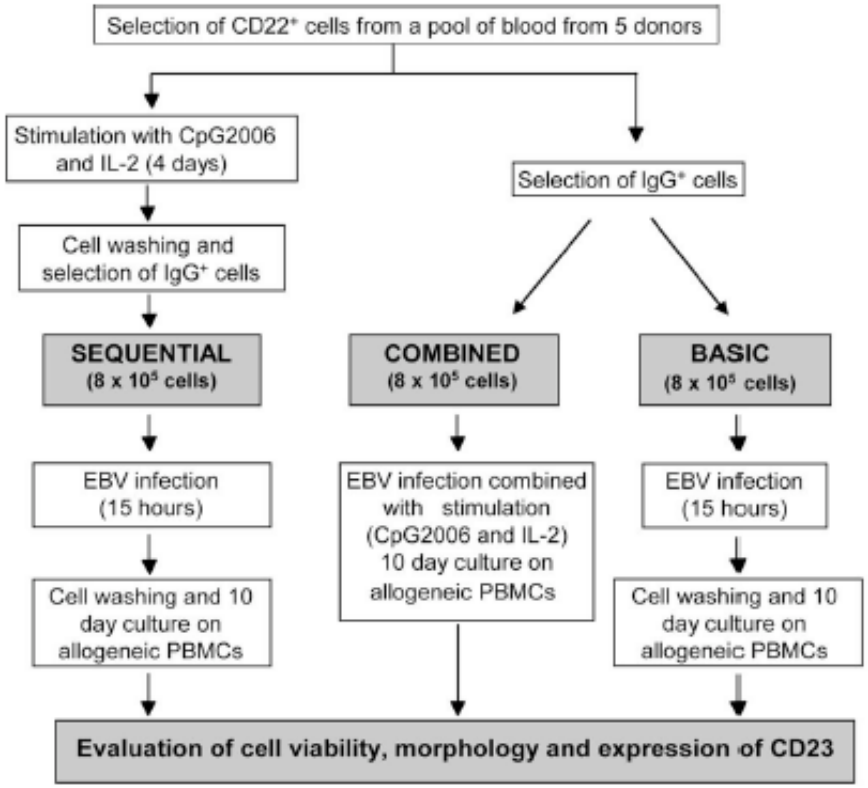
BioMed Central

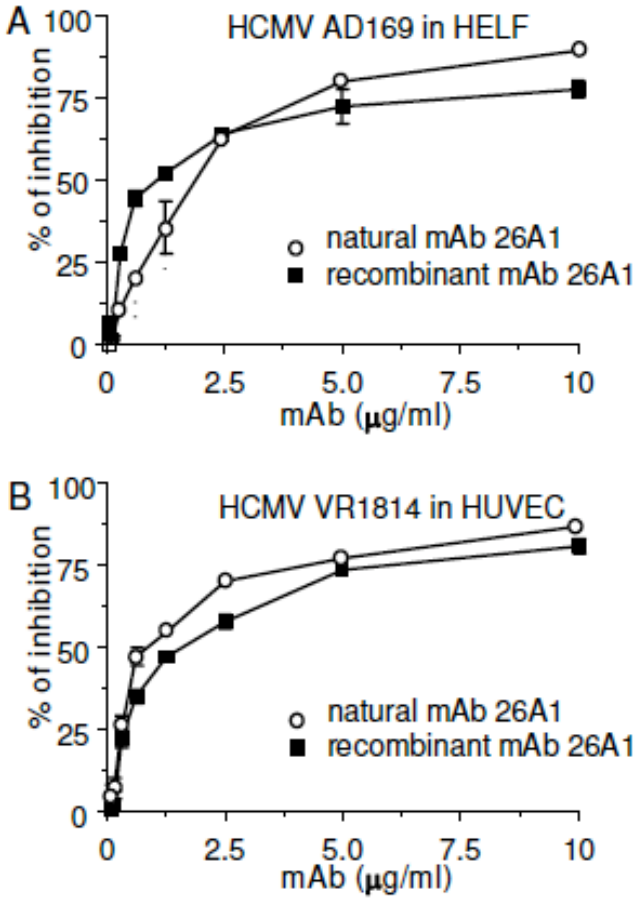
Research article

Open Access

Generation of potent neutralizing human monoclonal antibodies against cytomegalovirus infection from immune B cells

Ada Funaro*[1,2], Giorgio Gribaudo[3], Anna Luganini[3], Erika Ortolan[1,2], Nicola Lo Buono[1], Elisa Vicenzi[4], Luca Cassetta[4], Santo Landolfo[3], Richard Buick[5], Luca Falciola[6], Marianne Murphy[6], Gianni Garotta[6] and Fabio Malavasi[1,2]



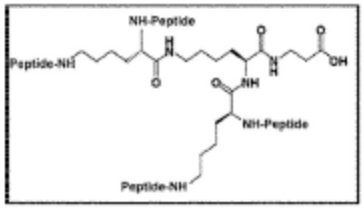


ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3231–3239 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.00149-11

Inhibition of Herpes Simplex Virus Type 1 and Type 2 Infections by Peptide-Derivatized Dendrimers▽†

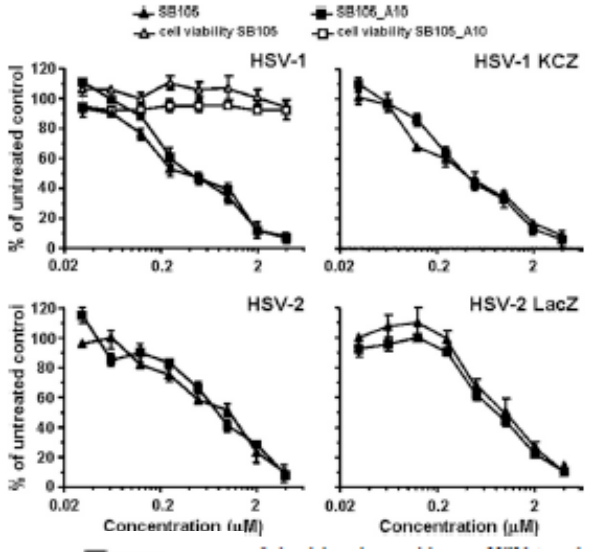
Anna Luganini,[1] Silvia Fabiole Nicoletto,[2] Lorena Pizzuto,[2] Giovanna Pirri,[2] Andrea Giuliani,[2] Santo Landolfo,[1] and Giorgio Gribaudo[1]*

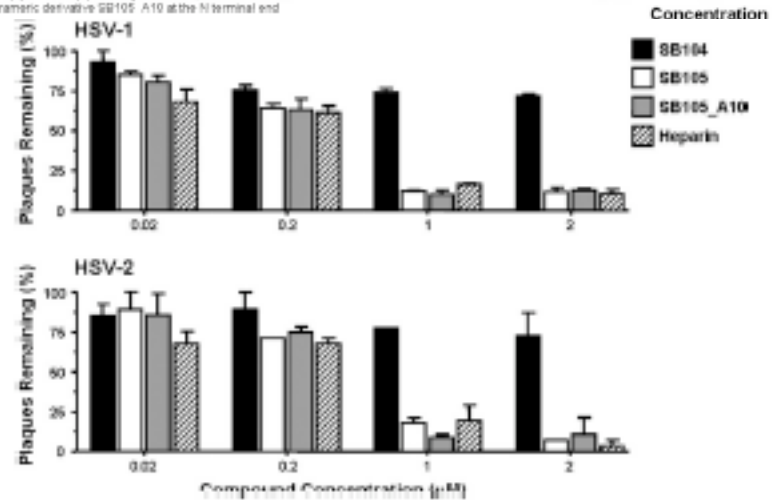
Department of Public Health and Microbiology, University of Turin, Turin,[1] and Spider Biotech S.r.l., Colleretto Giacosa, Turin,[2] Italy



Compound name	Peptide sequence
SB101[a]	QKKIRVRLSA
SB102	QKKIRVRL
SB103	QKKIRVRWSA
SB104	NKKIRVRL
SB105	ASLRVRIKKQ
SB105_LIN[b]	ASLRVRIKKQ
SB105_A10	ASLRVRIKK
SB105_A10-FAM[c]	(N$^{\alpha}$-5(6)FAM)-K-ASLRVRIKK
SB106	FKKIRVRL
SB107	QKKIRVRISA
SB108	QKKIRVRLSW
SB109	QKKIRVRFSA
SB110	QKKIRFRLSA
SB111	QKKIRIRLSA
SB112	QKKIRWRLSA
SB113	QKKFRVRLSA

a the linear peptide sequence is linked to the dimeric core Lys-β-Ala-OH
b the SB105_LIN is a 10-mer linear amido peptide, having the SB105 peptide sequence
c 5(6)-FAM is 5(6)-carboxyfluorescein, used to label the tetrameric derivative SB105_A10 at the N terminal end



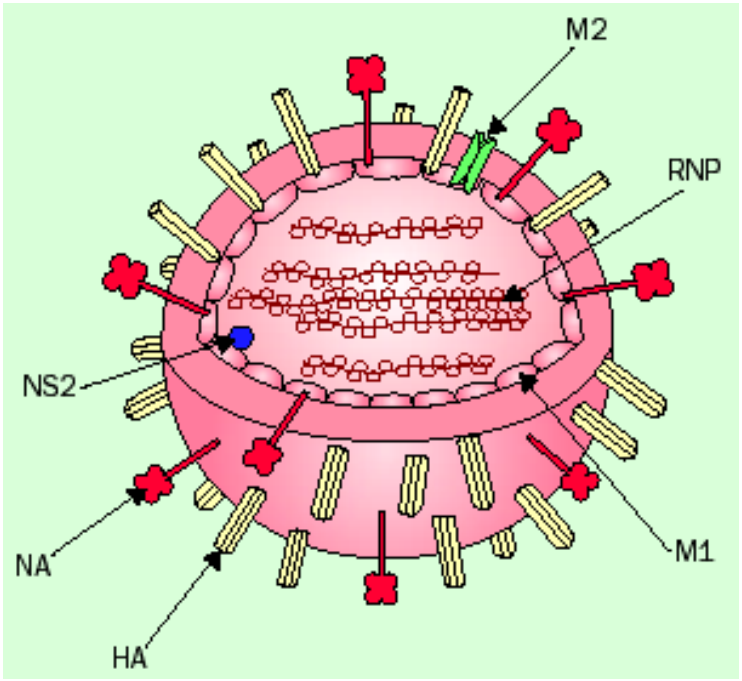


Examples of viral targets for antiviral drugs

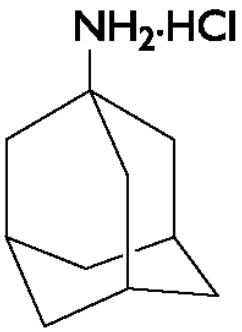
- ✓Attachment
- ✓Entry and uncoating
- ✓Viral gene expression
- ✓Replication of viral genome
- ✓Assembly
- ✓Maturation and release

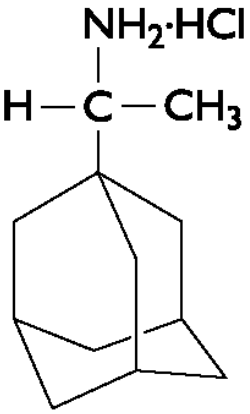
Entry and uncoating

Agent	Virus
Amantadine, rimantadine	Influenza A virus
Pleconaril	Picornavirus
Enfuvirtide	HIV



Amantadine and Rimantadine





✓They are ion channel blockers that inhibit viral RNA uncoating by blocking the function of the envelope M2 protein

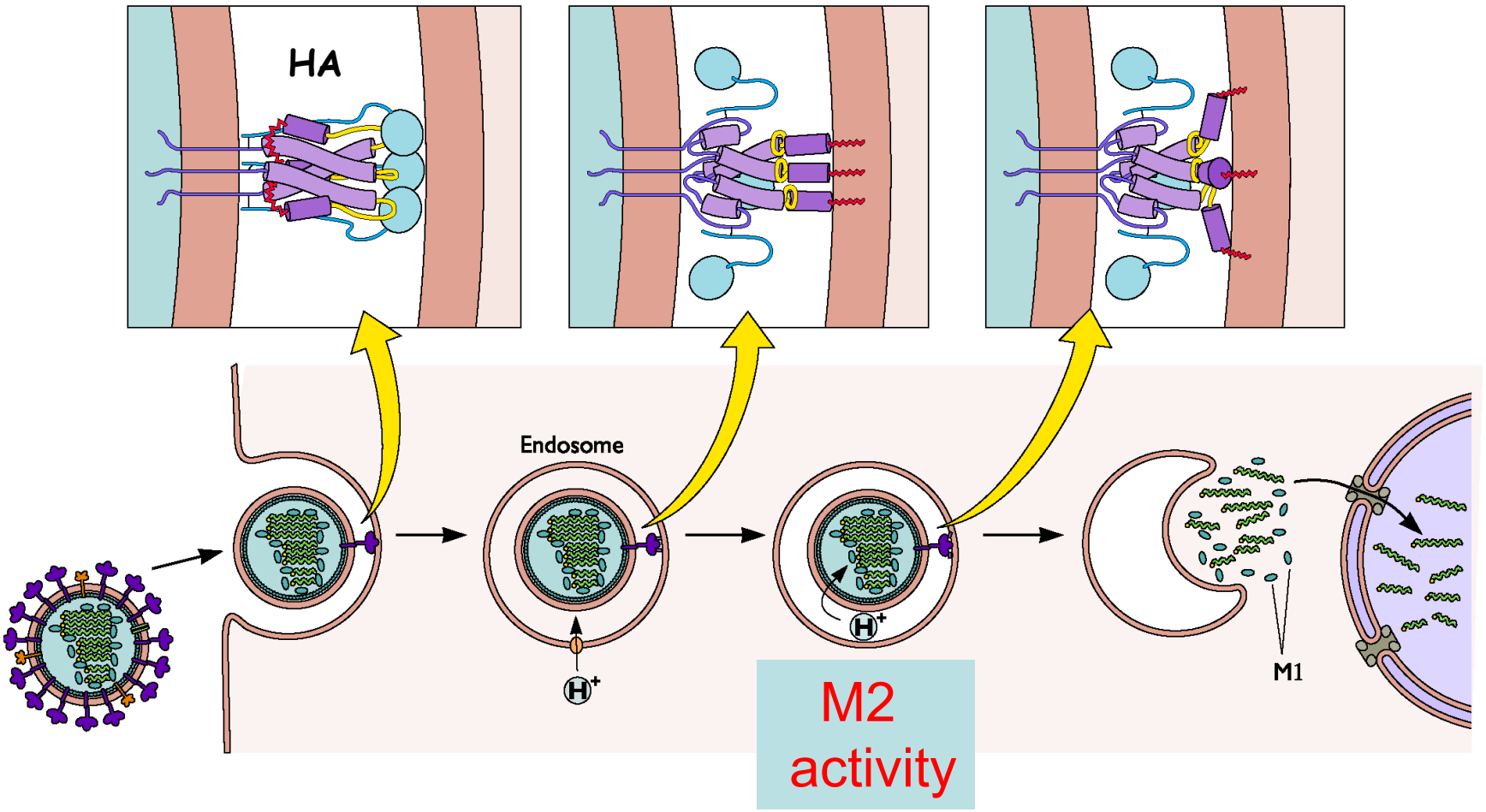
✓Useful in therapy and prevention of influenza A infections

✓They must be given within 24-48 hrs from symptoms onset

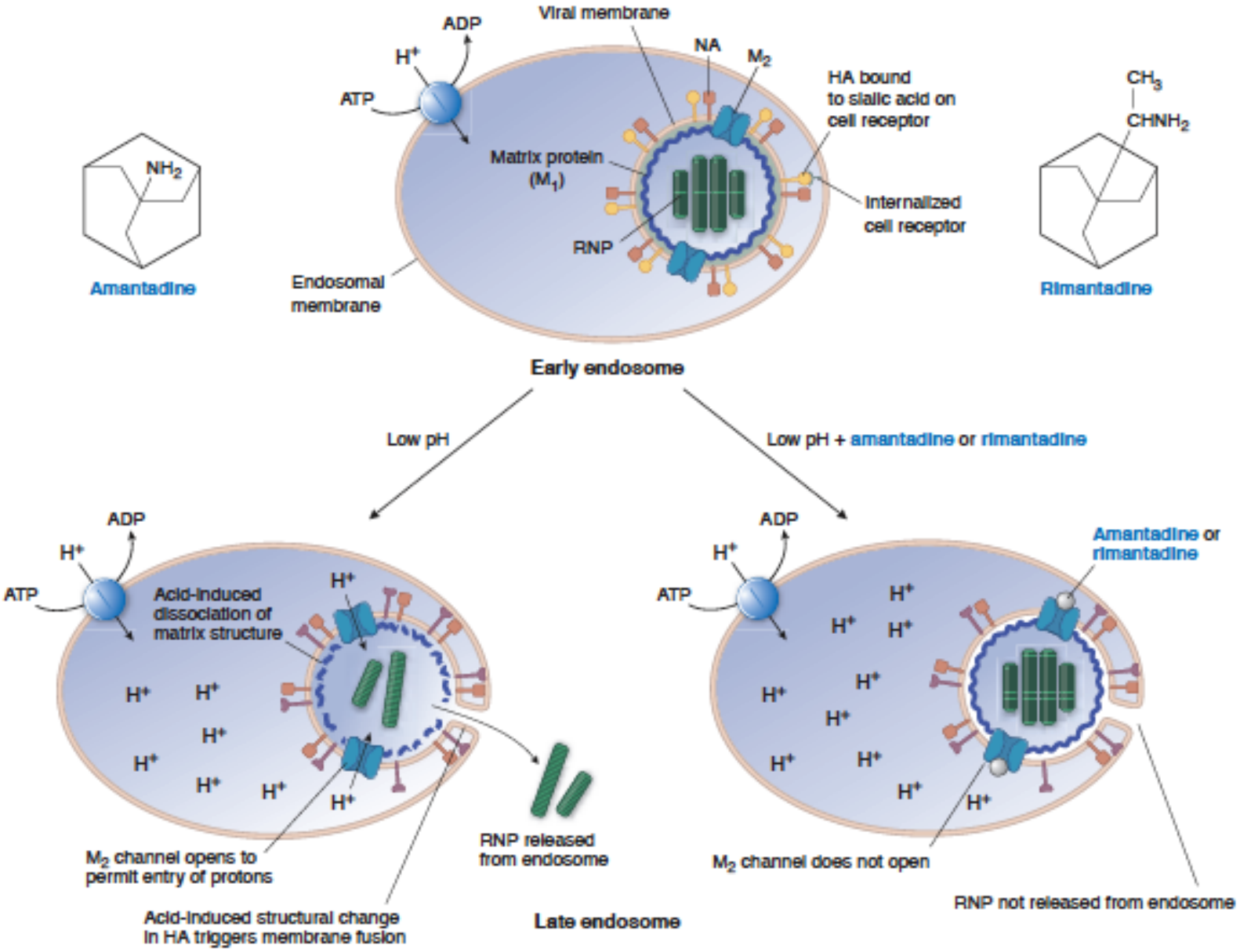
✓High frequency of resistant virus due to M2 or HA mutations

Amantadine and Rimantadine: mechanism of action

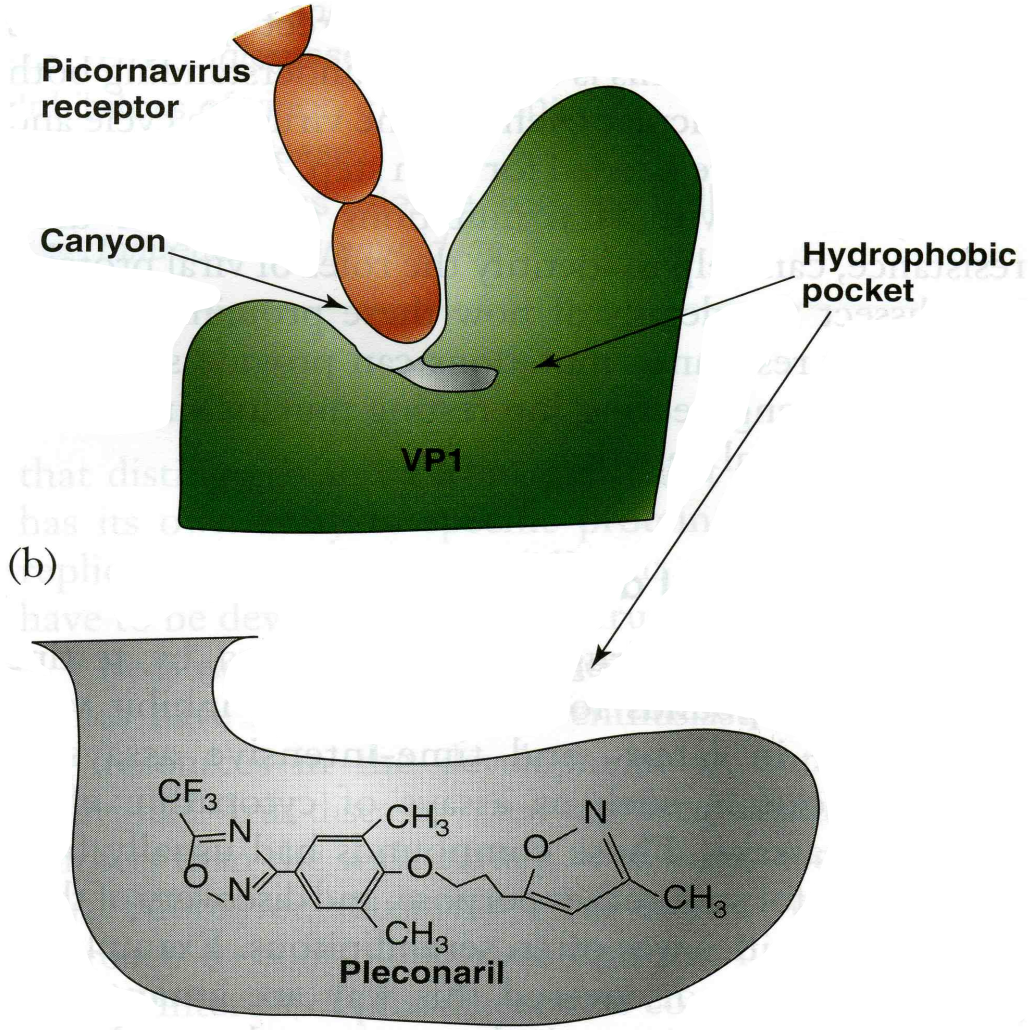
Role of M2 protein in Influenza A virus uncoating



Model for uncoating Influenza A virus and effect of amantadine and rimantadine



Pleconaril : mechanism of action



Examples of viral targets for antiviral drugs

- ✓**Attachment**
- ✓**Entry and uncoating**
- ✓**Viral gene expression**
- ✓**Replication of viral genon**
- ✓**Assembly**
- ✓**Maturation and release**

Viral gene expression

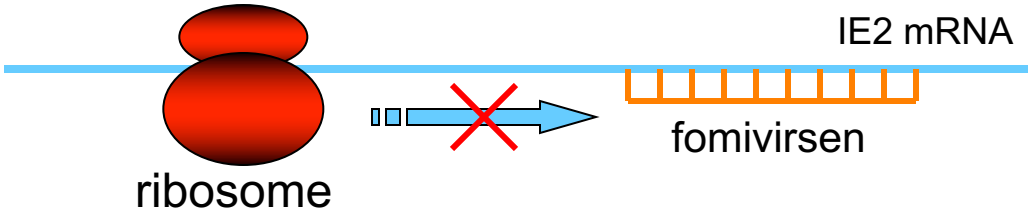
Agent	Virus
Interferons	**HAV, HBV, HCV, HPV**
Antisense oligos	**HCMV**

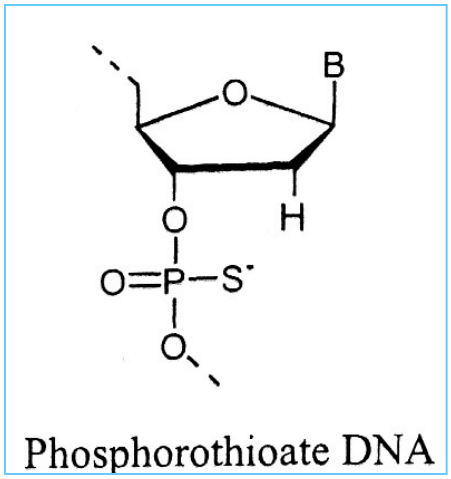
An example of an antiviral antisense oligodeoxynucleotide: Fomivirsen

- It is an antisense phosphorothioate oligodeoxynucleotide complementary to a segment of HCMV IE2 mRNA

21 nt
GCG TTT GCT CTT CTT CTT GCG

- It inhibits viral E and L gene expression, viral DNA synthesis, and viral replication by blocking translation of IE2 mRNA





Phosphorothioate DNA

- Fomivirsen (Vitravene) was used for intravitreal treatment of HCMV retinitis in AIDS patients resistant to other anti-HCMV drugs. No longer marketed.

- Fomivirsen was the first antisense oligo approved for use in humans (FDA, 1998)

Examples of viral targets for antiviral drugs

- ✓Attachment
- ✓Entry and uncoating
- ✓Viral gene expression
- ✓Replication of viral genome
- ✓Assembly
- ✓Maturation and release

Replication of viral genome

Agent	Virus
Nucleoside analogs	Herpes, HIV, HBV
Nonnucleoside inhibitors	Herpes, HIV
Ribavirin	RSV, HCV, HEV

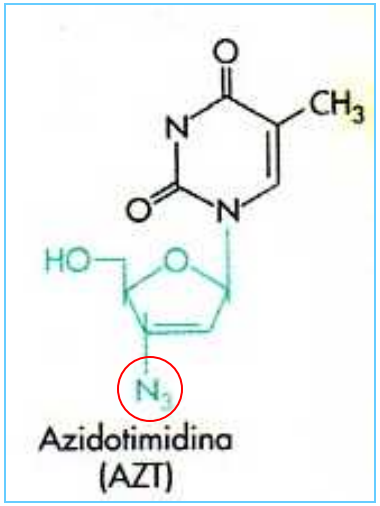
Antiviral nucleoside and nucleotide analogs

- ✓ The most widely used antiviral drugs
- ✓ They target viral DNA polymerases and viral reverse transcriptases
- ✓ They bear a base and/or a sugar modification
- ✓ Their activation to dNTPs requires phoshorylation mediated by cellular and/or viral kinases
- ✓ Viral polymerases are selectively inhibited because they bind the modified dNTP with higher affinity (100-fold) than cellular enzymes.

Nucleoside and nucleotide analogs:mechanism of action

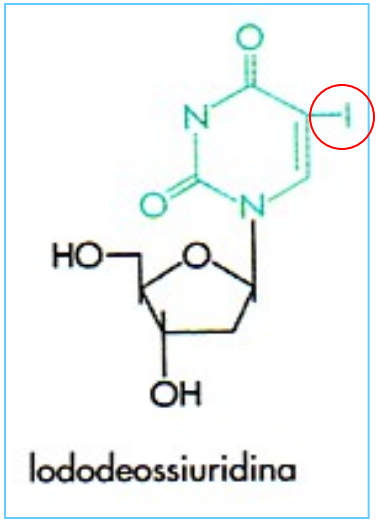
Lack of the di un 3’-OH group of the sugar

Growing DNA chain terminates and further DNA replication is blocked

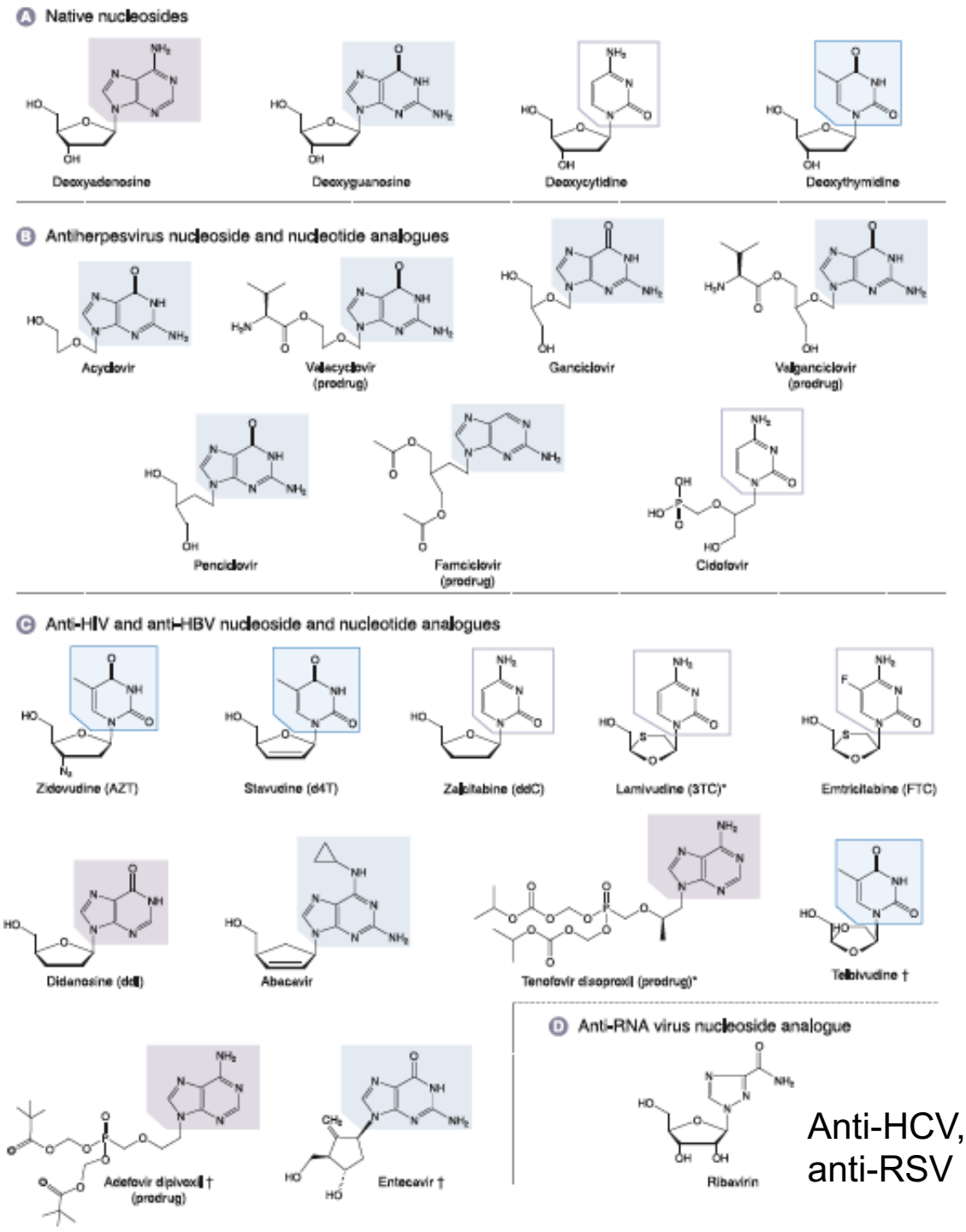


Base modification

Alteration of base pairing

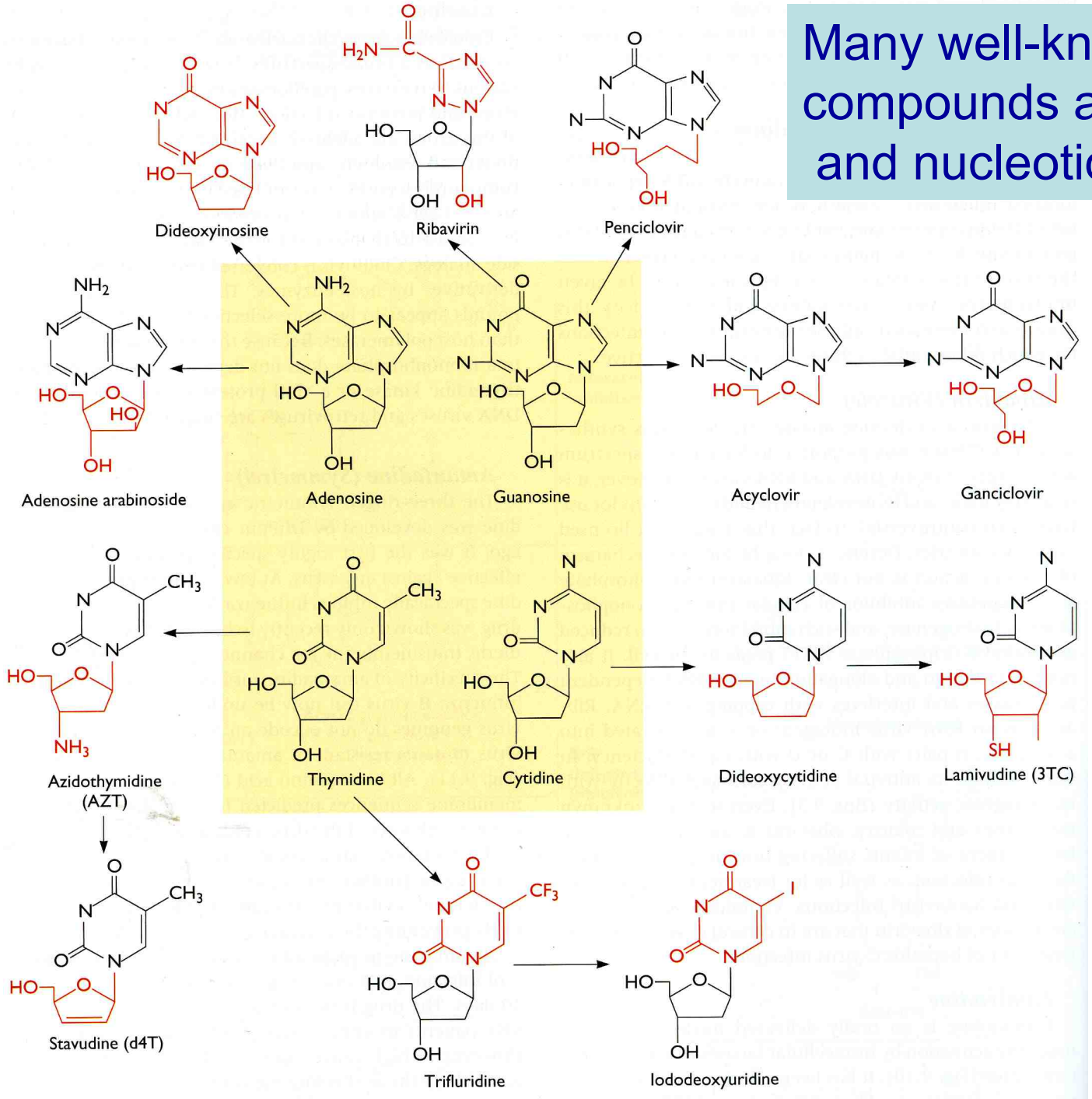


Structures of antiviral nucleoside analogs

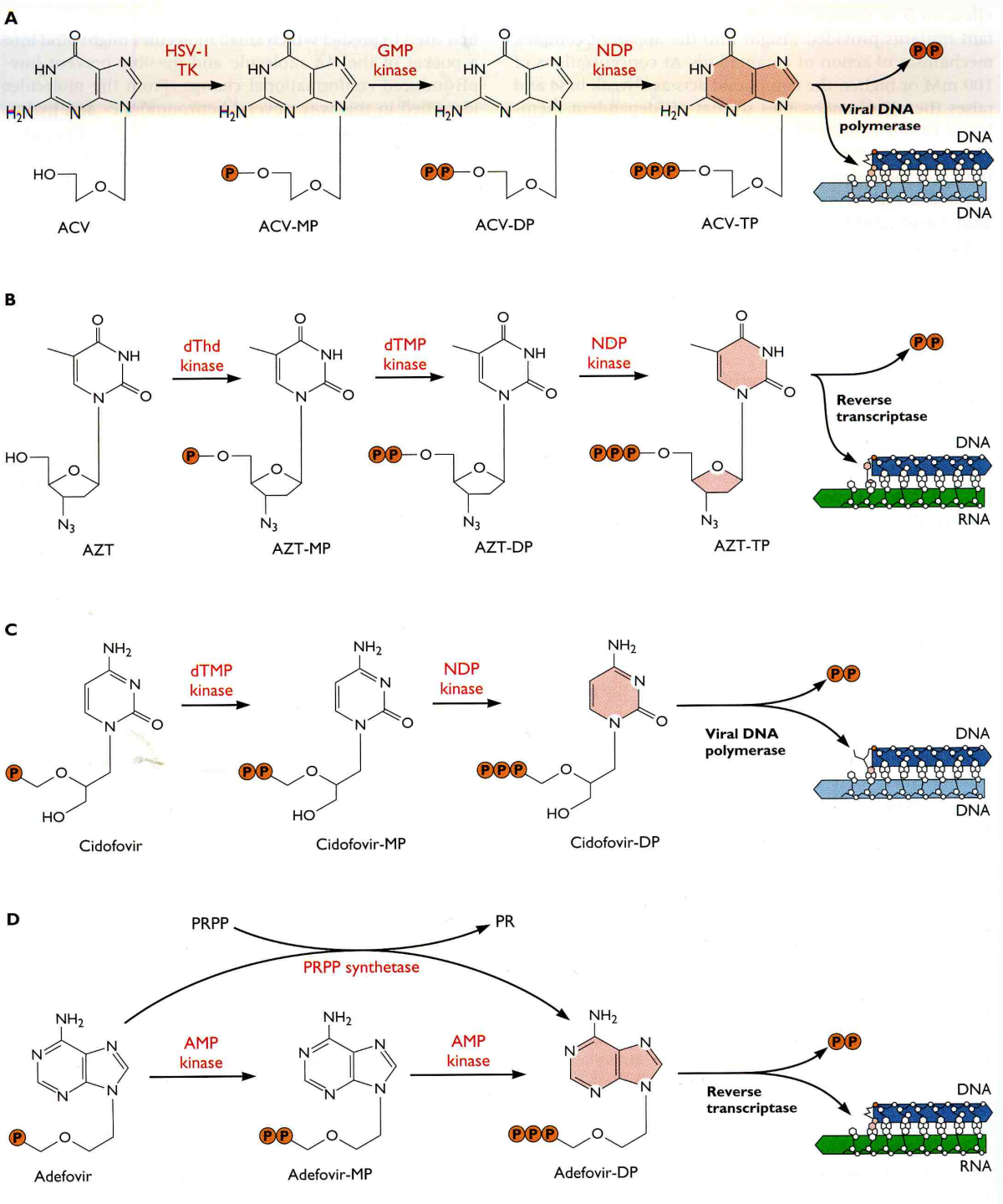


Anti-HCV, anti-RSV

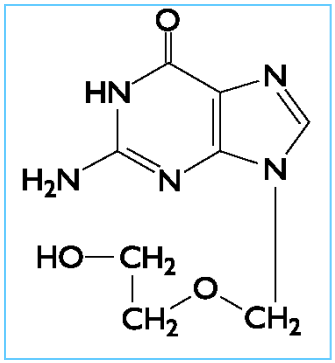
Many well-known antiviral compounds are nucleoside and nucleotide analogs



Chain termination by acyclic nucleoside analogs ACV and AZT, and by acyclic nucleotide analogs Cidofovir and Adefovir



Acyclovir (Zovirax)



✓Acyclovir is a nucleoside analog similar to guanosine, but contain an acyclic sugar group (hydroxyetossimethyl) in place of the deoxyribose. It is a **prodrug** – a precursor of the active antiviral compound

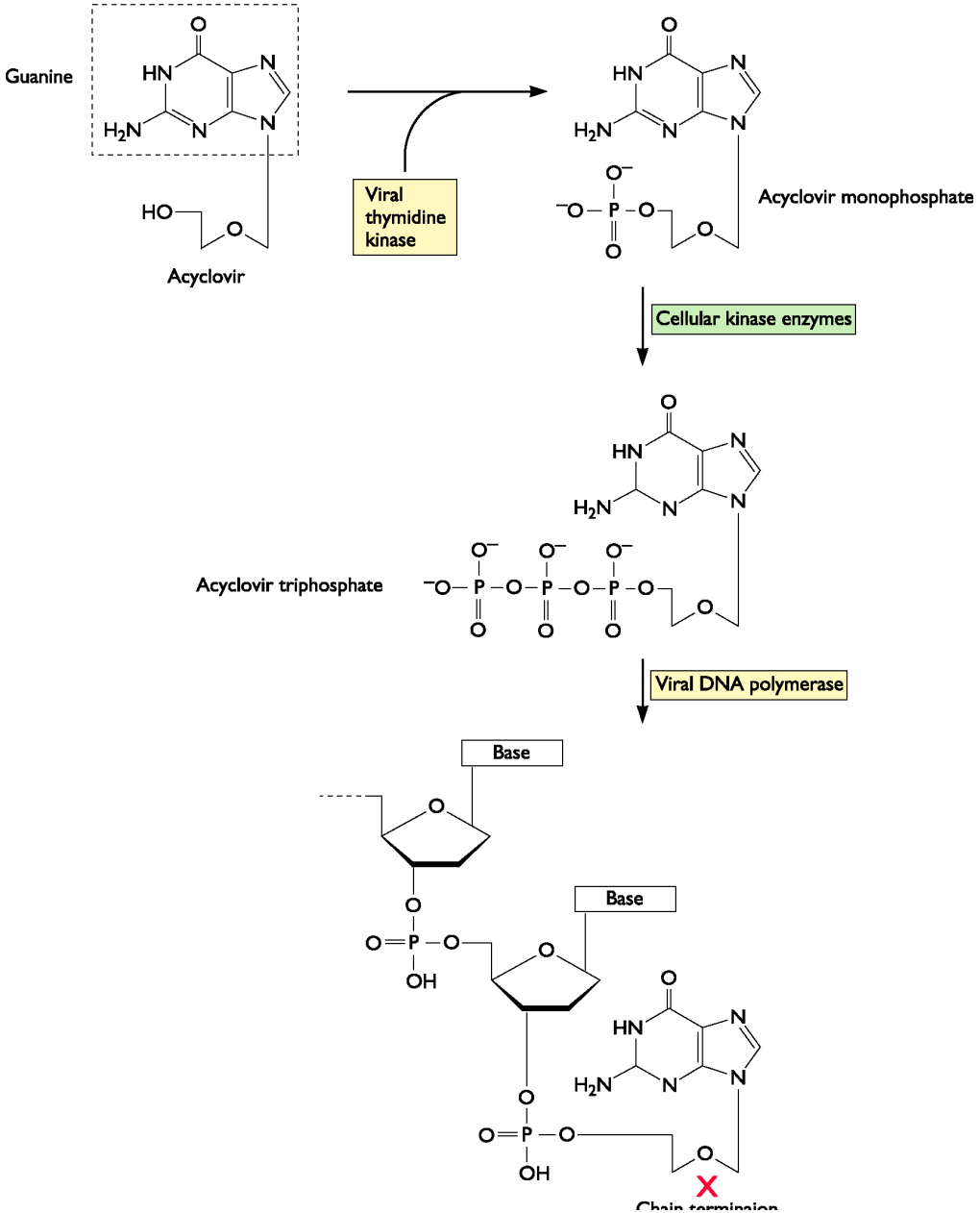
✓Acyclovir is active against HSV and VZV (to some extent)

✓Useful for acute primary diseases and to prevent reactivations

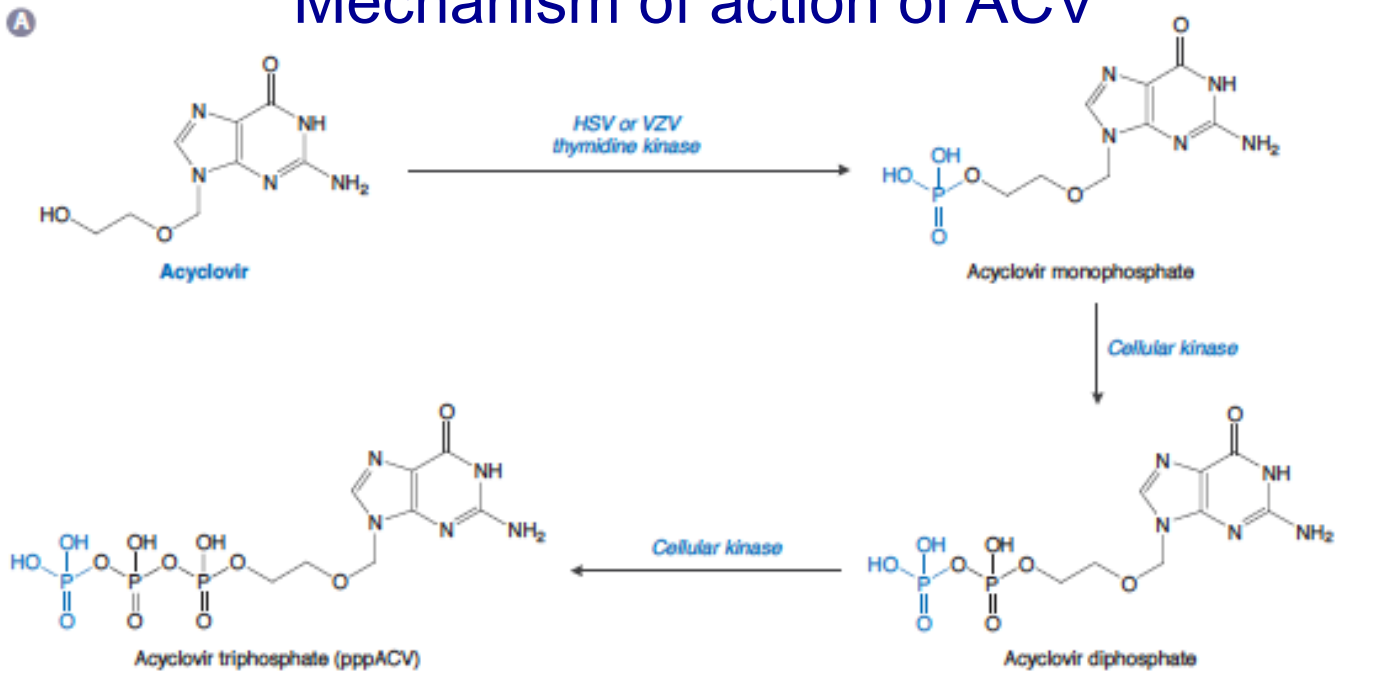
✓It does not eliminate latent infections

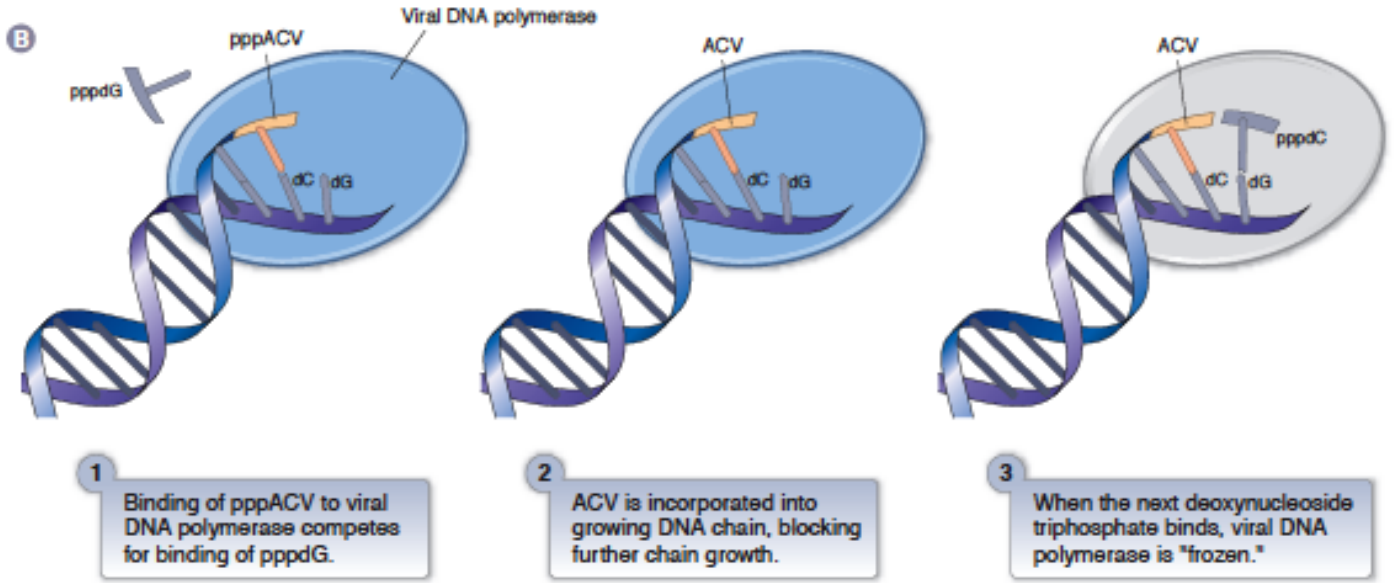
✓Resistance stems from mutations of the viral thymidine kinase (TK) and/or DNA polimerase

Acyclovir: mechanism of action



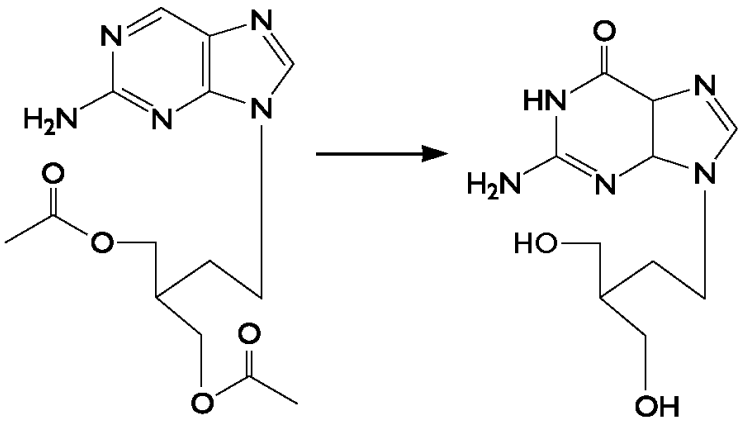
Mechanism of action of ACV





ACV derivatives: Famciclovir and Valaciclovir

Famciclovir:
9-(4-acetoxy-3-acetoxymethylbut-1-yl)guanine (Famvir)



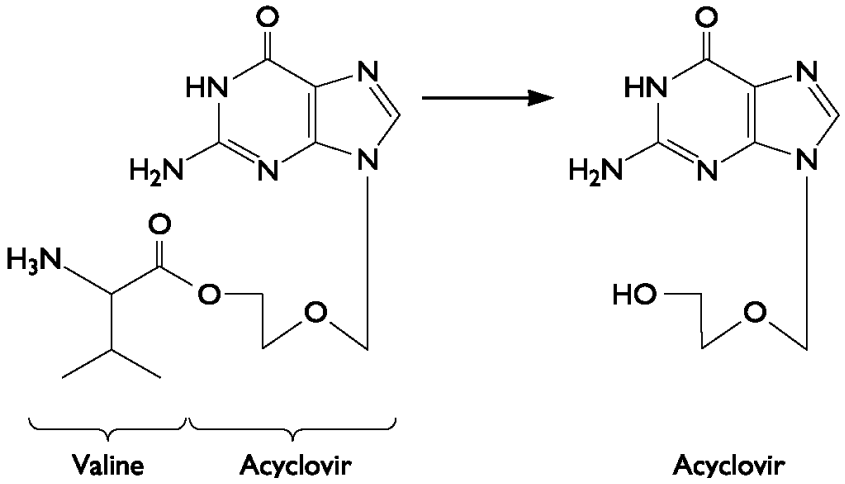
Famciclovir Penciclovir

✓They are **proprodrugs** that require two activation events in a row:

- cleavage of the side chain
- phoshorylation

✓Fivefold-higher oral bioavailability than ACV

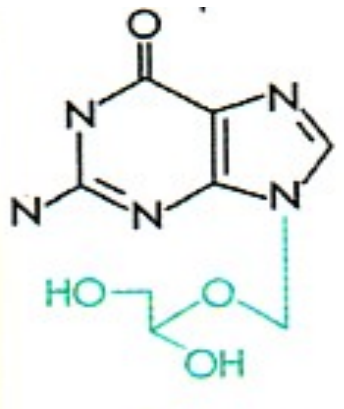
Valacyclovir:
L-valyl ester of acyclovir (Valtrex)



Valine Acyclovir Acyclovir

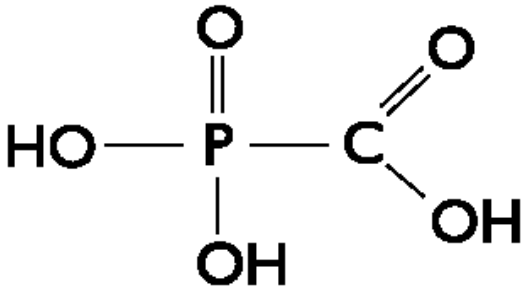
✓Active against HSV and VZV

Ganciclovir (Cytovene)



- ✓GCV is a guanosine analog with an acyclic sugar group (dihydroxyipropoximethyl) in place of deoxyribose
- ✓It is selectively active agaisnt HCMV
- ✓Useful for over HCMV diseases, prophylaxis and preemptive therapy
- ✓Intially given intravenously, quite toxic, used only for life-threatening HCMV infections
- ✓Valgancicovir, a valyl ester prodrug of GCV can be taken orally and is less toxic
- ✓Resistance stems from mutations of the viral kinase (pUL97) and/or DNA polimerase

Foscarnet (Foscavir)



✓Foscarnet it is the only nonnucleoside DNA replication inhibitor of herpesviruses. It is a pyrophosphate analog (phoshonoformic acid)

✓Foscarnet it is a noncompetitive inhibitor of the pyrophoshate-binding site of herpesvirus DNA polymerases

✓It is active against HCMV (HSV, HBV, HIV)

✓Useful for therapy of HCMV GCV-resistant strain infections

✓It accumulates in bone and causes kidney toxicity, recommended only for life-threatening infections for which other anti-HCMV drugs are no longer effective

✓Resistance stems from mutations of the viral DNA polimerase

Examples of viral targets for antiviral drugs

- ✓Attachment
- ✓Entry and uncoating
- ✓Viral gene expression
- ✓Replication of viral genome
- ✓Assembly
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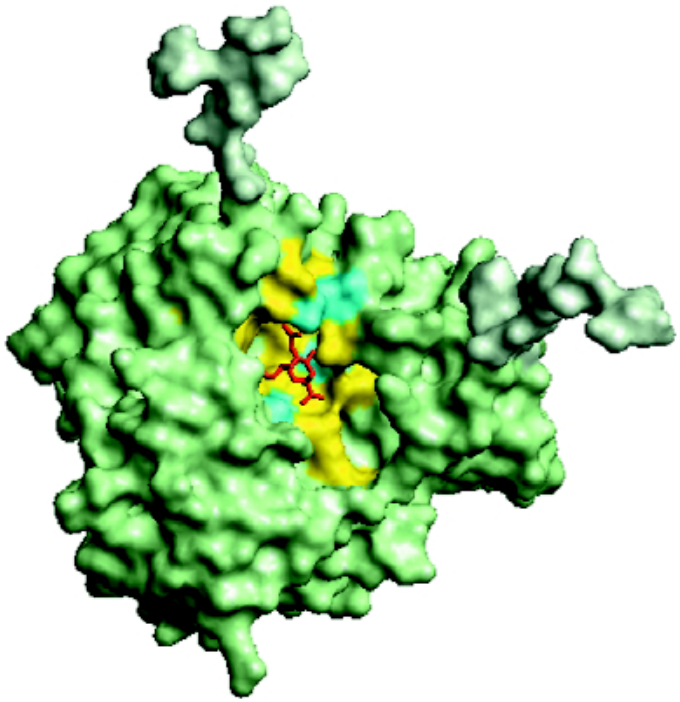
Maturation and release

Agent	Virus
Peptidomimetics, substrate analogs	HIV
Neuraminidase inhibitors	Influenza

Neuroaminidase inhibitors:
an example of structure-based design in antiviral drug discovery

Neuraminidase: structure and function



In light blue and yellow,
NA active site invariant amino acids
In red, neuraminic acid

✓NA is a tetrameric envelope glycoprotein

✓NA cuts the the a-chetosidic bond between terminal neuraminic (sialic) acid and the oligosaccharide chain

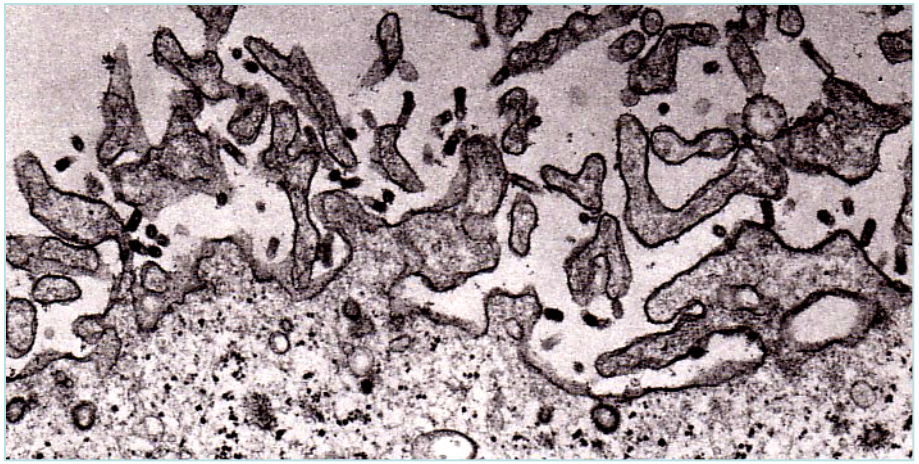
✓NA destroys HA cell surface receptors

✓NA promotes virus penetration through sialic acid-rich secretions

✓NA mediates release of viral progenies by cutting sialic acid residues of the surface of infected cells and viral envelope

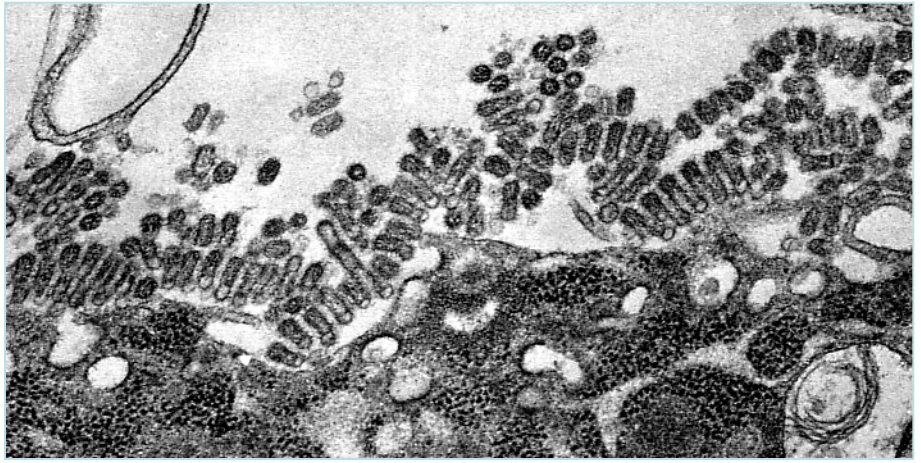
Function of Influenza virus neuraminidase

P. Palese, M. Ueda, K. Tobita, R.W. Compans. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology 61:397–410 (1974).



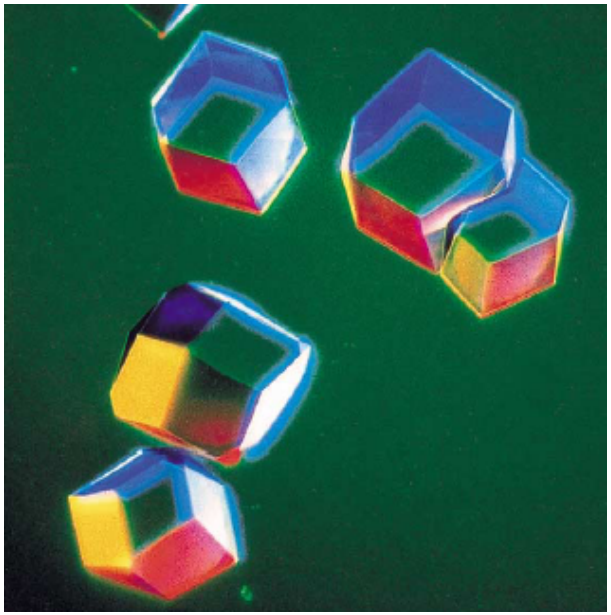
33° C
WT phenotype
Release of infectious free virions

39.5° C
NA- phenotype
Non-infectious virions aggregates



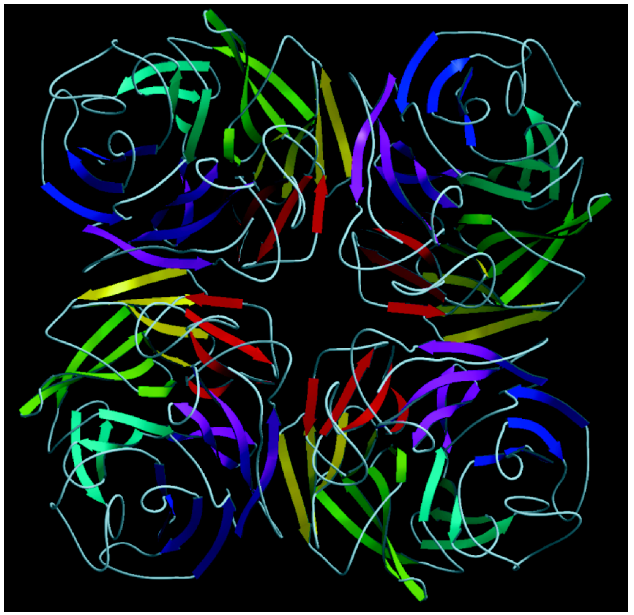
Development of neuroaminidase inhibitors

1



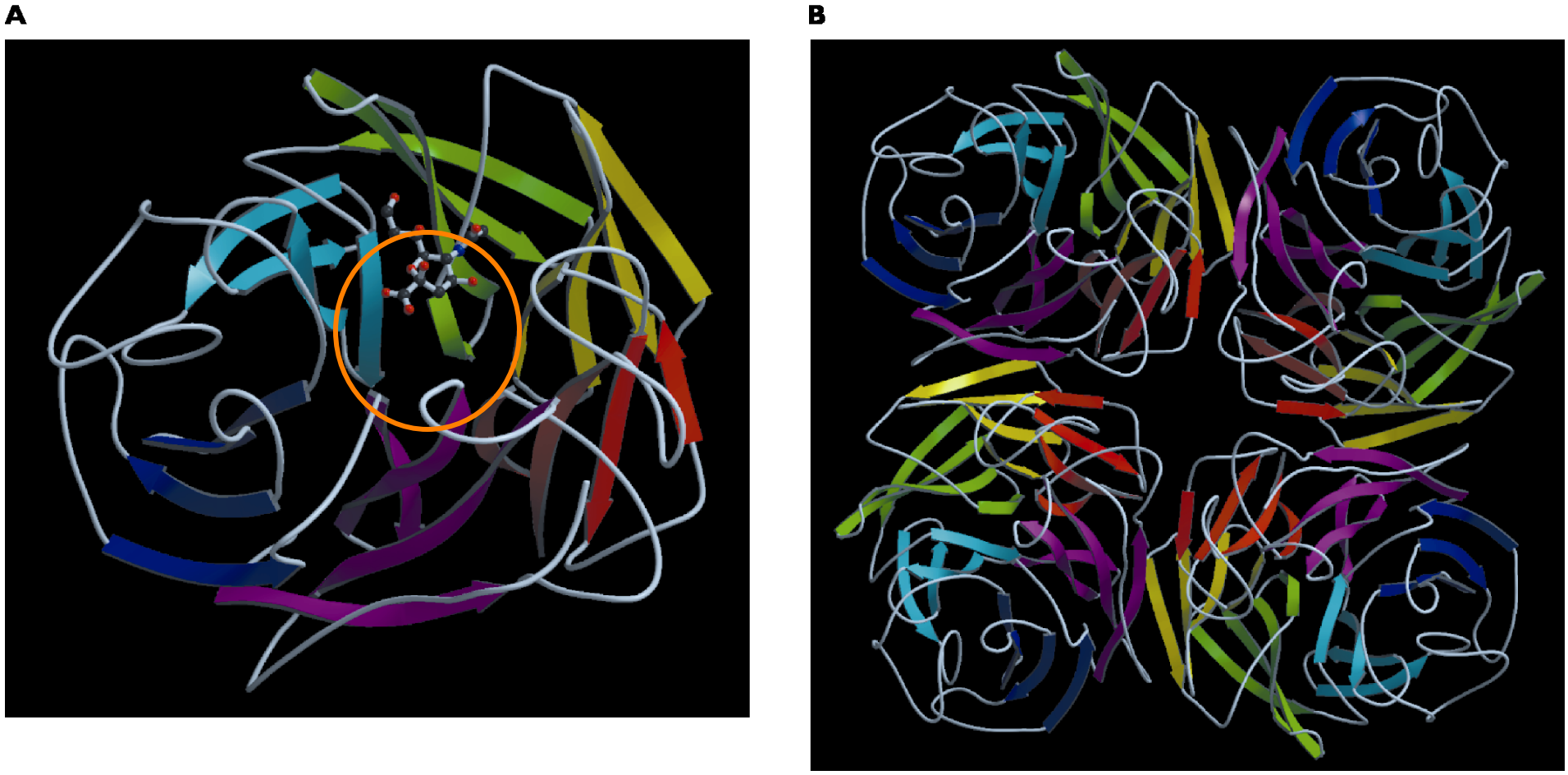
Crystallization

2



Structural analysis

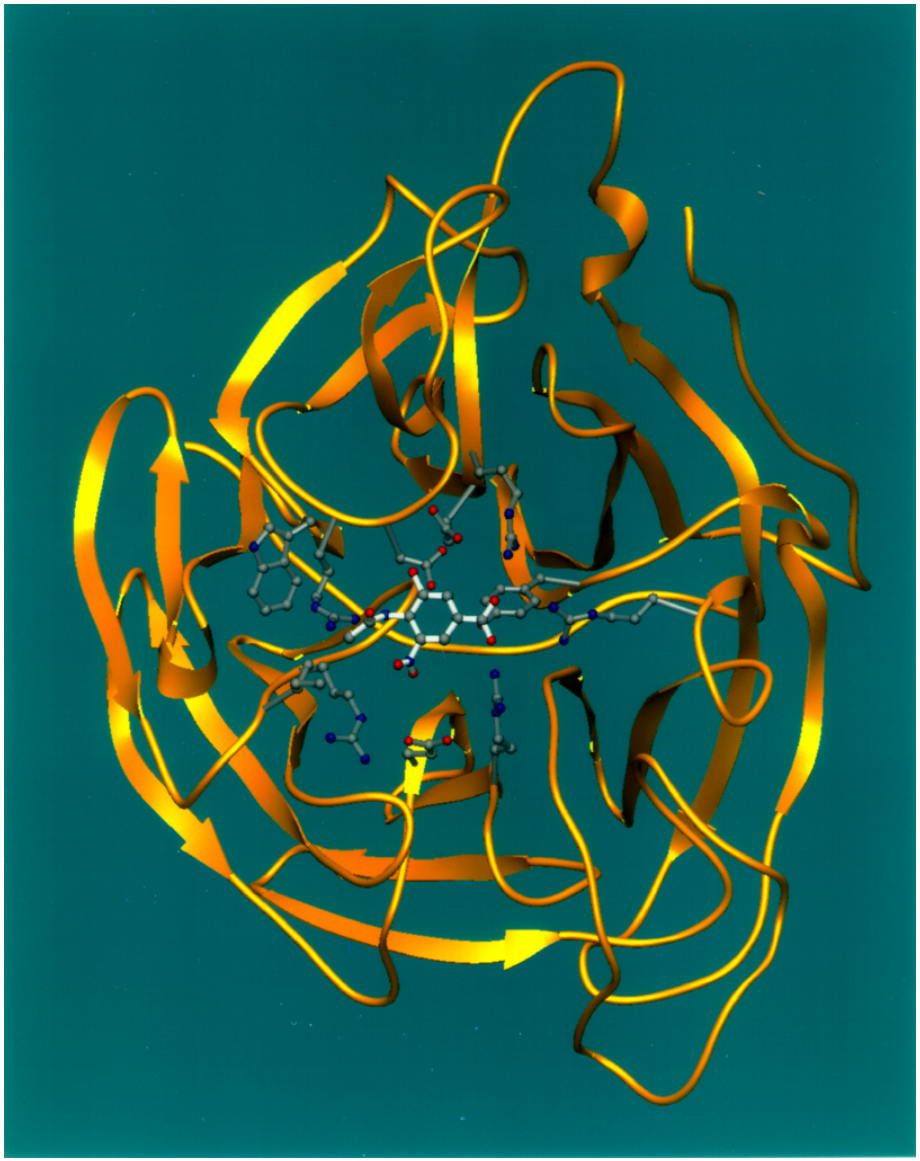
Structure of influenza A virus neuroaminidase

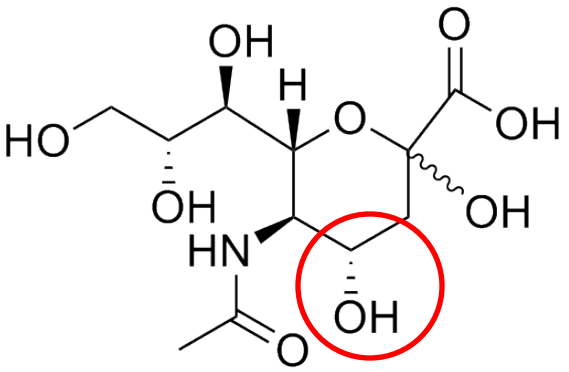


The binding site of the enzyme does not vary from strain to strain

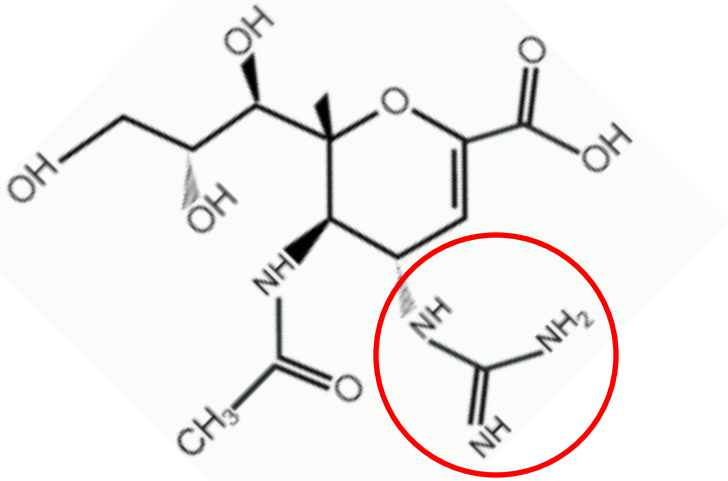
- It consists of 18 amino acid residues of which 12 are in direct contact with the bound sialic acid analogue (and presumably with sialic acid in catalytically active situations).
- Four of these 12 are positively-charged arginines, while another 4 are **negative-charged glutamic** and **aspartic acid** residues. The remainder are neutral (tyrosine, asparagine, isoleucine and tryptophan).

Structure of the influenza A neuraminidase N9 bound to an analogue of sialic acid has been determined by X-ray crystallography



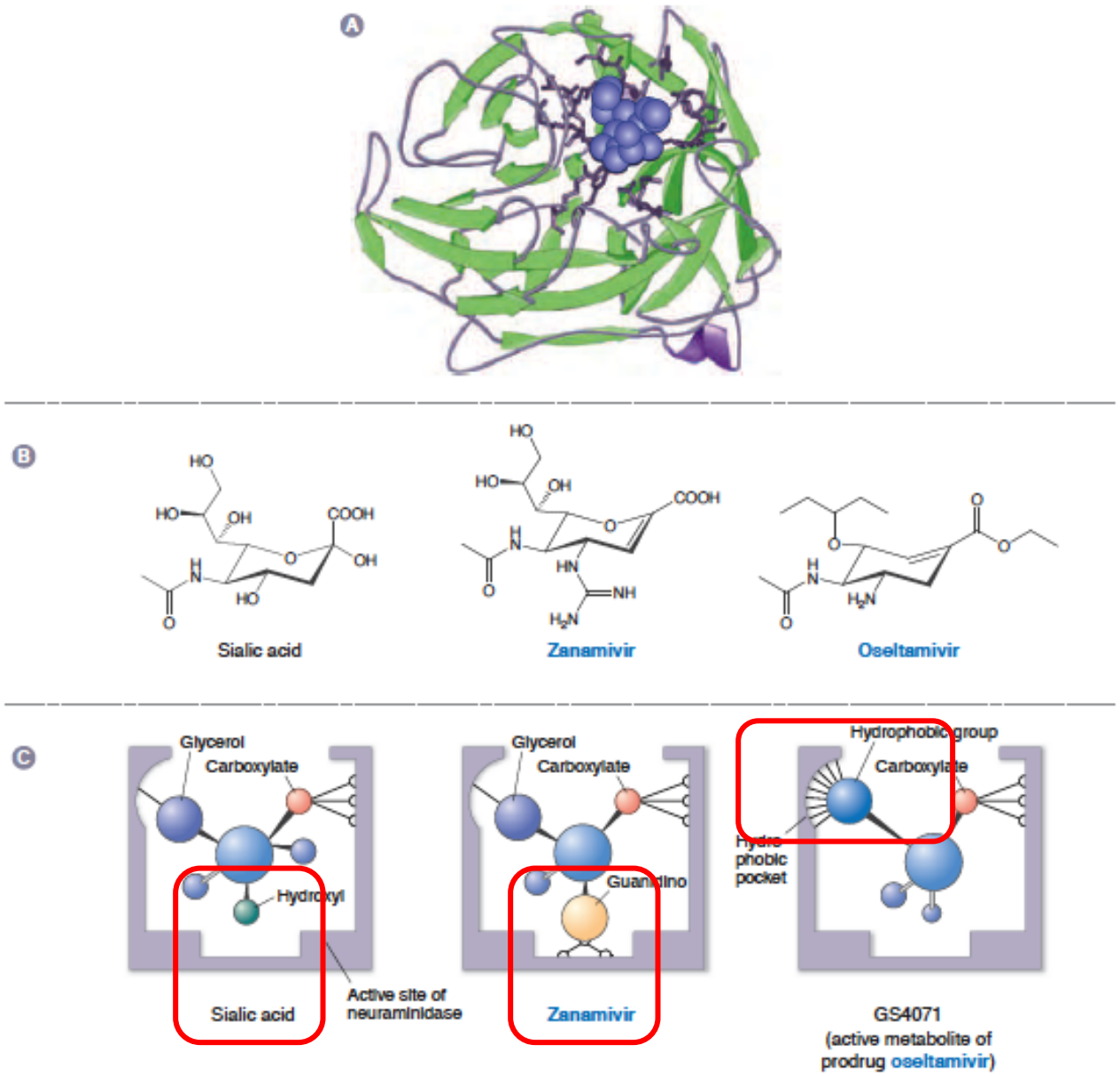


Sialic acid
(*N*-acetyl neuraminic acid)

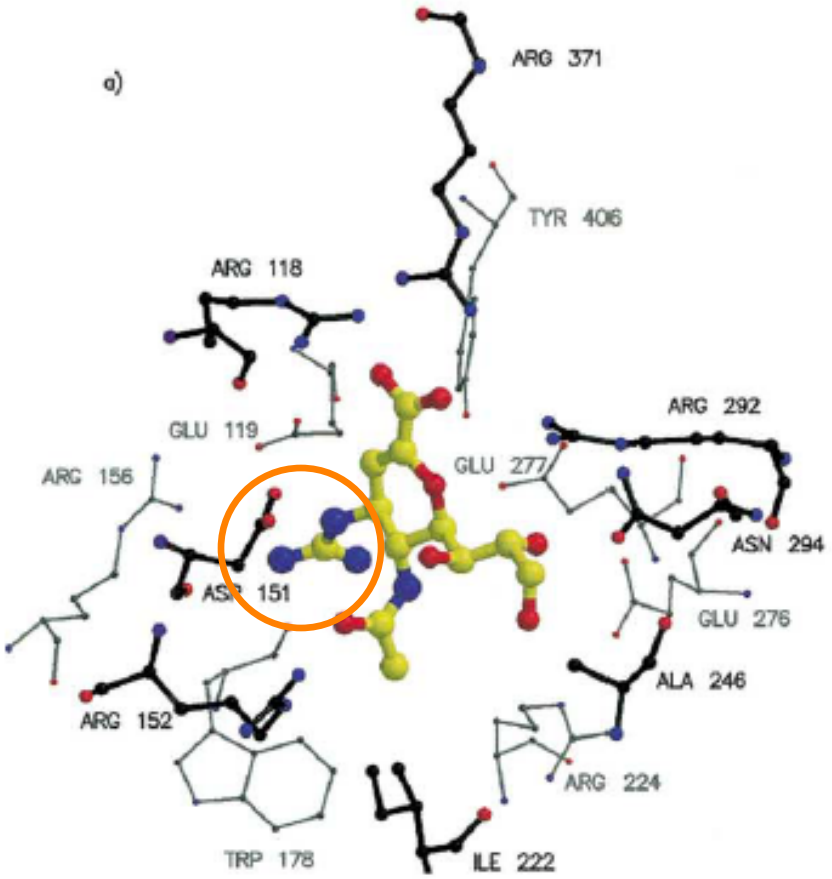


Zanamivir

Strucure-based design of inhibitors of IV A and B neuraminidase



Model of part of the active site of influenza neuraminidase bound to zanamivir

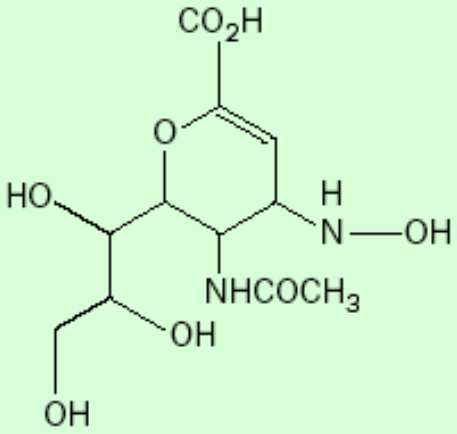


Crystallographic structure of the influenza A neuraminidase with an inhibitor (Zanamivir) bound to the active site

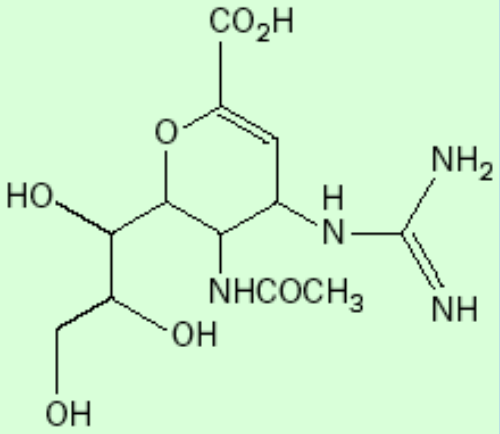
Rational drug design

Development of neuroaminidase inhibitors

Neu5Ac2en

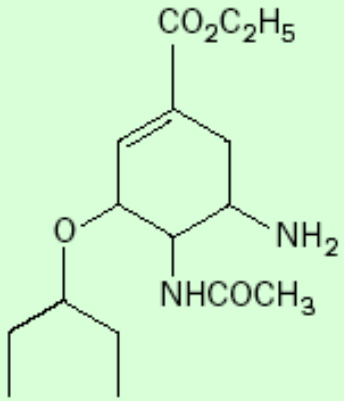


Zanamivir



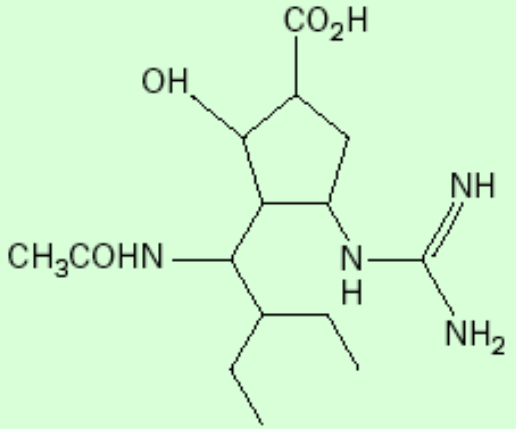
Relenza
(Biota/Glaxo)
FDA approved
Inhalation

Oseltamivir



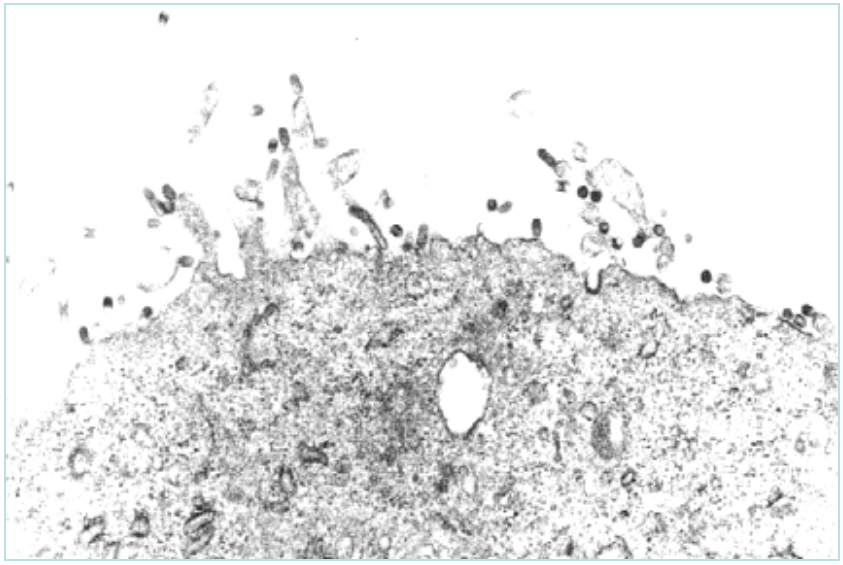
Tamiflu
(Gilead/Hoffmann
La Roche)
FDA approved
Oral

RWJ-270201

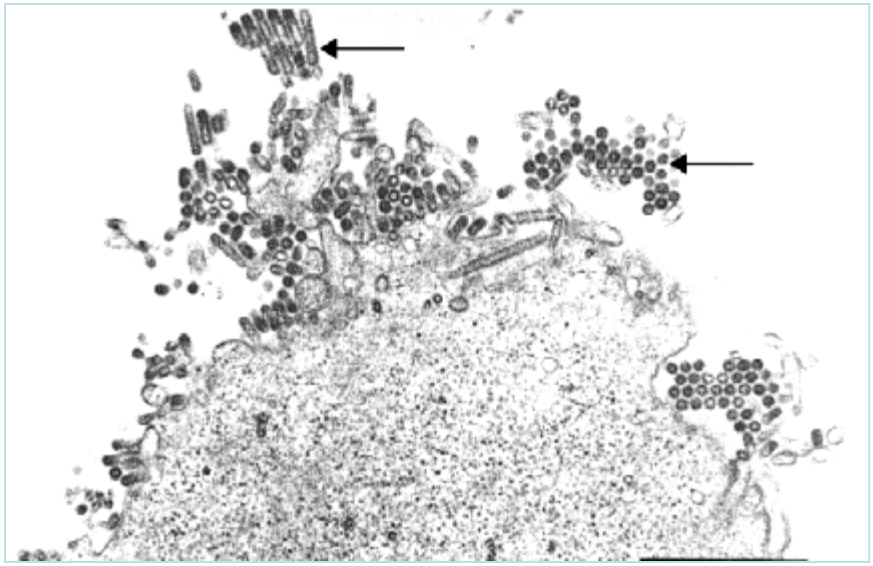


(BioCryst/
Johnson & J)
Clinical trials
Oral

Development of neuroaminidase inhibitors: *in vitro* activity



Untreated cells:
the virus is normally assembled and released from infected cells



Cells treated with a NA inhibitor:
the virus forms large non-infectious aggregates on the surface of infected cells

Zanamivir (*Relenza*) and Oseltamivir (*Tamiflu*)

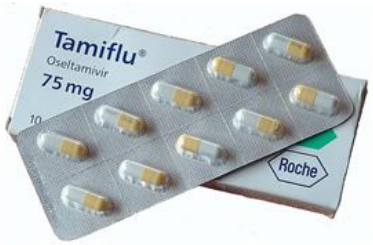
✓Neuraminic (sialic) acid analogs

✓They specifically inhibit NA of influenza and B viruses

✓Useful in therapy and prophylaxis of influenza infections. If given within 48, they will reduce disease's symptoms and duration.

Zanamivir: inhalation by Diskhaler

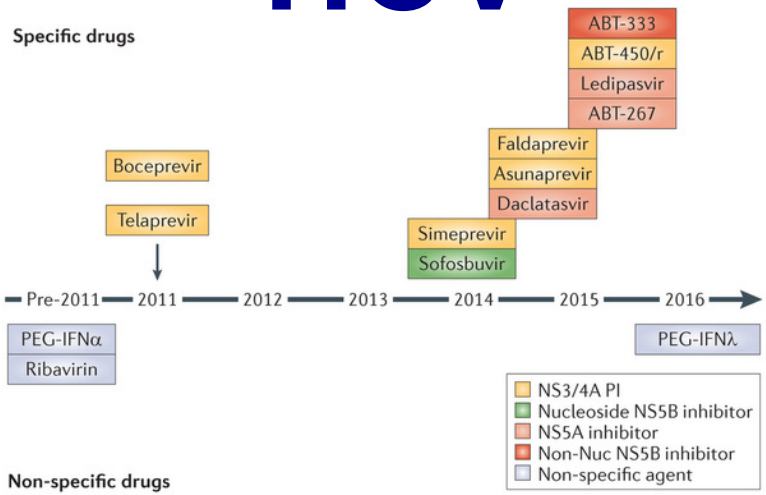
Oseltamivir: oral

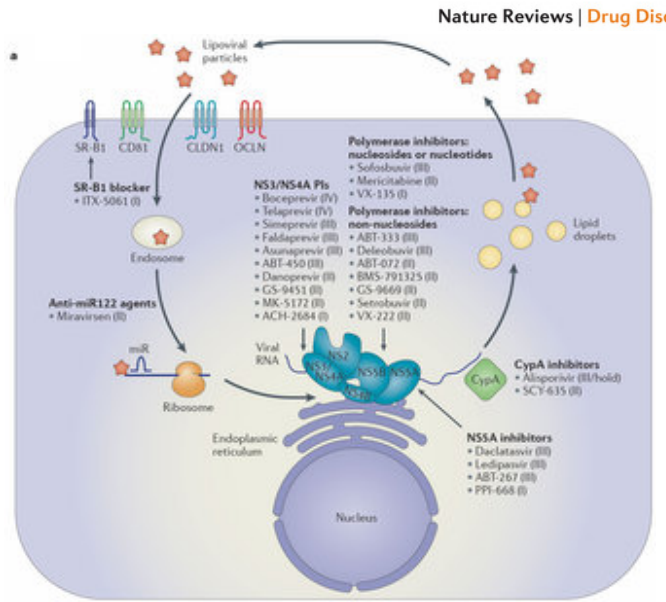


✓Resistance stems from NA mutations

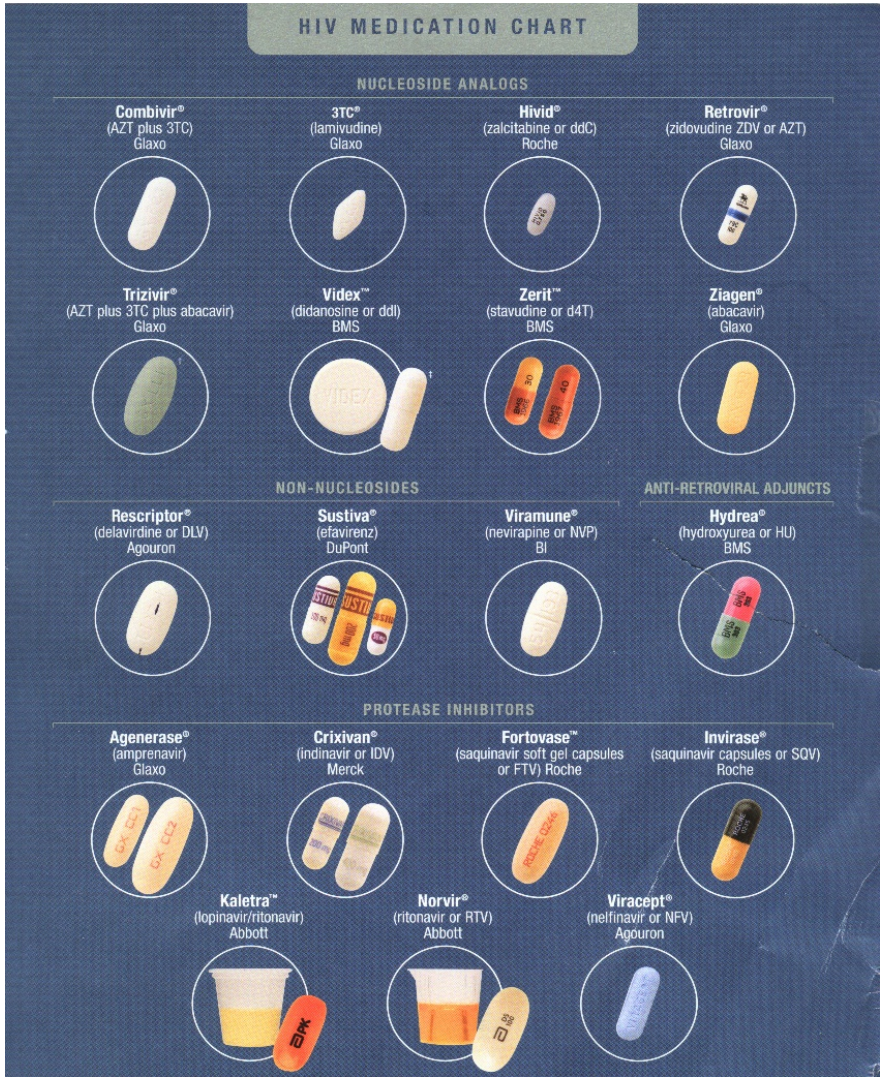
Two Success Stories

HCV





HIV



Hepatitis C Virus

Flaviviruses (Hepatitis C)

Virus	Vector	Disease
Hepacivirus • Hepatitis C virus	None	Hepatitis

Epidemiology

Transmission
- Blood
- Sex

Distribution
- Worldwide
- No seasonal incidence

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

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

Pathogenesis

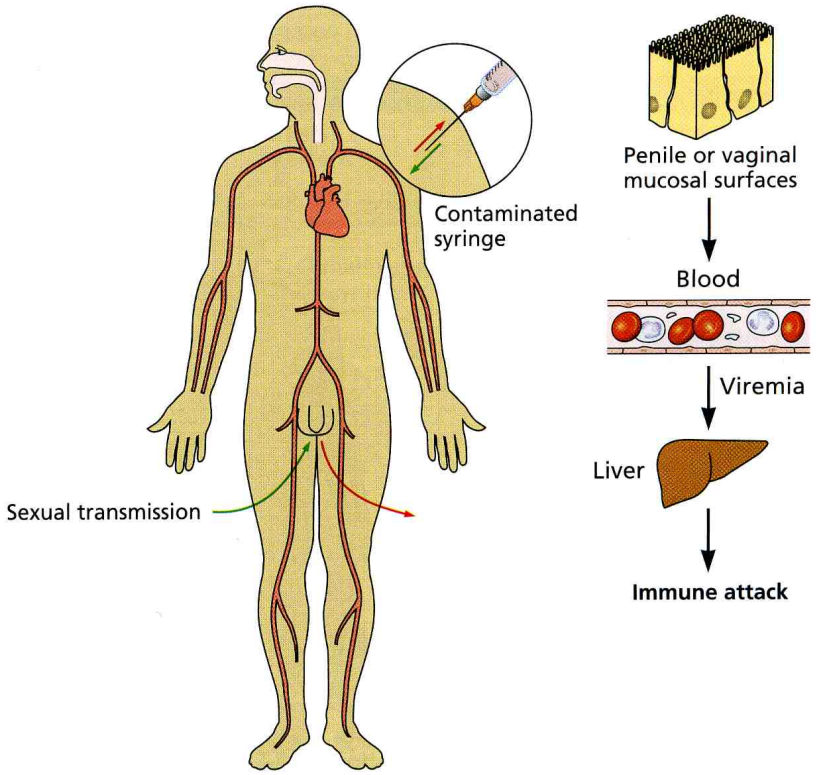
Viruses are noncytolytic and chronic

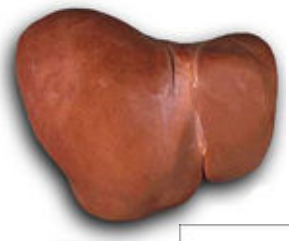
Disease caused by ongoing immune response

Liver cancer can result from chronic cirrhosis

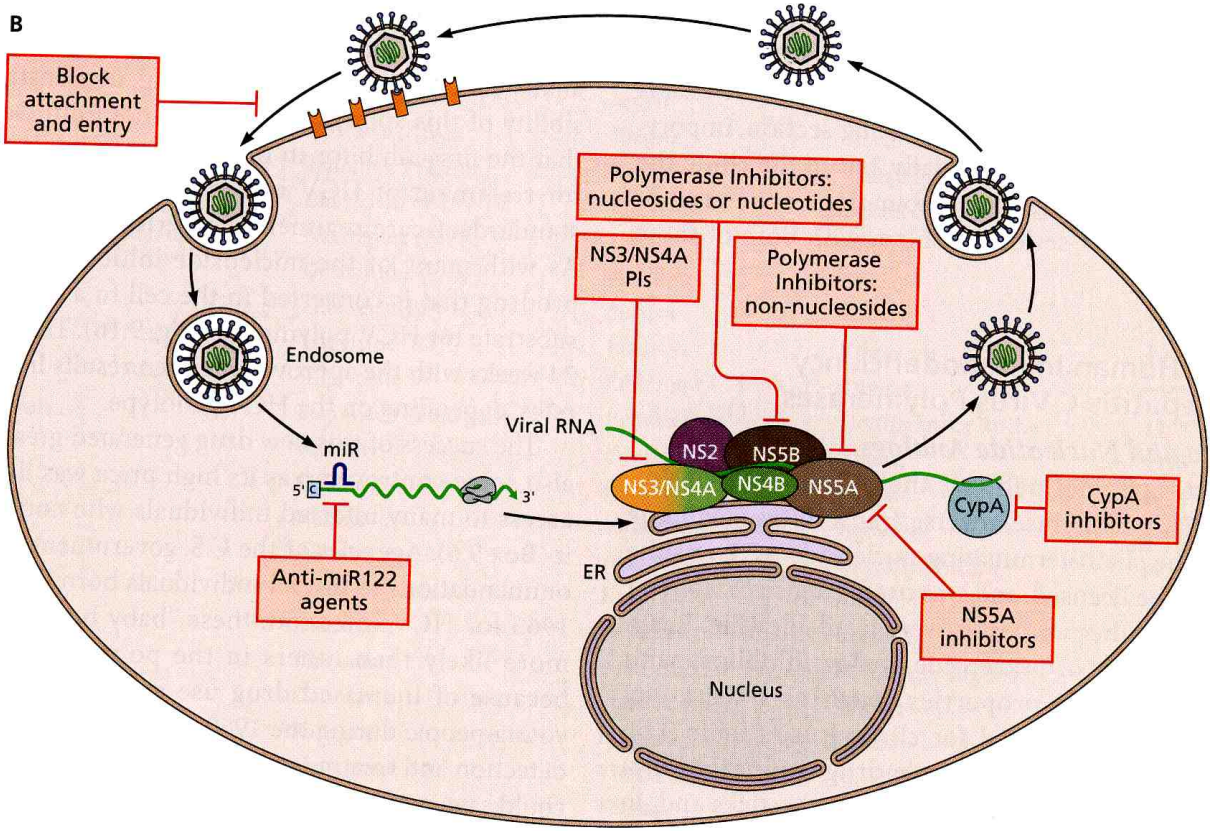
Human Infections

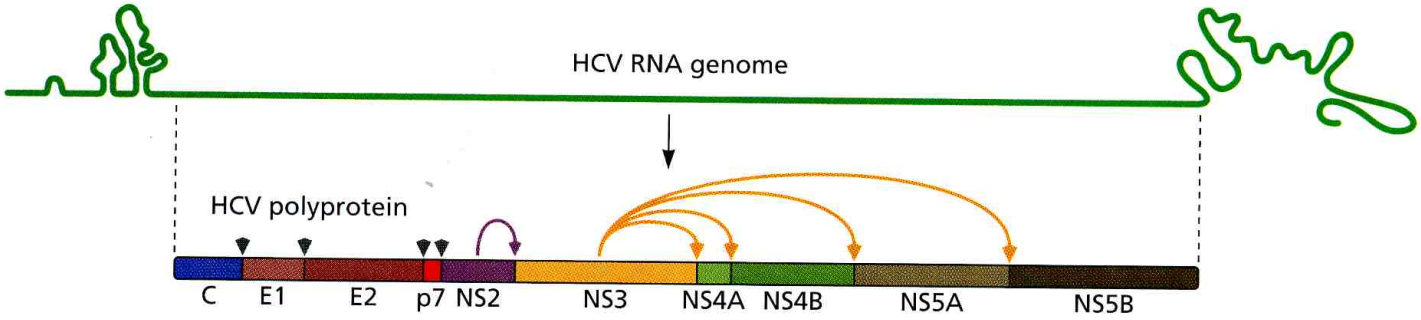
Persons living with hepatitis C
Worldwide: 130 million–150 million
In United States: 3.2 million

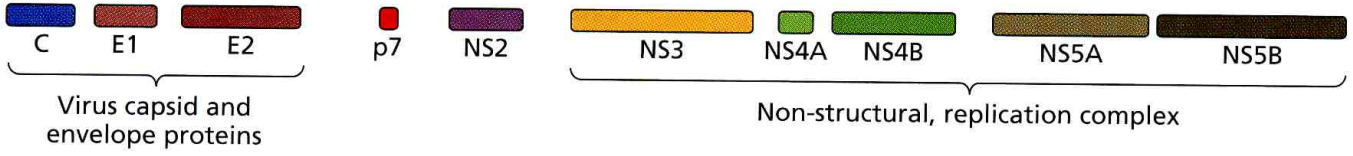




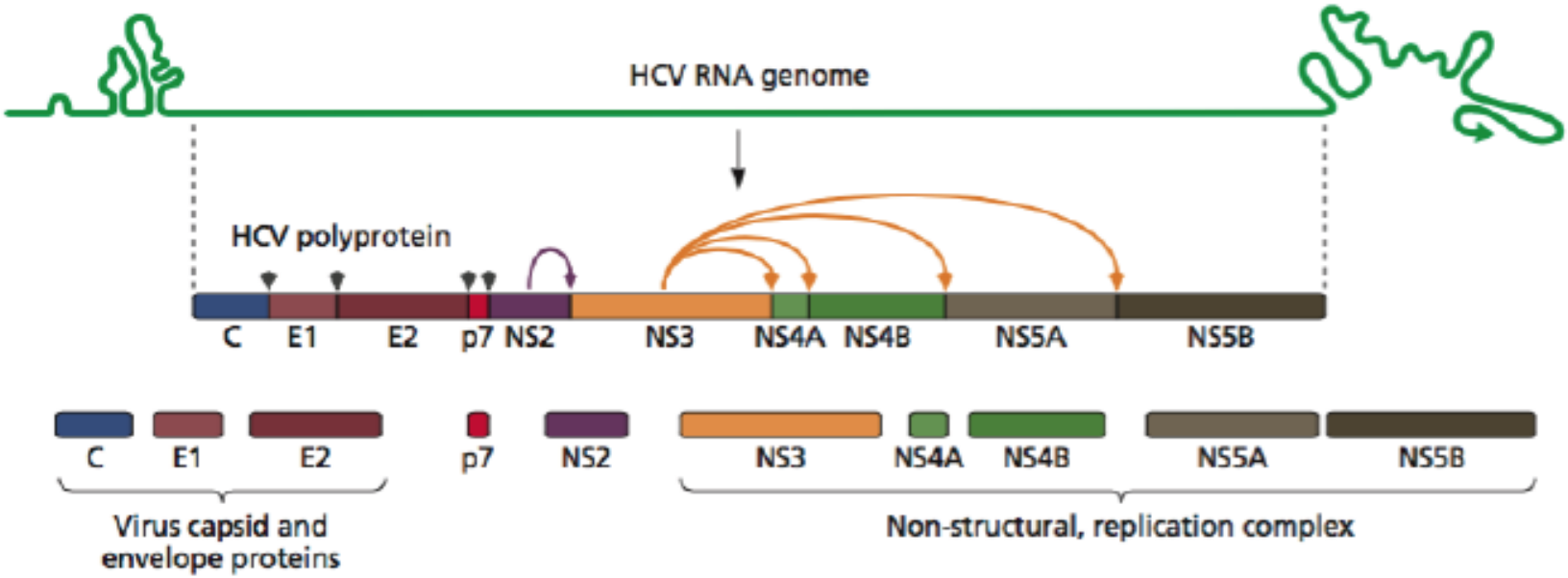
HCV replication cycle

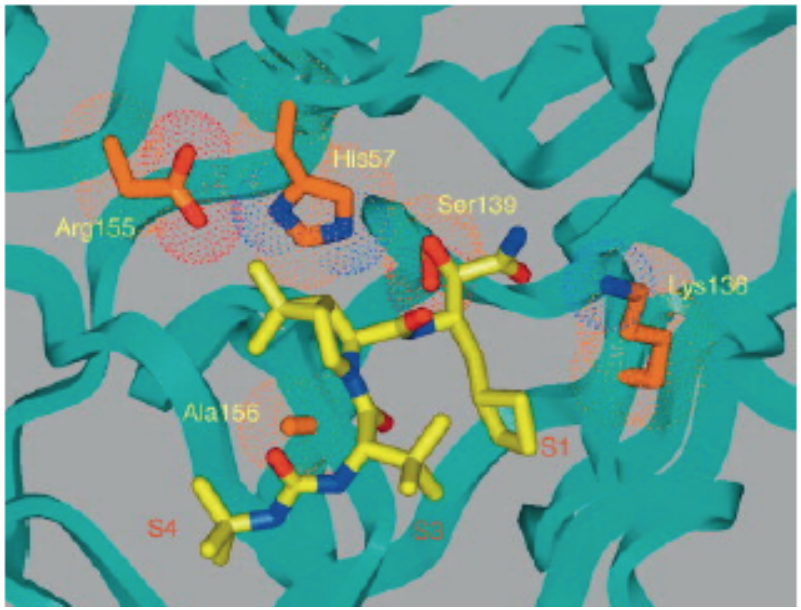


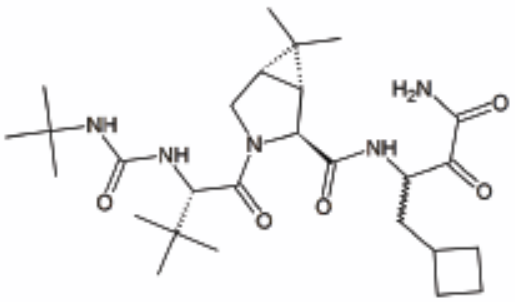




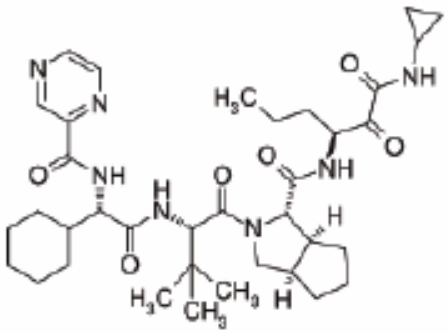
New HCV drugs







Boceprevir

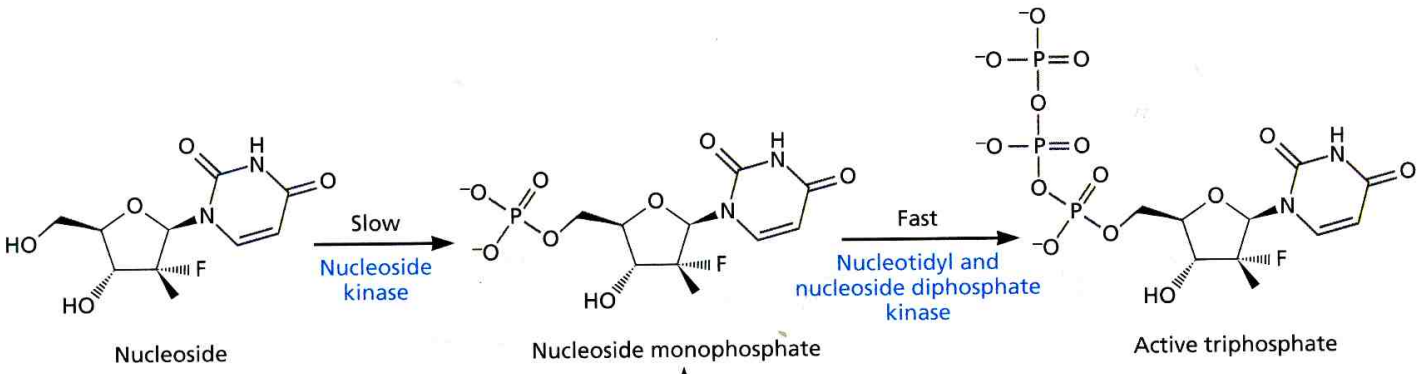


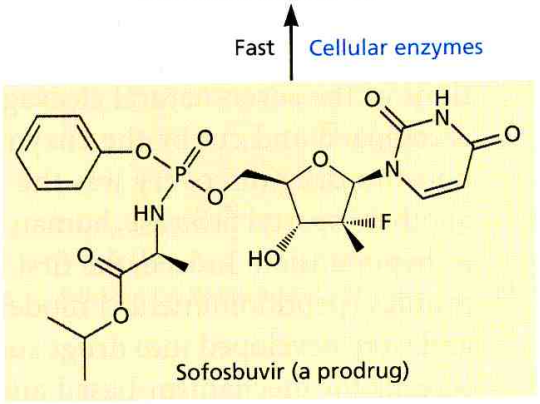
Telaprevir

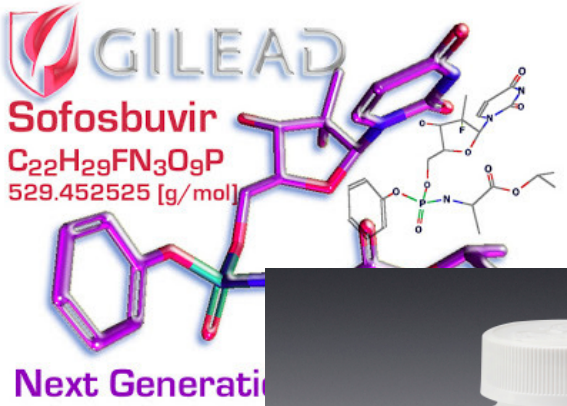
Examples of drugs targeted against HCV

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

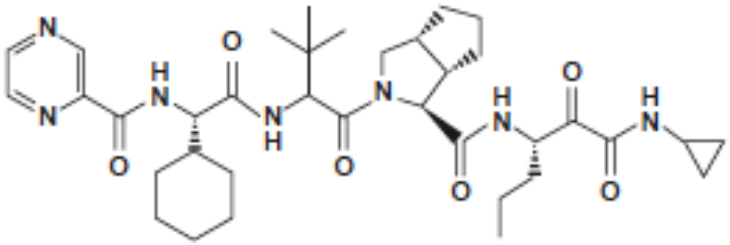
New HCV drugs: the prodrug sofosbuvir, its structure and activation



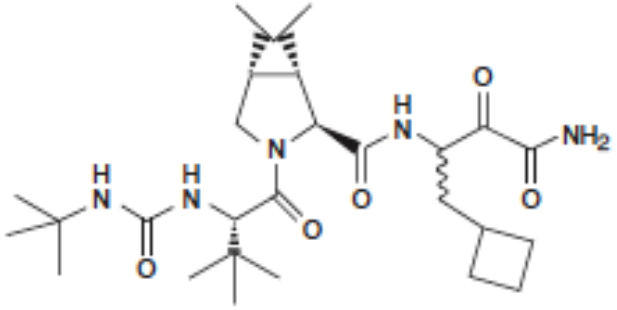




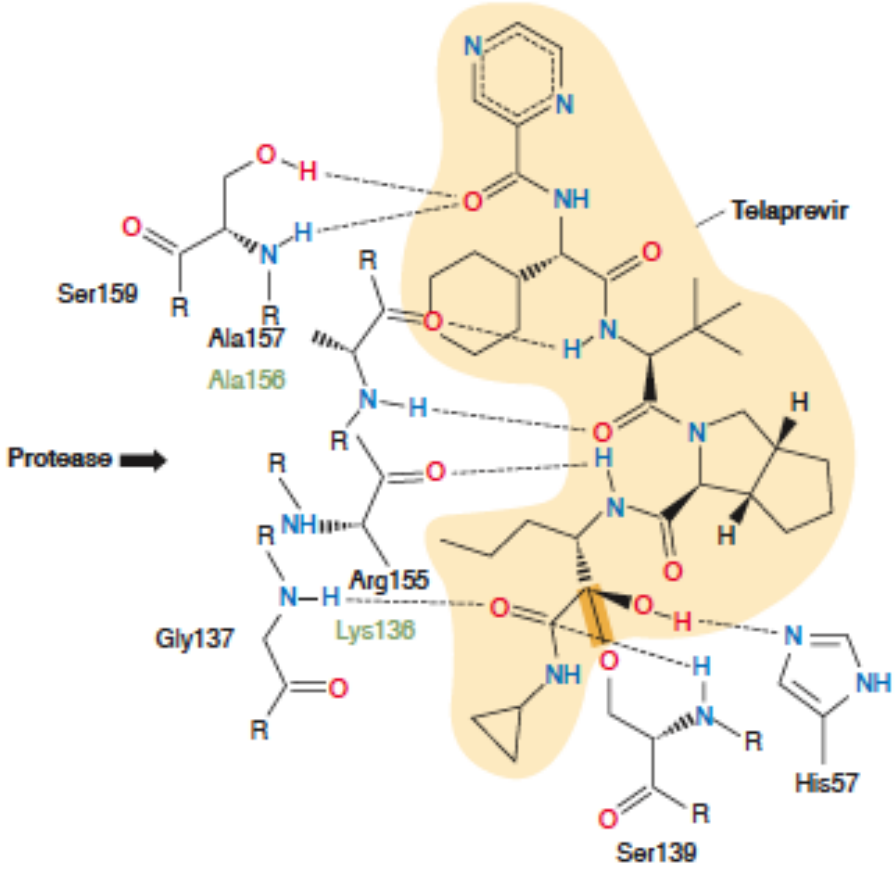
New HCV drugs: protease inhibitors



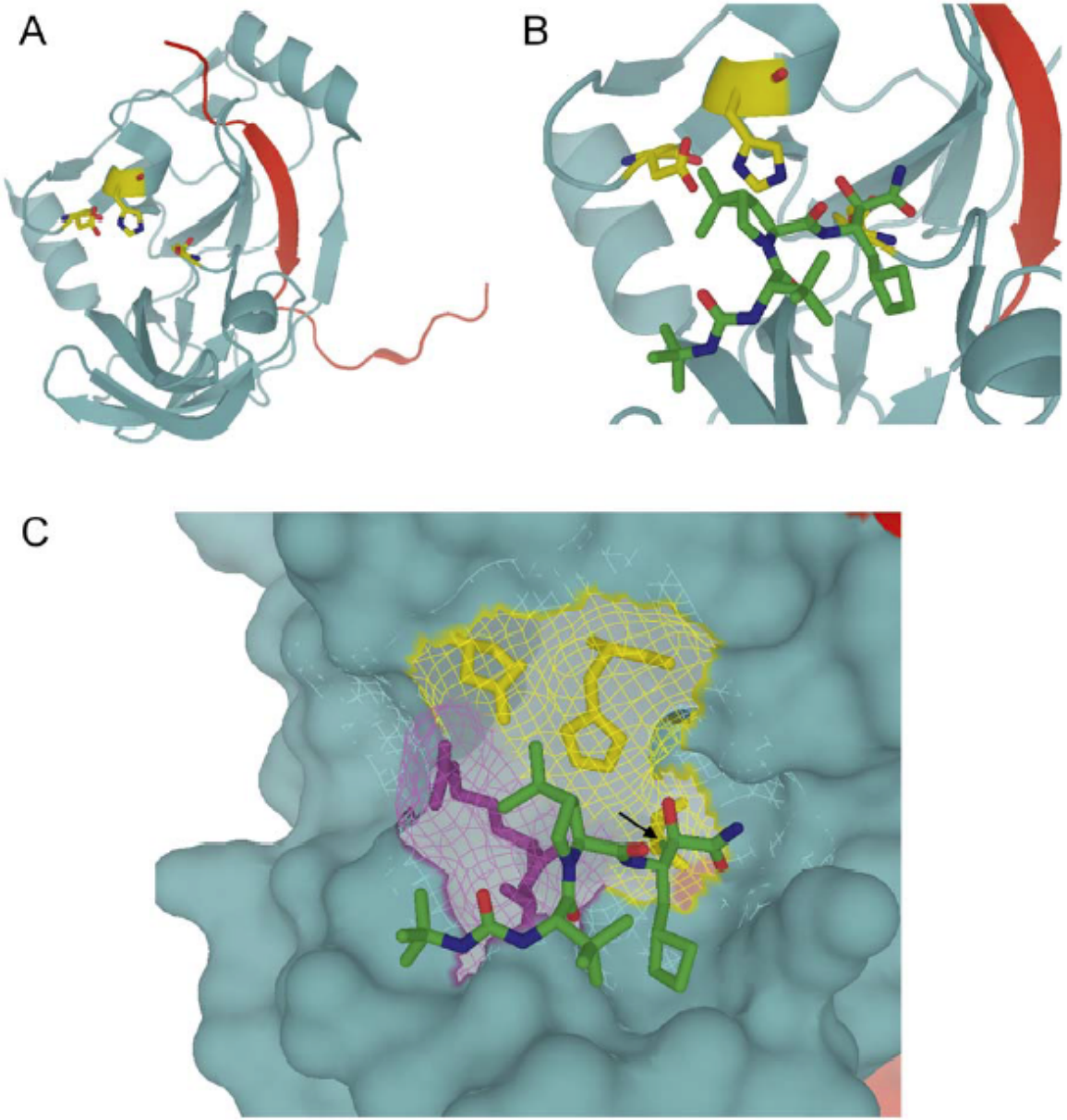
Telaprevir



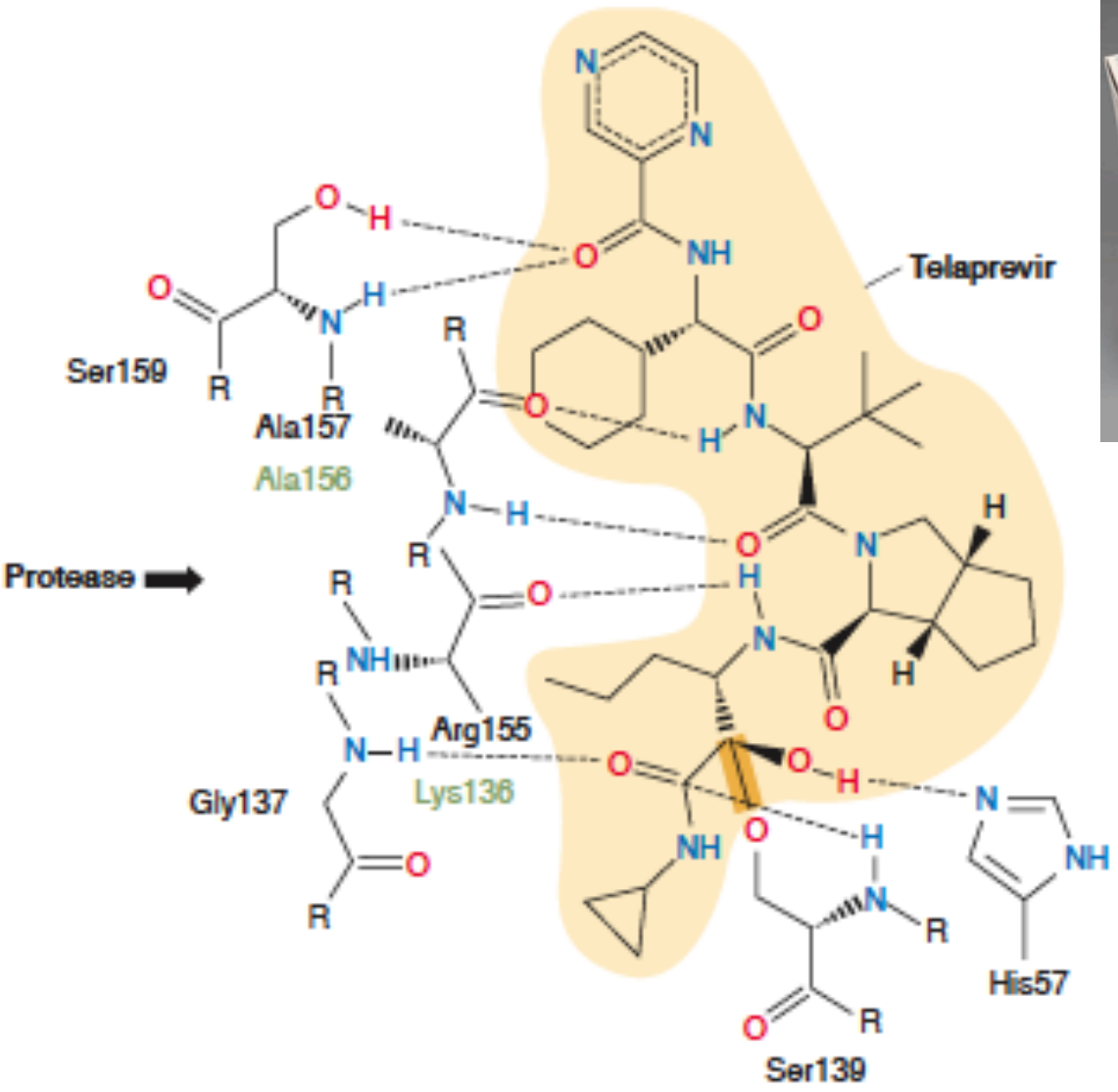
Boceprevir



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



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



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

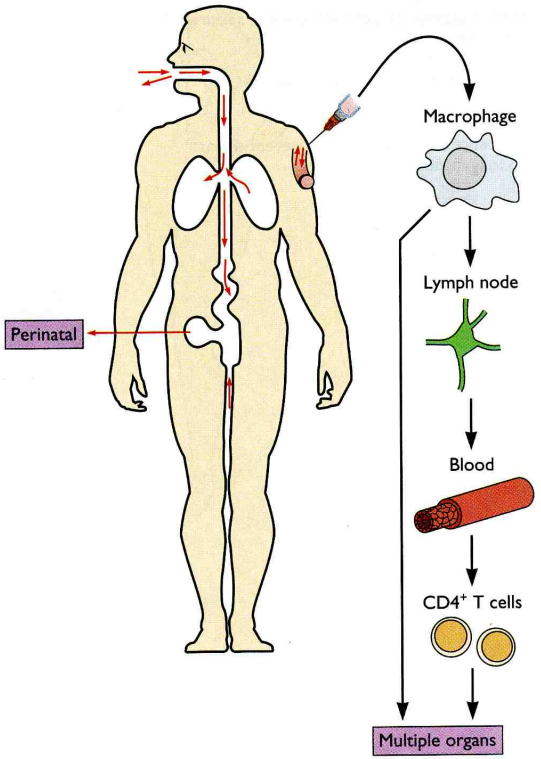
Infects mainly $CD4^+$ T cells and macrophages

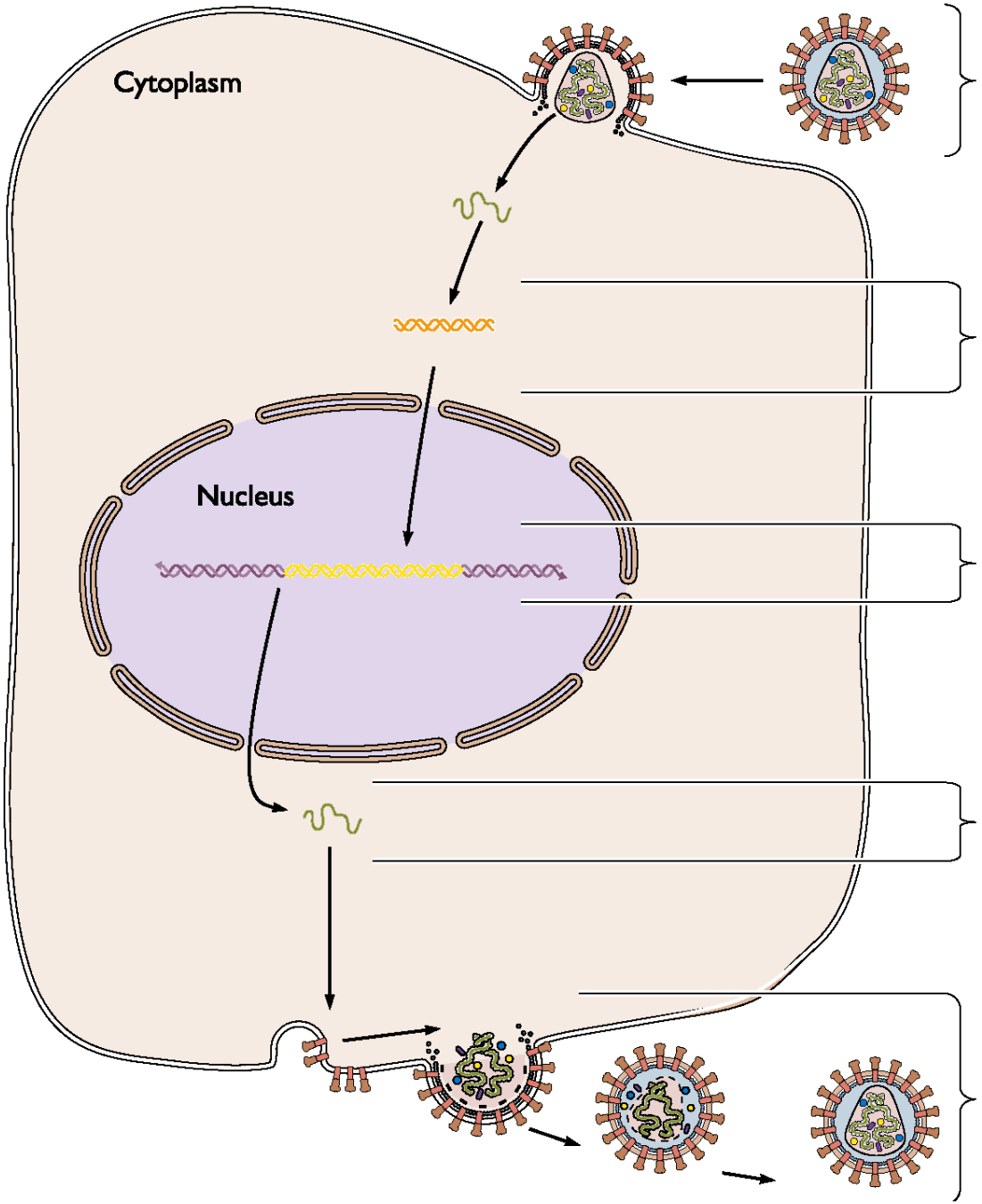
Lyses $CD4^+$ T cells, persistently infects macrophages

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

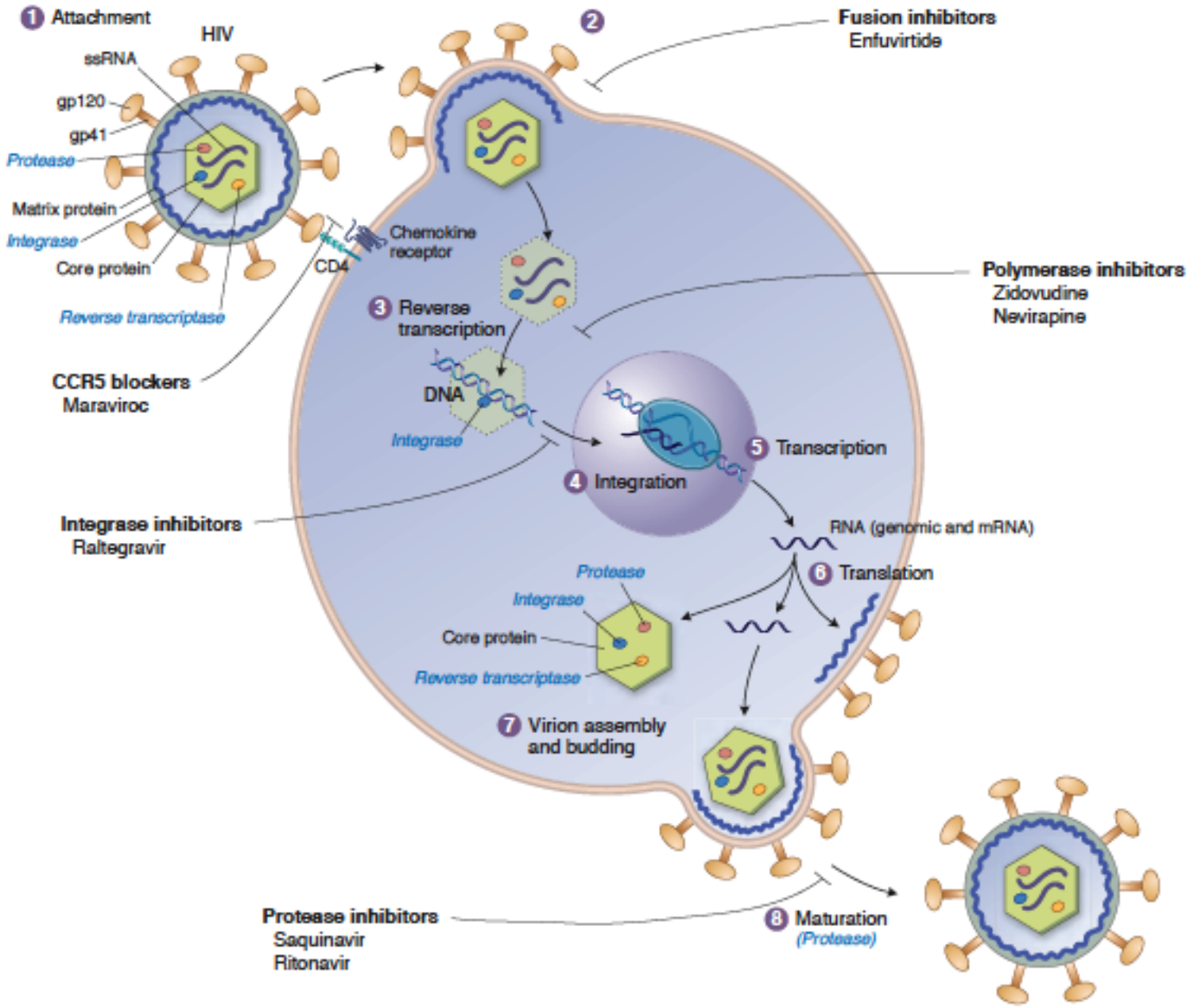
Reverse transcription
- Nucleoside analog reverse transcriptase inhibitors: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, acyclic nucleoside phosphonates
- Nonnucleoside reverse transcriptase inhibitors: nevirapine, delavirdine, loviride, efavirenz

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.

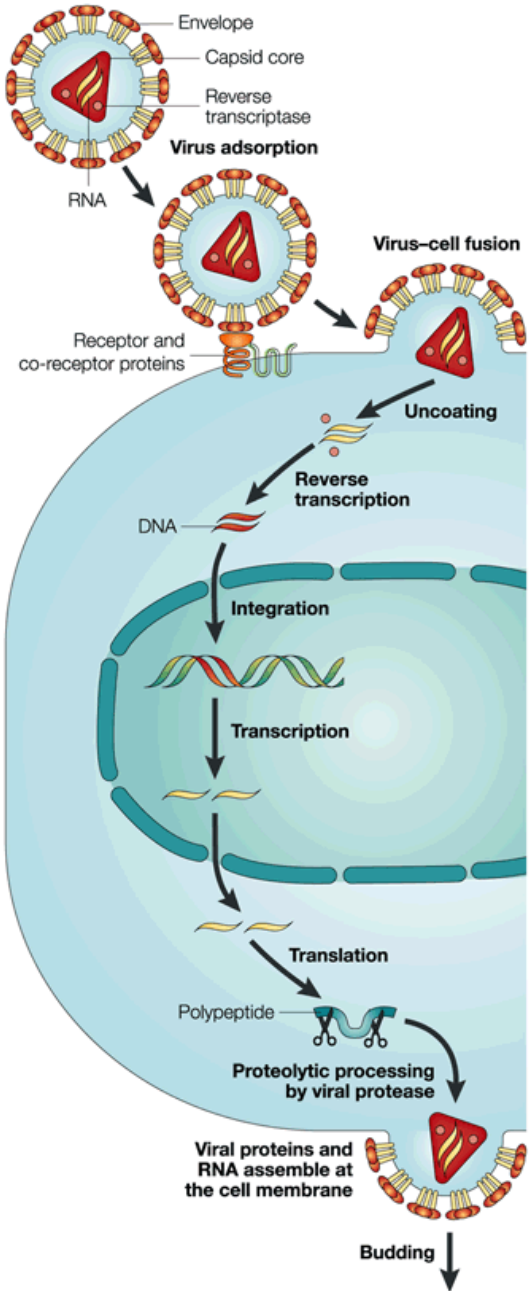
Target or mechanism	Generic name	Brand name	Manufacturer	Yr approved
Nucleoside reverse transcriptase inhibitors	Zidovudine (AZT, ZDV)	Retrovir	GlaxoSmithKline	1987
	Didanosine (ddI)	Videx	Bristol-Myers Squibb	1991
	Zalcitabine (ddC)	Hivid	Roche	1992
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	1994
	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

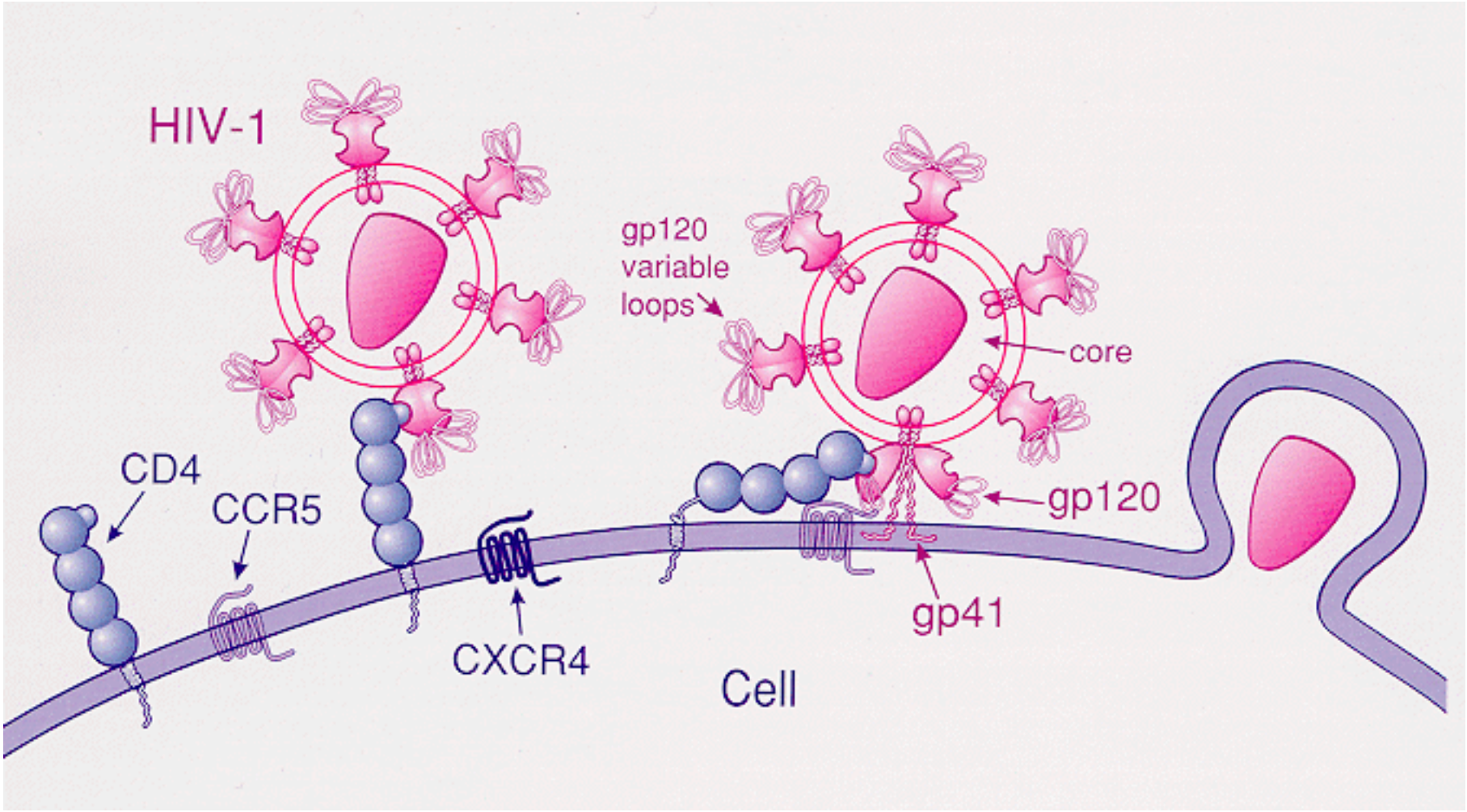
Target	Generic name	Brandname	Manufacturer	Year
Reverse transcriptase	Zidovudine (AZT)	Retrovir	GlaxoSmithKline	1987
Nucleos(t)ide inhibitors	Didanosine (ddI)	Videx	Bristol-Myers Squibb	1991
	Zalcitabine (ddC)	Hivid	Hoffmann-La Roche	1992
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	1994
	Lamivudine (3TC)	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

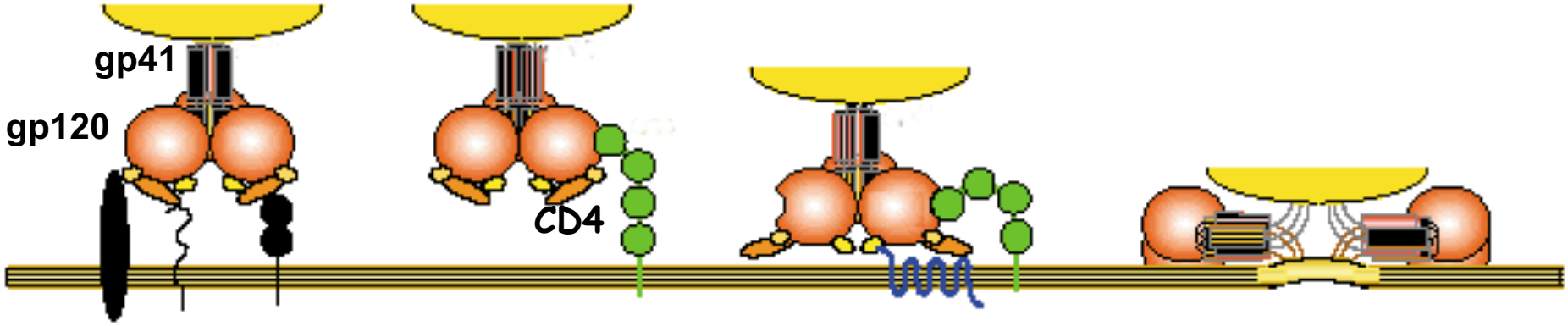


HIV adsorption and virus-cell fusion

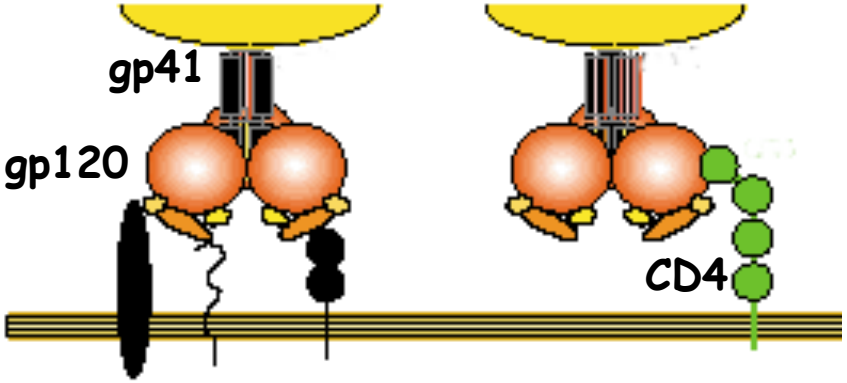


HIV adsorption and virus-cell fusion

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



HIV adsorption: binding inhibitors



CD4

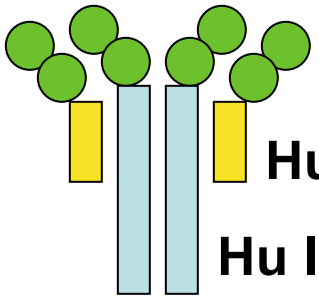
It is the high affinity cell receptor on:

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

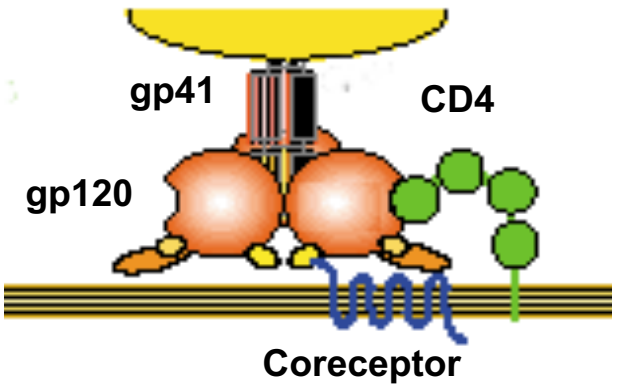


CD4 D1-D2

Hu k-light chain FC

Hu IgG2 heavy chain FC

HIV binding to co-receptors

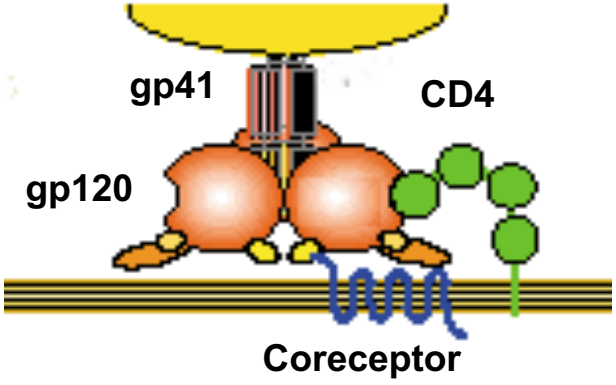


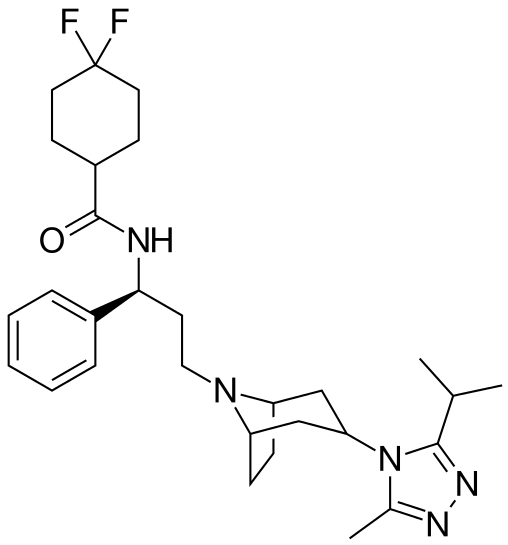
- ✓ **HIV coreceptors are chemokine cell receptors**
- ✓ **They belong to the G-coupled 7TMDs receptor superfamily**
- ✓ **The CD4-gp120 interaction promotes gp120 binding to coreceptors**

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**, or **Celsentri**) 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

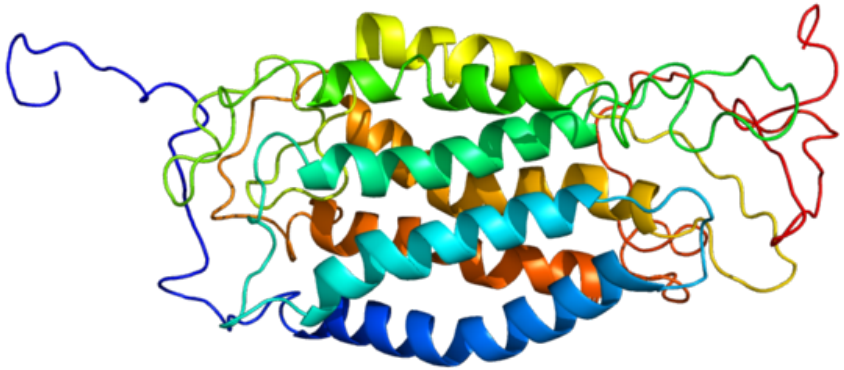
The co-receptor story

- ✓ **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

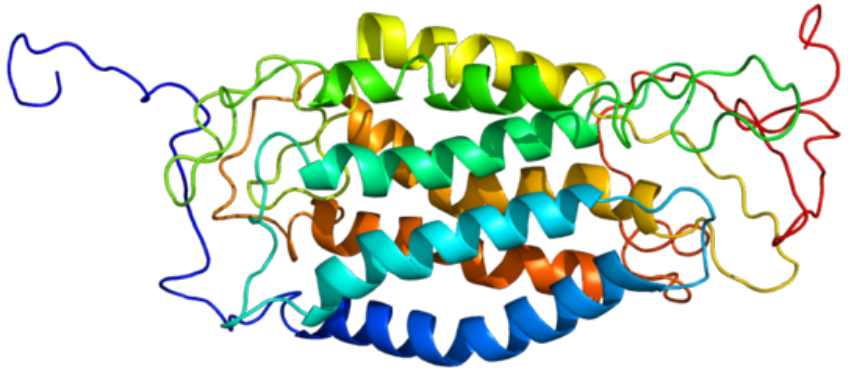
- ✓ To verify this hypothesis, Samson et al., sequenced CCR5 genes of three long term survivors and in one, they found a deletion defined to as **Δ32**

- ✓ This mutation has not a negative effect on the functions of T cells, 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.**

- ✓ In fact, the presence of this mutation decreases the number of CCR5 proteins present on the membrane of target cells, thus affecting the rates of progression of HIV infection.

- ✓ 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.

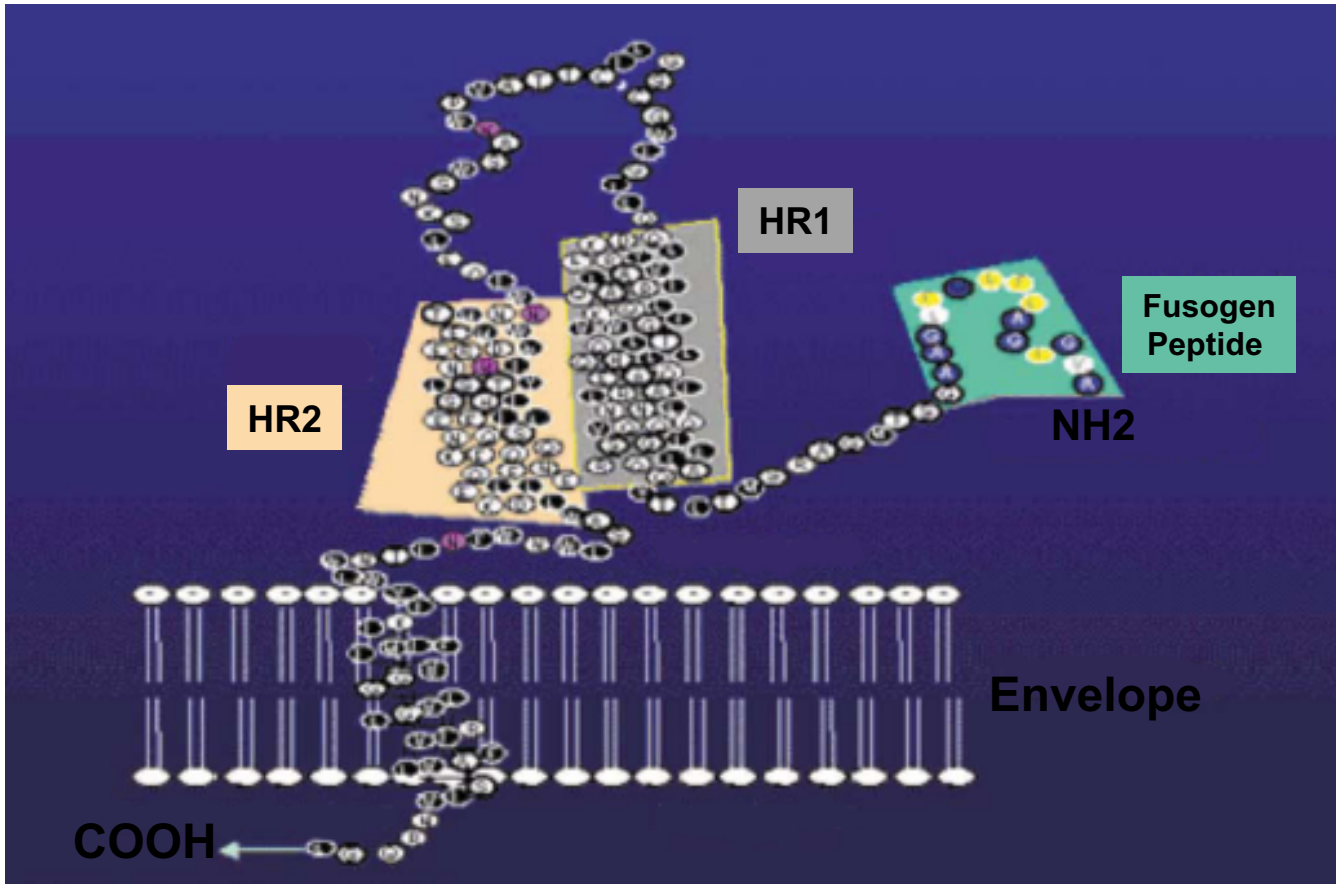
- ✓ Two hypotheses: genetic drift and natural selection

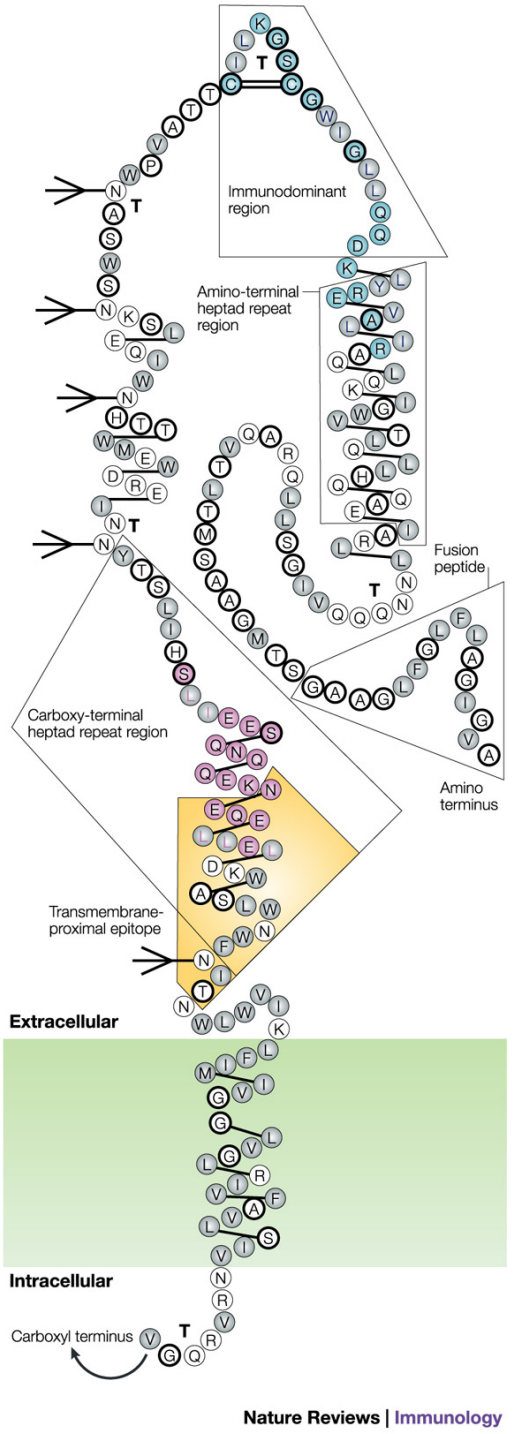
  During the plague of the 14th century in Europe, the CCR5 Δ32 allele would be selected because it conferred resistance to this infection. However, more recent studies have ruled out that possibility, revealing the absence of a protective role in this context . It is believed now that Δ32 mutation may 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 from a donor with the "Δ32" mutation on the CCR5.
- ✓ He received two stem cell 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





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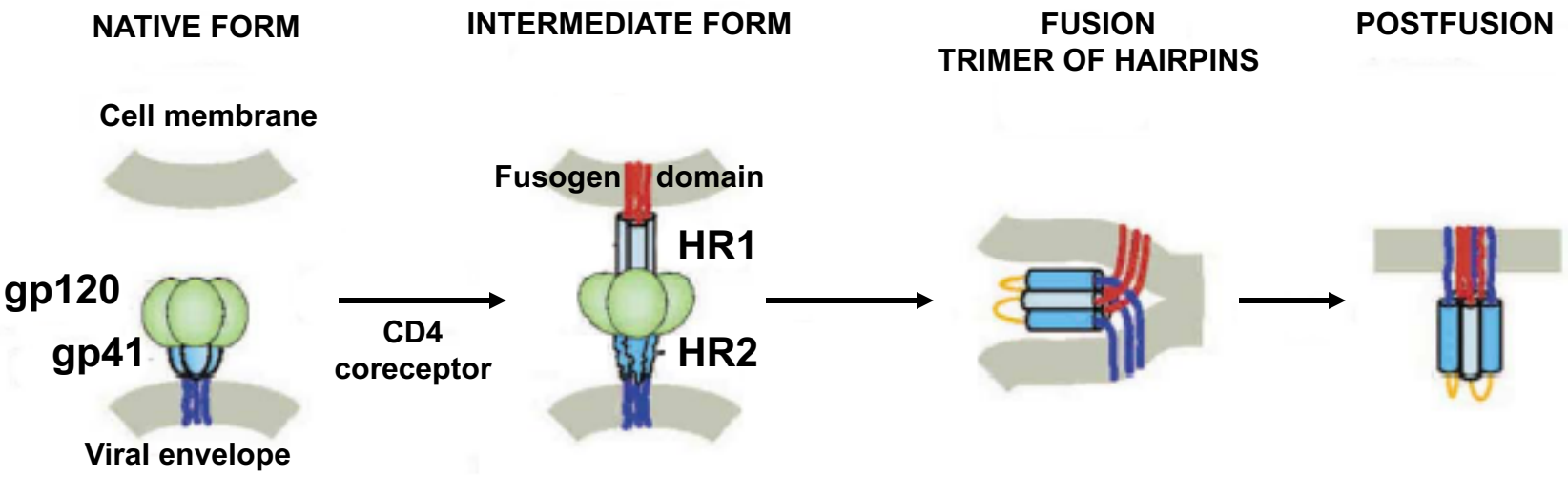
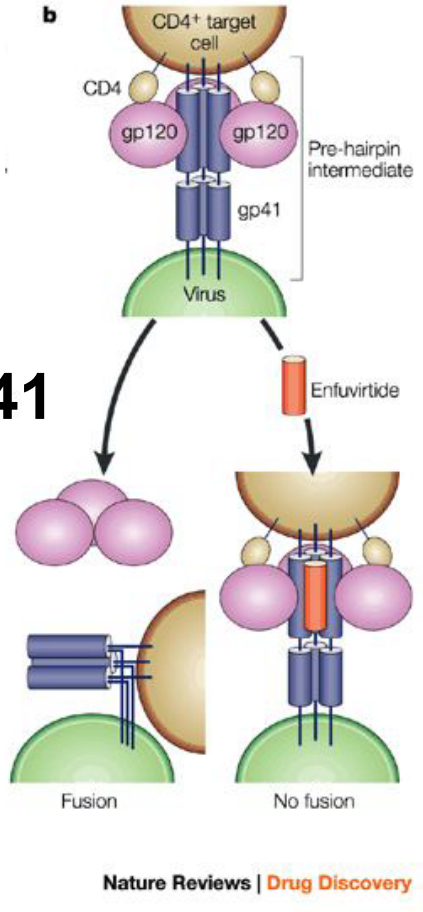
HIV entry: the gp41 role



Fusion inhibitors

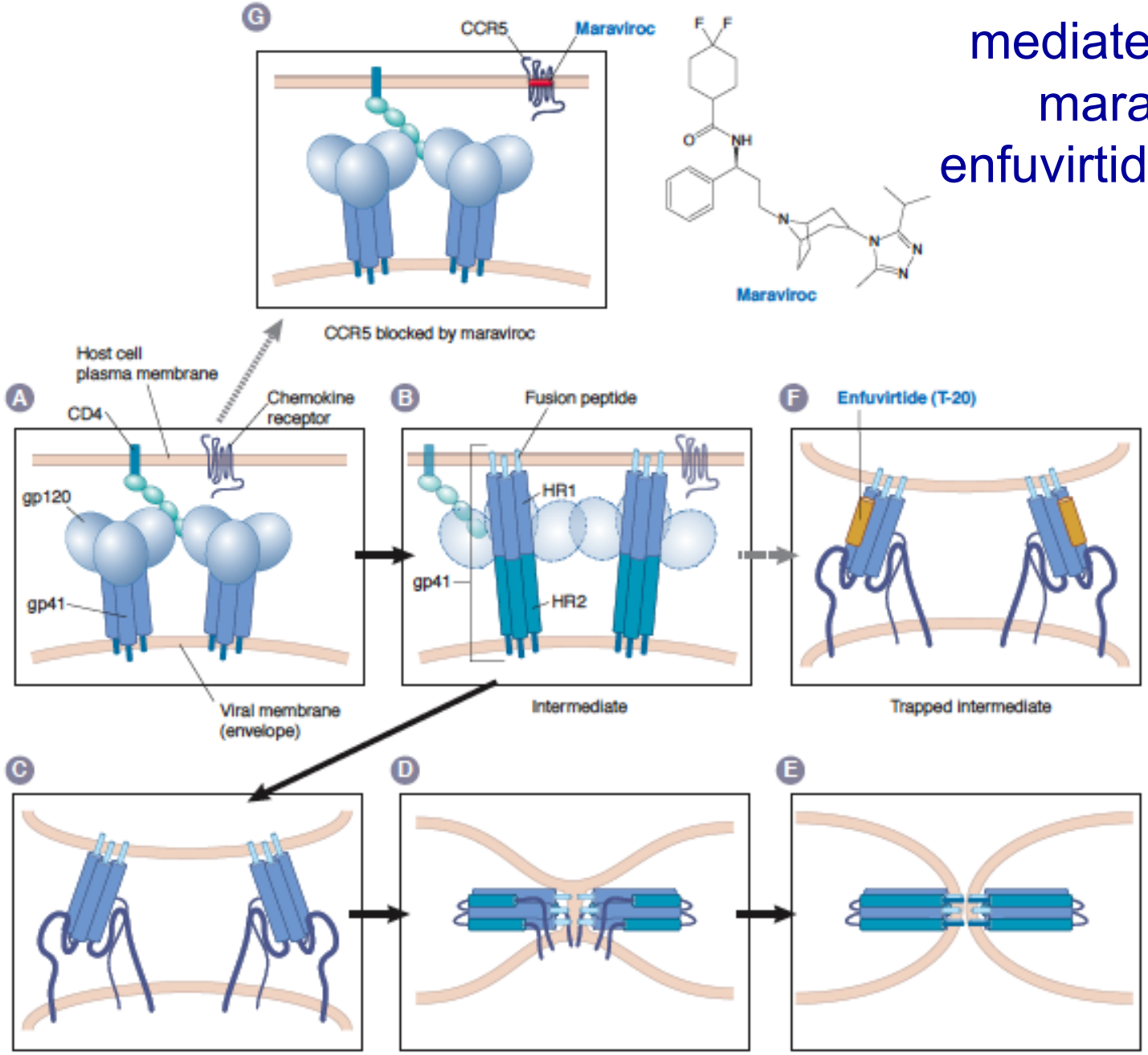
- ✓ They interact with the intermediate form of **gp41**



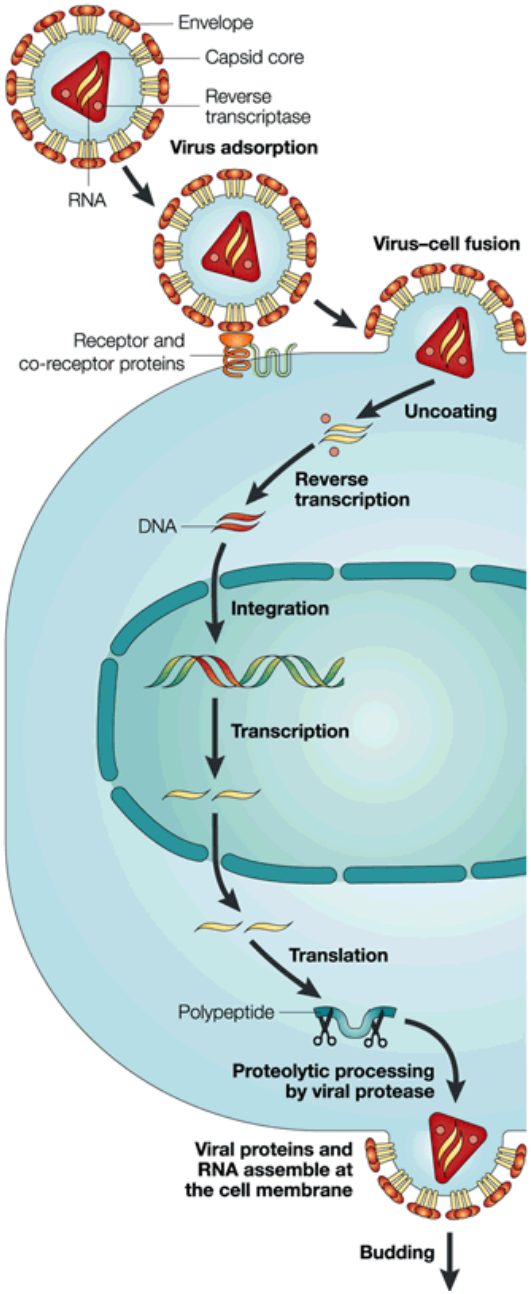
- ✓ **Enfuvirtide (T20)**

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



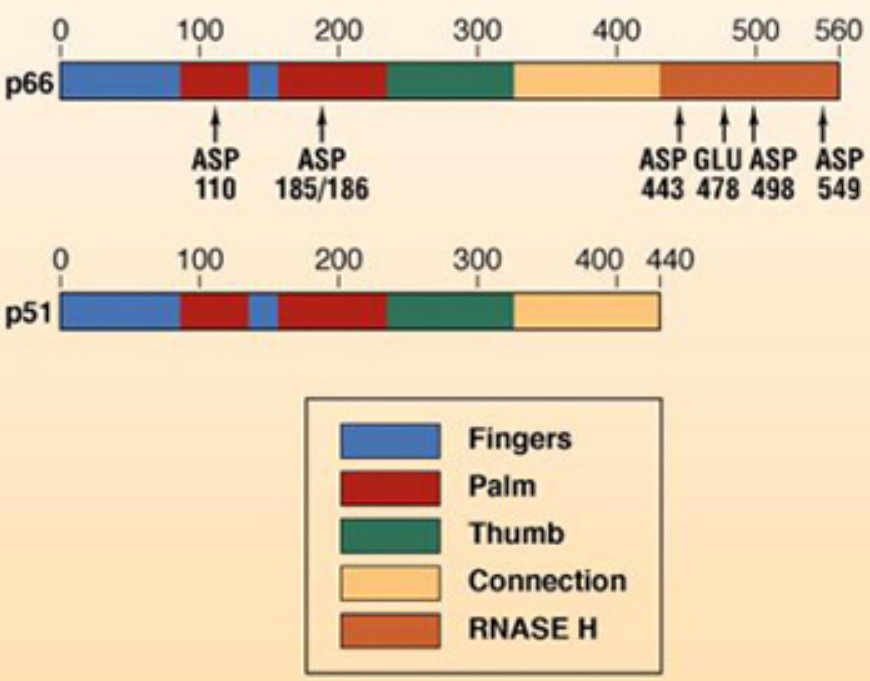
Druggable targets in HIV replication cycle

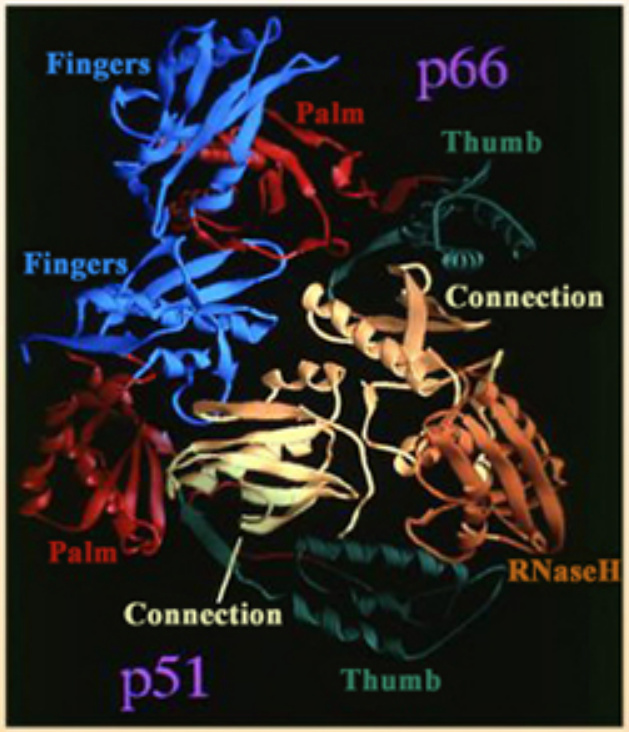


Reverse Transcription

Structure of HIV reverse transcriptase (RT)

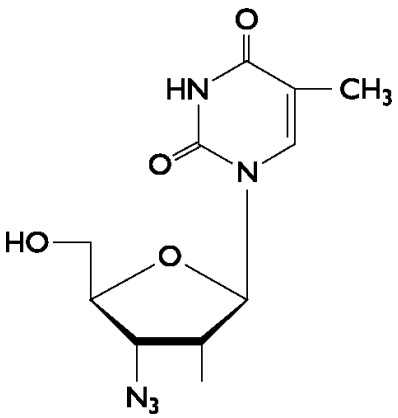
Heterodimer of **p51** and **p66** subunits



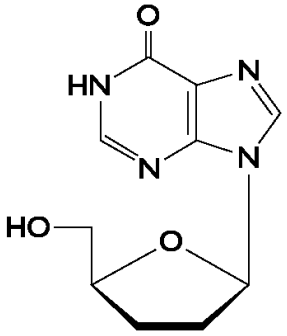


HIV RT inhibitors

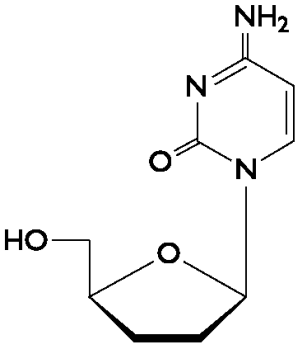
Nucleoside analogs (NRTI):



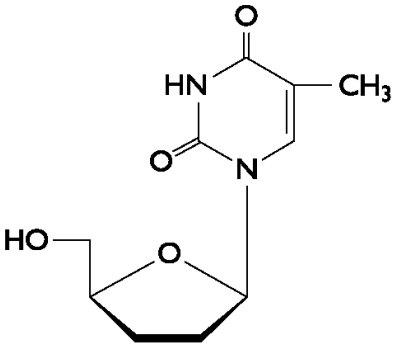
Zidovudine
(AZT)



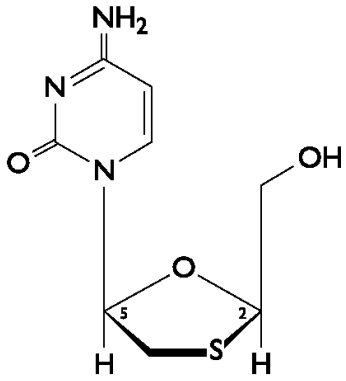
Didanosine
(ddI)



Zalcitabine
(ddC)



Stavudine
(d4T)



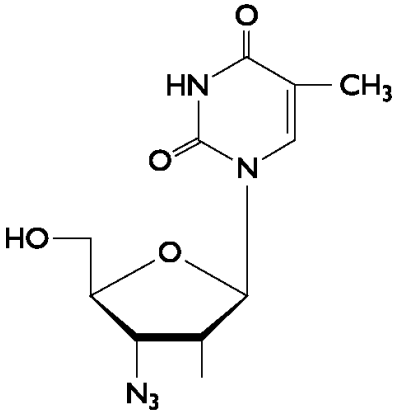
Lamivudine
(3TC)

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

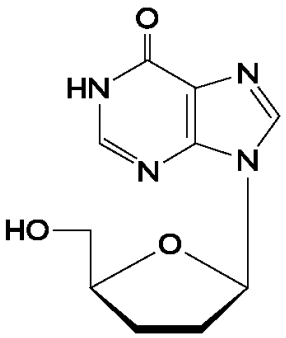
✓ Their selectivity of action stems from their affinity for RT that is 100-folds higher than that for human DNA polymerases

HIV RT inhibitors

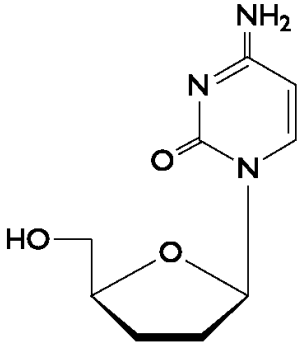
Nucleoside analogs (NRTI):



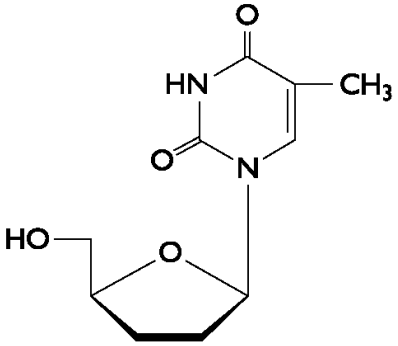
Zidovudine
(AZT)



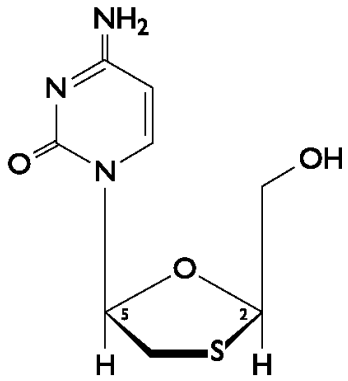
Didanosine
(ddI)



Zalcitabine
(ddC)



Stavudine
(d4T)



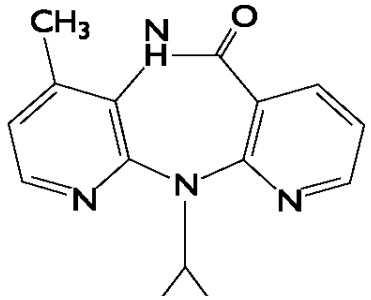
Lamivudine
(3TC)

- ✓ **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

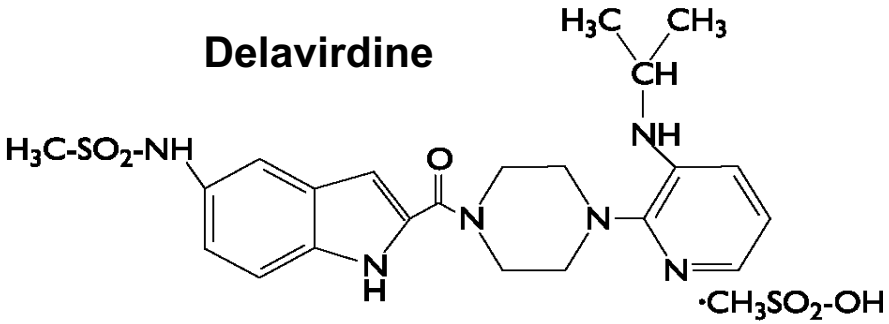
HIV RT inhibitors

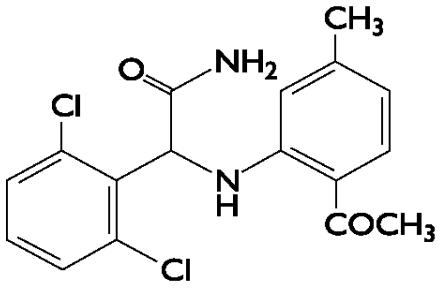
Non-nucleoside inhibitors (NNRTI):



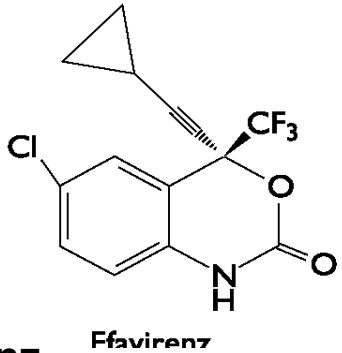
Nevirapine

Delavirdine





Loviride

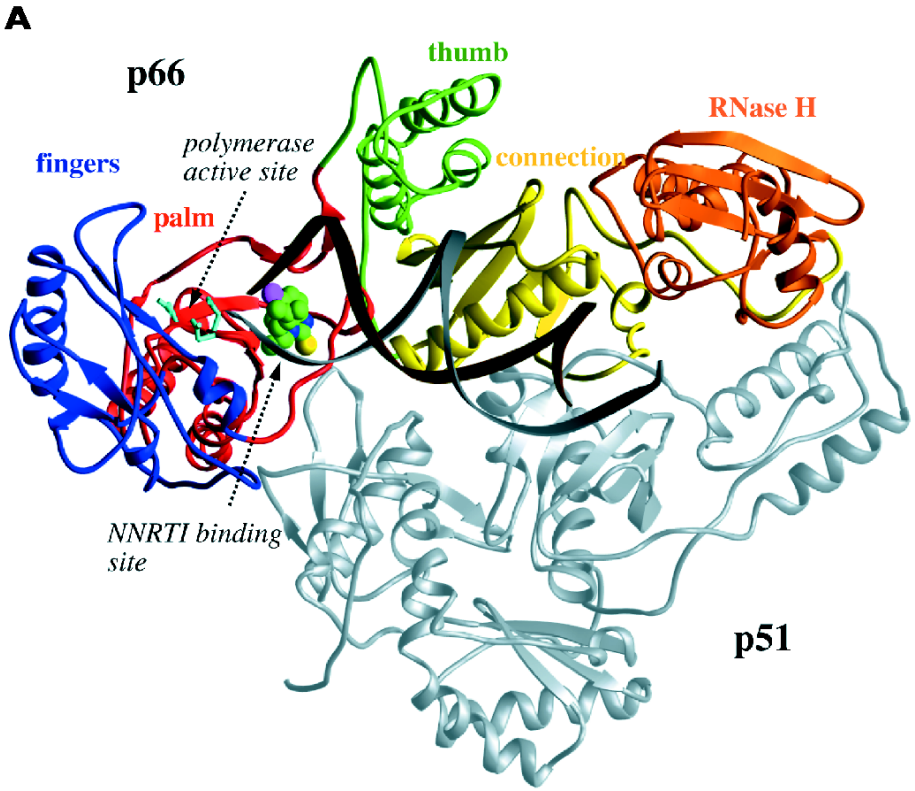


Efavirenz

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

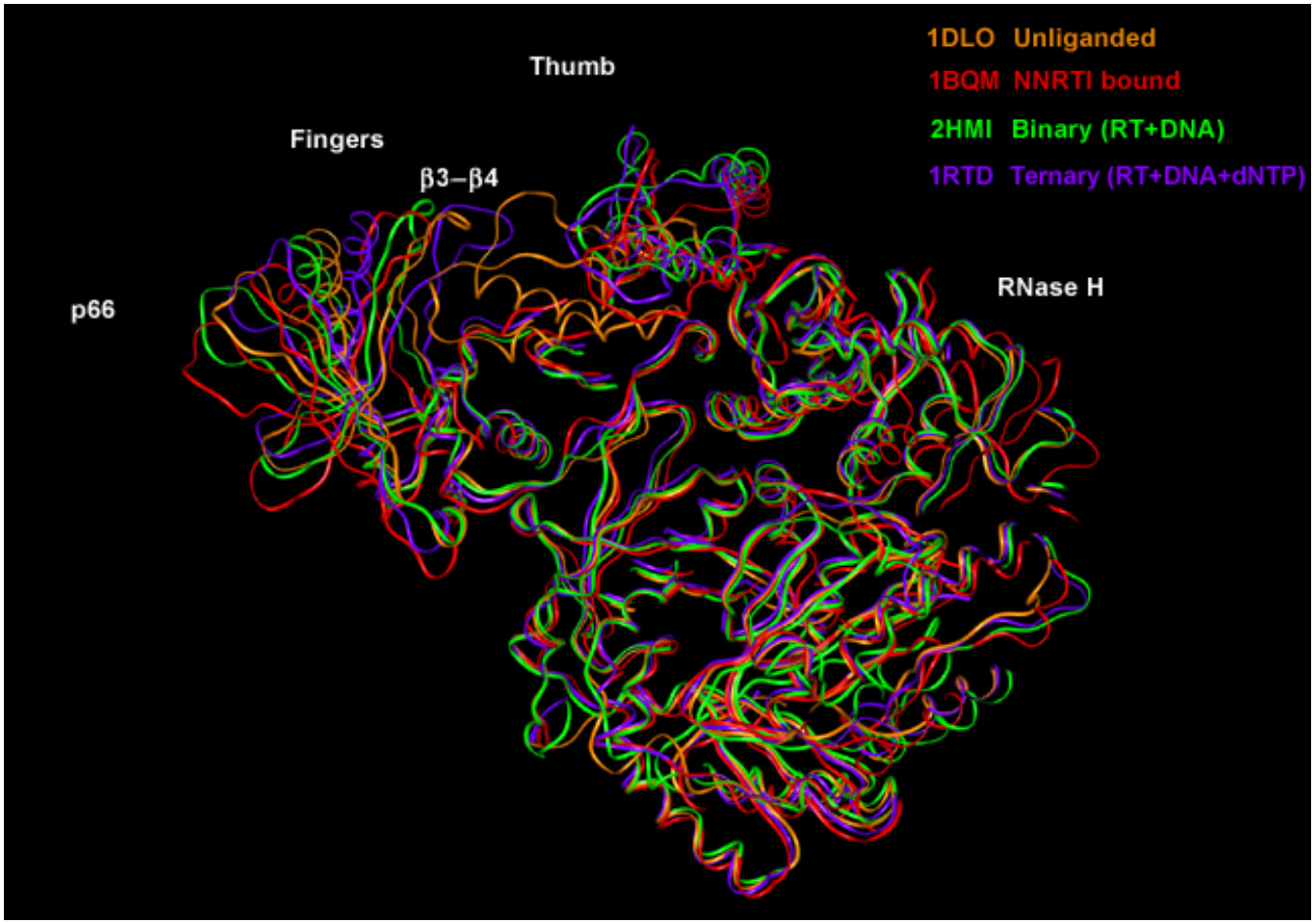
HIV RT inhibitors

Non-nucleoside inhibitors (NNRTI):



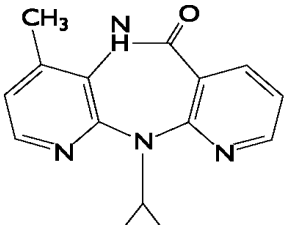
- ✓ They bind to a hydrophobic pocket close to the active site of RT (NNRTI pocket), thus blocking proviral DNA synthesis

Nonnucleoside inhibitors of HIV RT (NNRTI)

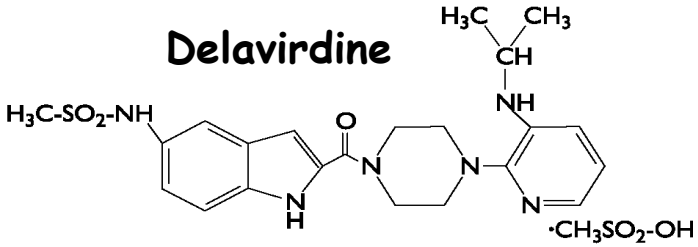


- ✓ The mechanism of action is unclear, however it is thought they alter the structure of the enzyme at the catalytic site, the binding site of template nucleic acid and of the binding site for dNTP

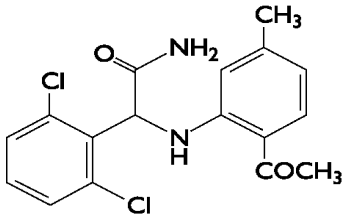
Nonnucleoside inhibitors of HIV RT (NNRTI)



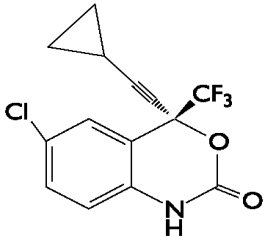
Nevirapine



Delavirdine



Loviride

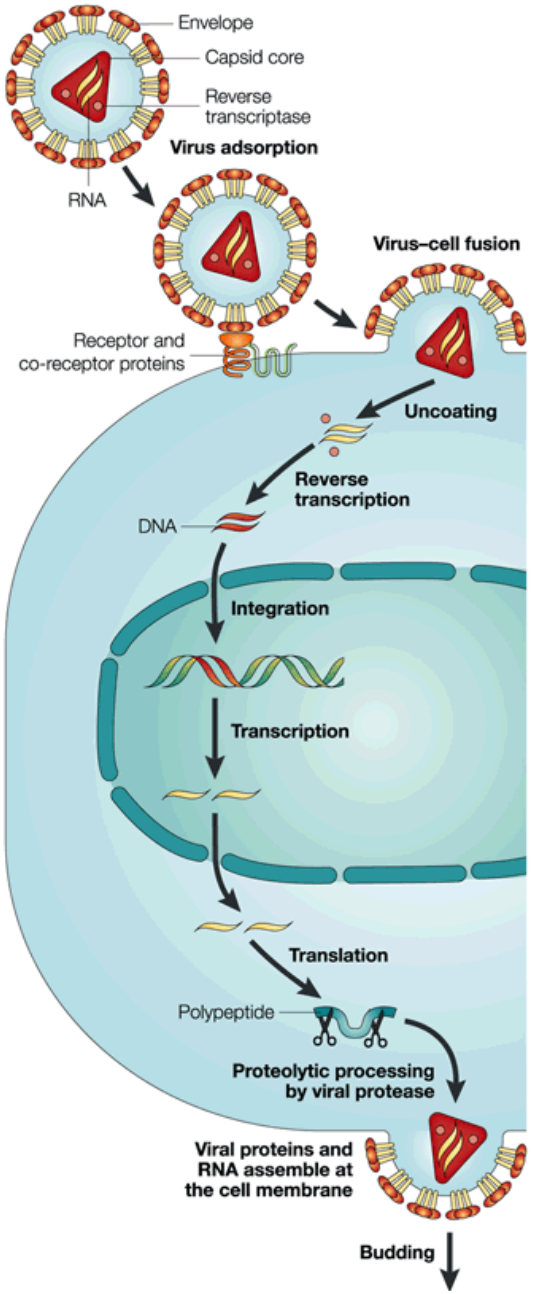


Efavirenz

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

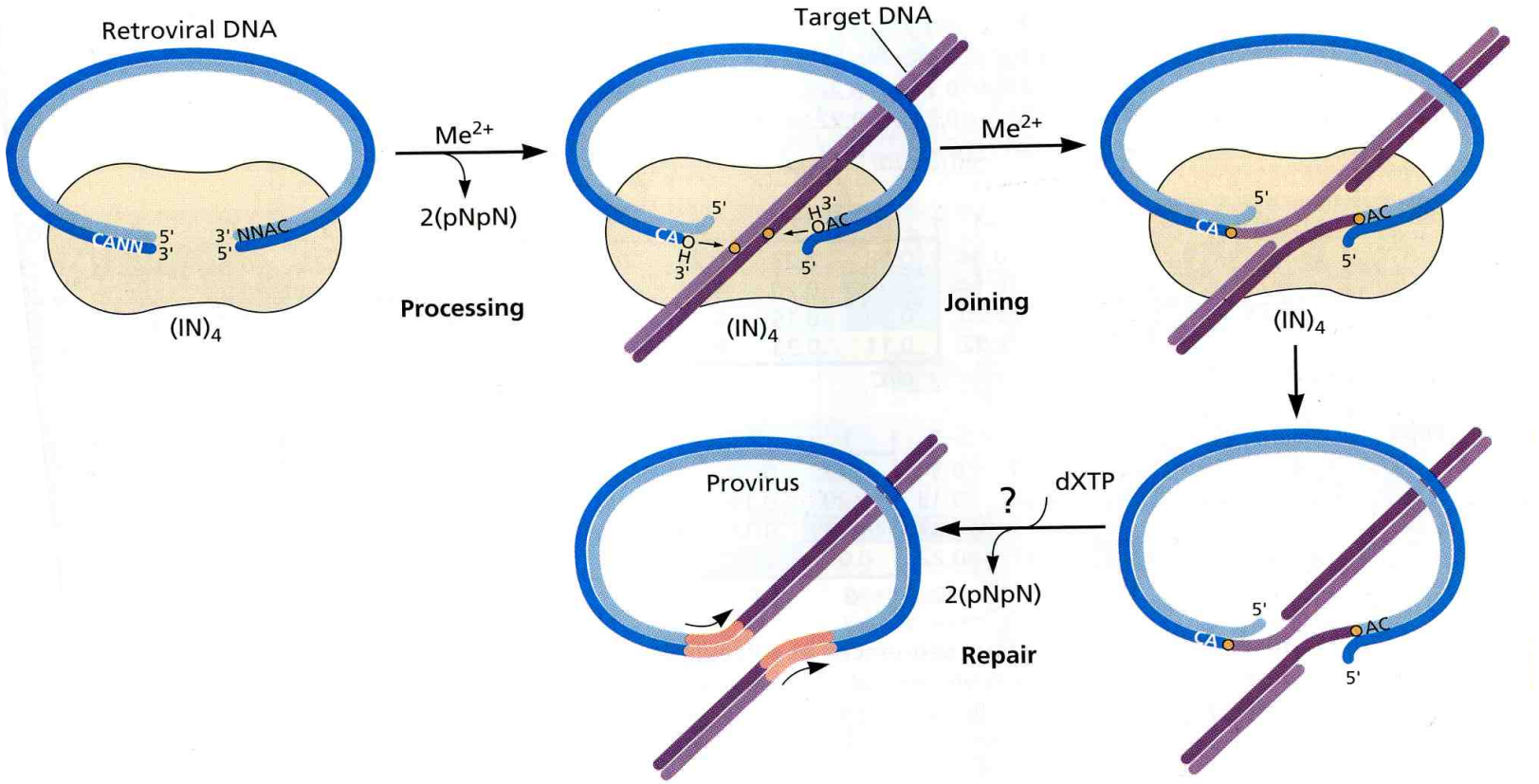
- ✓ **RESISTANCES:** Resistances and cross-resistances are frequent for point mutations in the RT. No reported cross-resistances with nucleoside analogues and protease inhibitors.

Druggable targets in HIV replication cycle



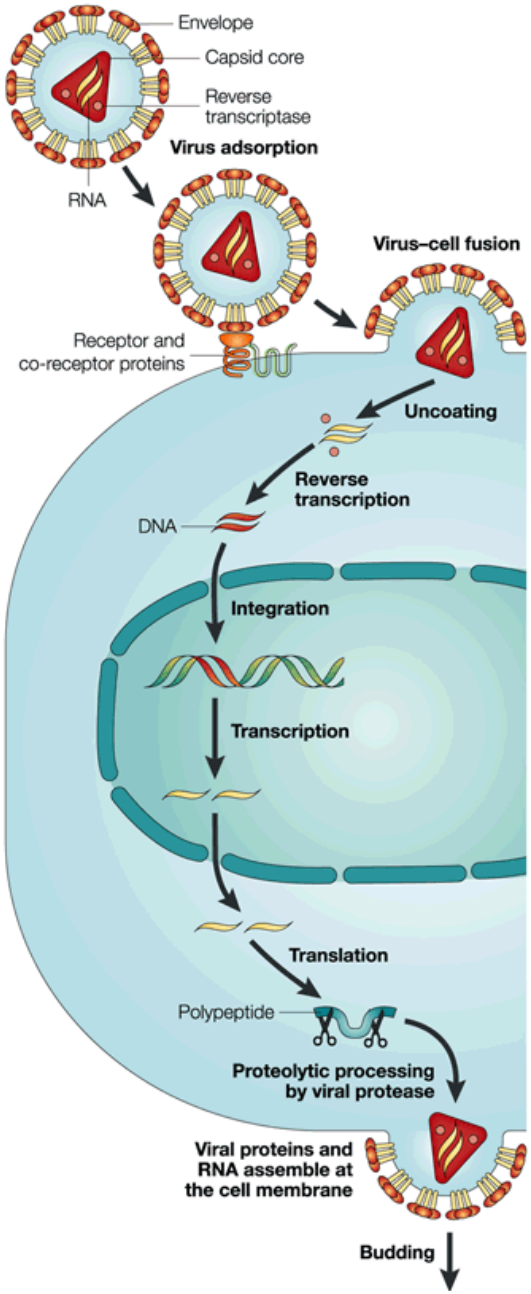
Integration

Development of HIV Integrase Inhibitors



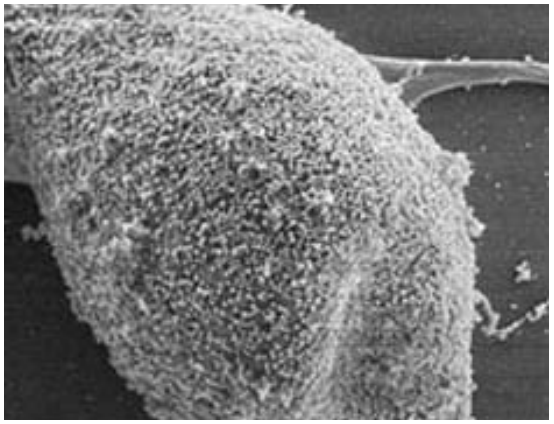
- ✓ The HIV integrase is an excellent drug target because it is a unique recombinase that mediates the insertion of retroviral DNA into host genome, where it is called a provirus
- ✓ HIV integrase is a tetrameric enzyme (crystal structure solved)
- ✓ These drugs act as strand transfer inhibitors that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

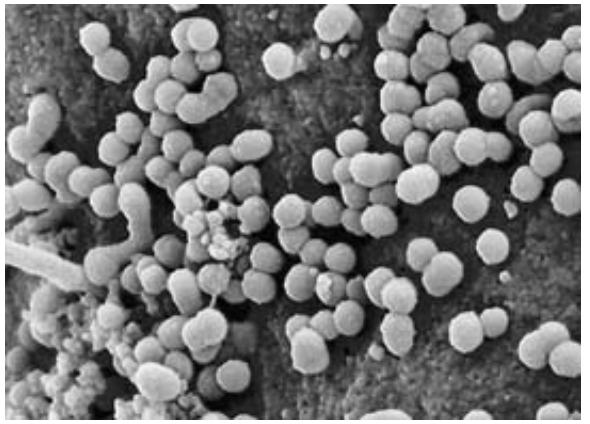
Druggable targets in HIV replication cycle

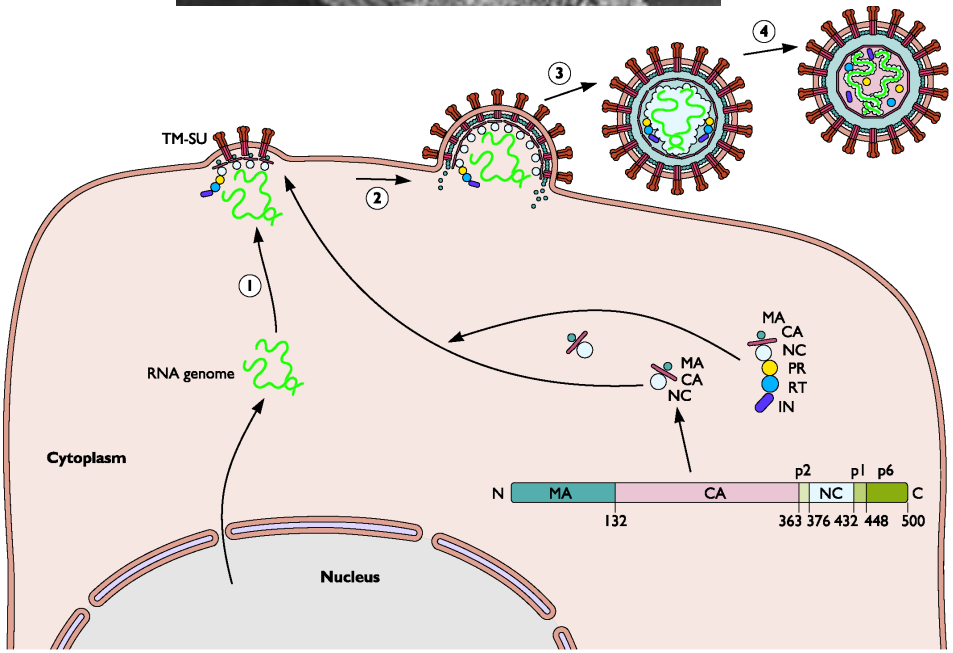


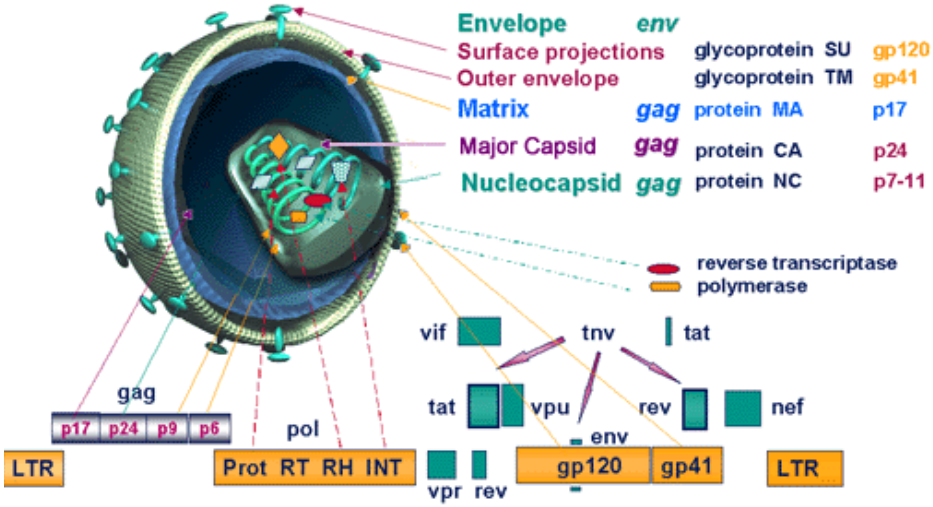
Maturation

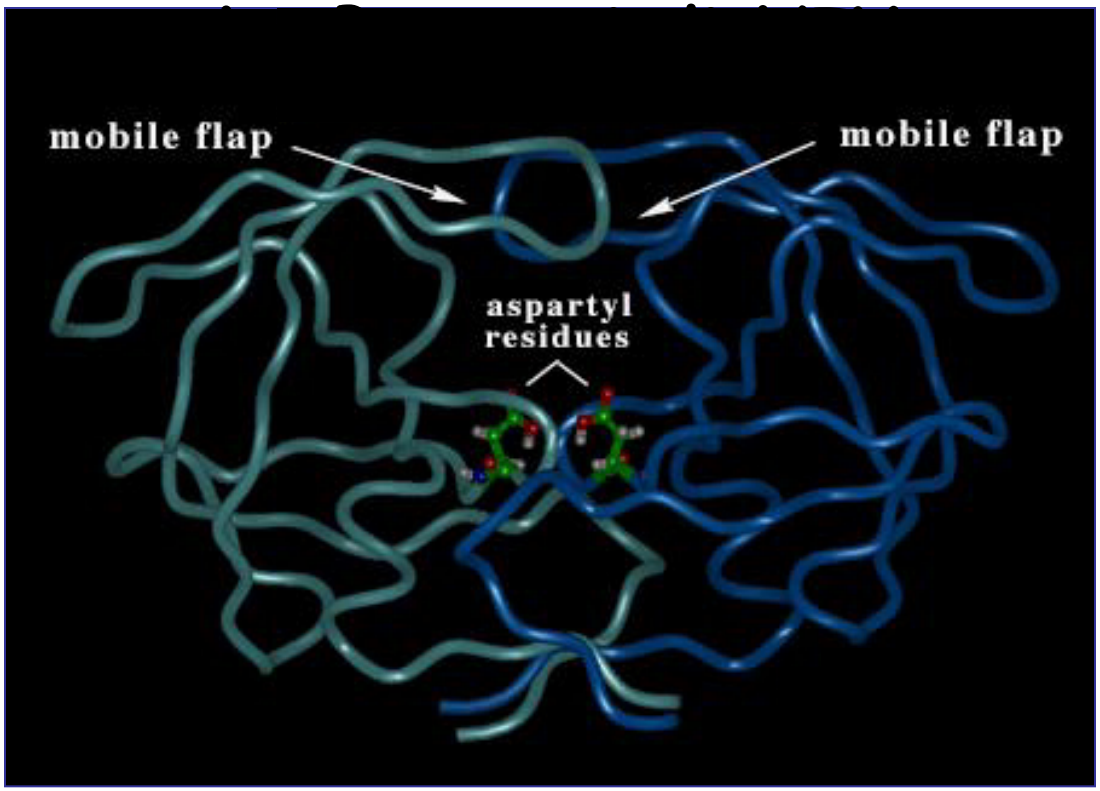
HIV egress by budding at the plasma membrane







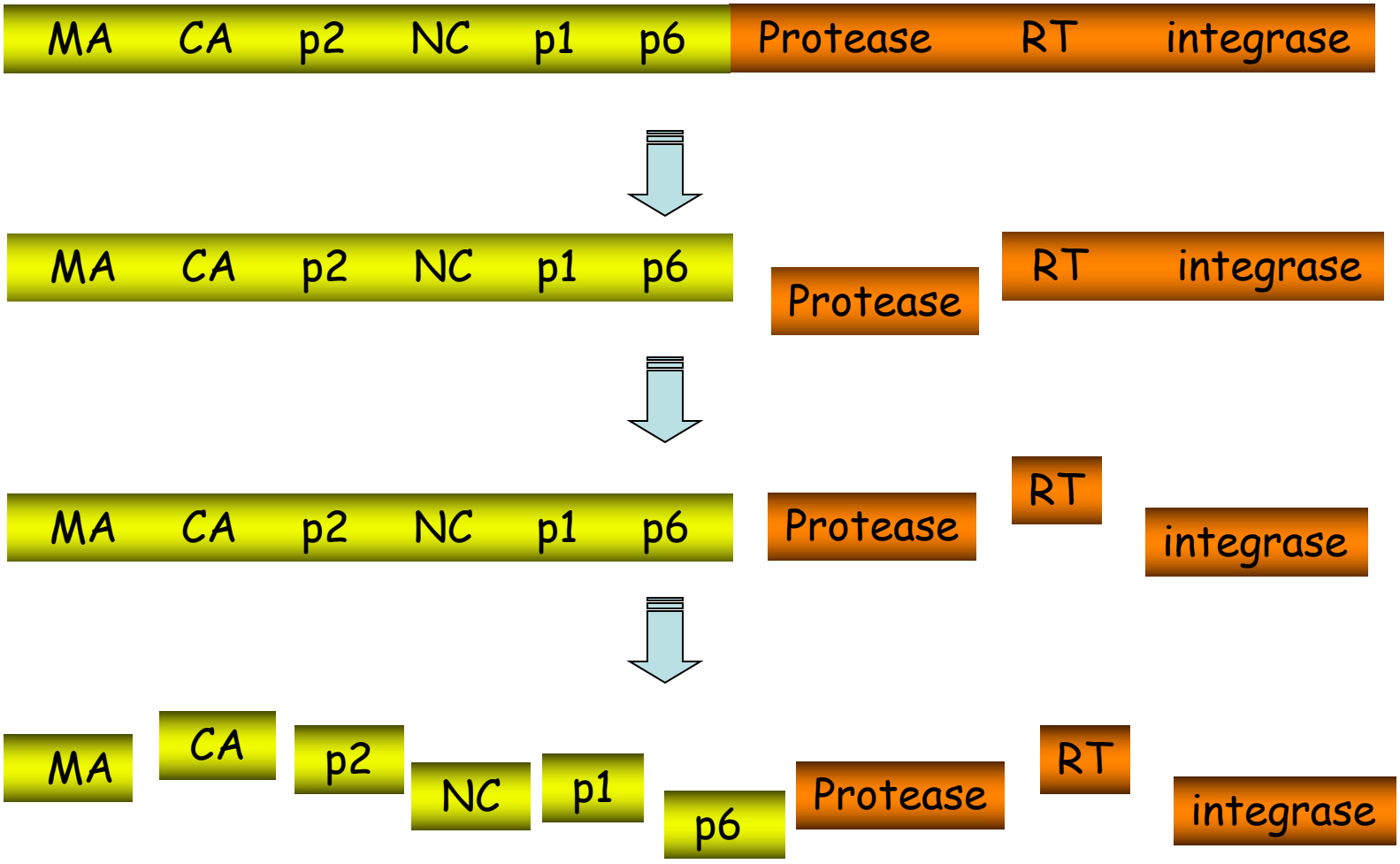




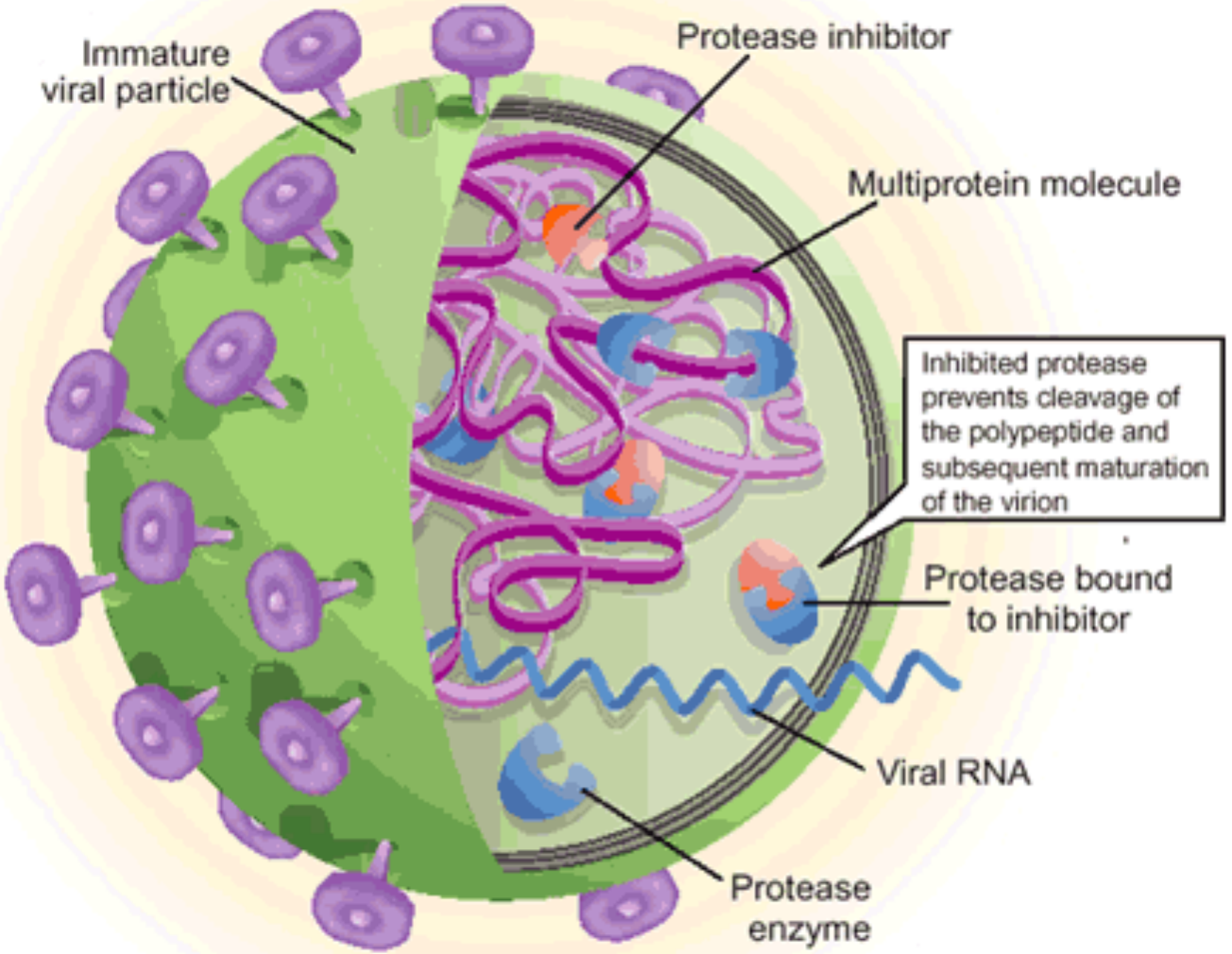
- ✓ The HIV-1 protease is a 99 aa protein encoded by ***pol***
- ✓ Dimeric
- ✓ Belongs to the aspartyl-proteases family (as rennin and pepsin)
- ✓ Asp 25 is crucial for enzyme activity
- ✓ HIV protease is essential for release of mature virions

Role of HIV protease

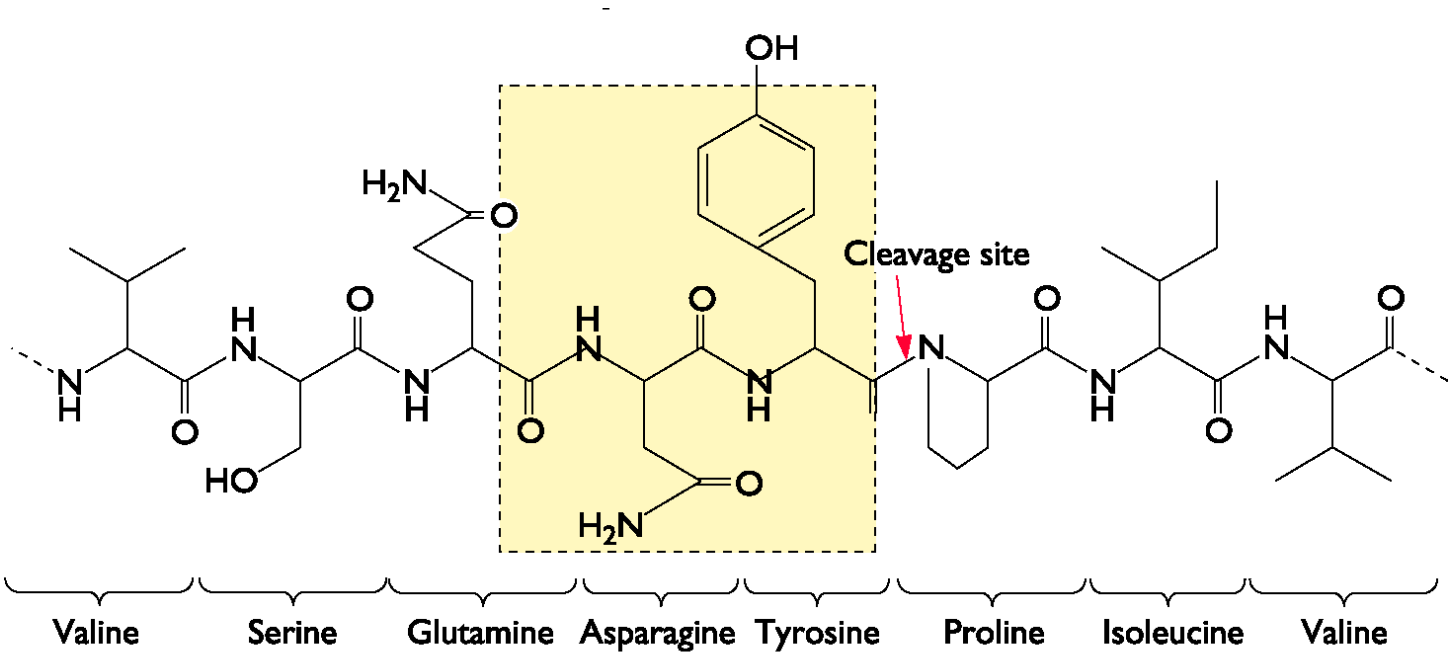
gag-pol polyprotein (9 cutting sites)



Role of HIV protease



Activity of HIV protease

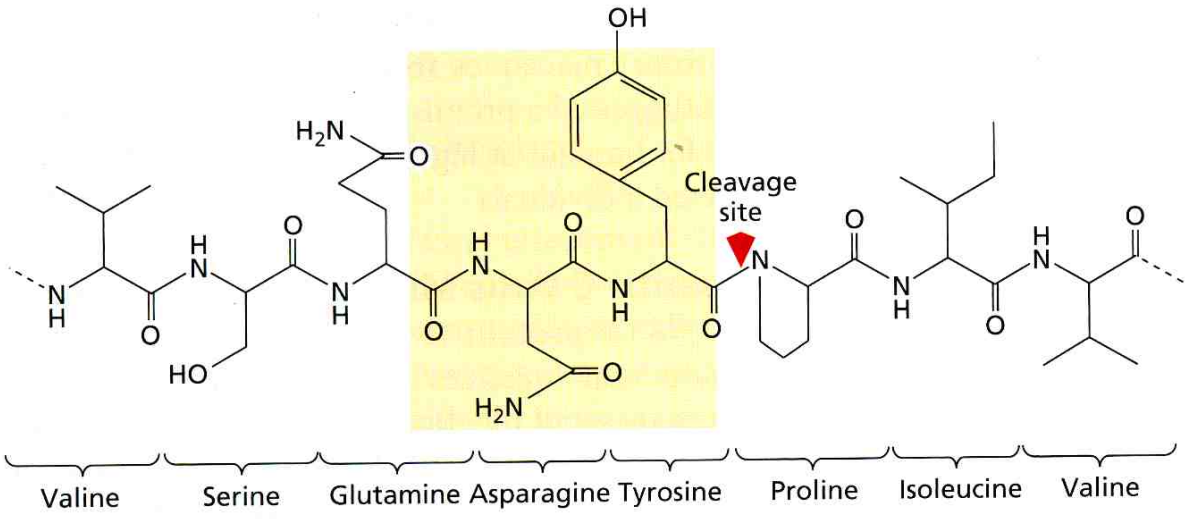


- ✓ HIV protease cuts between phenilalanine, or tyrosine, and a proline
- ✓ No human protease shows this specificity:

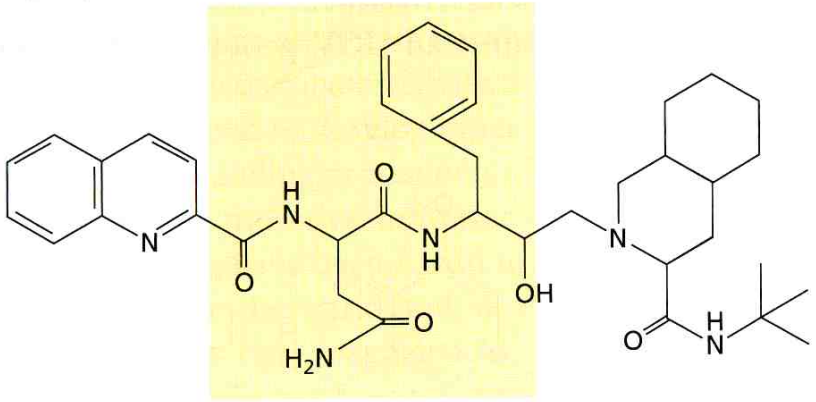
→ Thus, it possible to develop specific inhibitors!

Development of HIV PR inhibitors

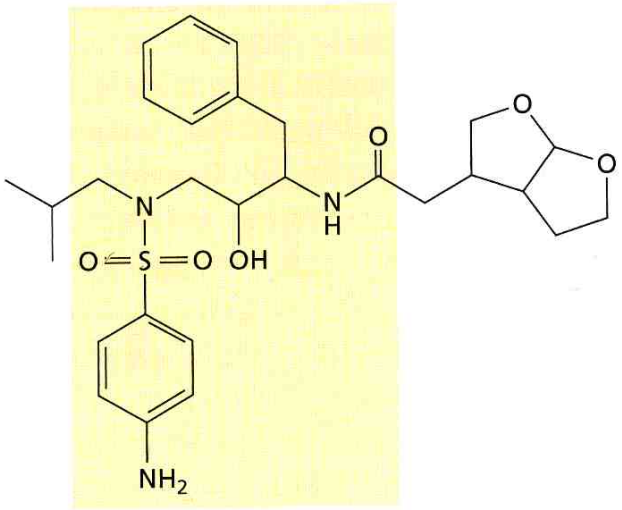
A Natural substrate of the HIV-1 protease



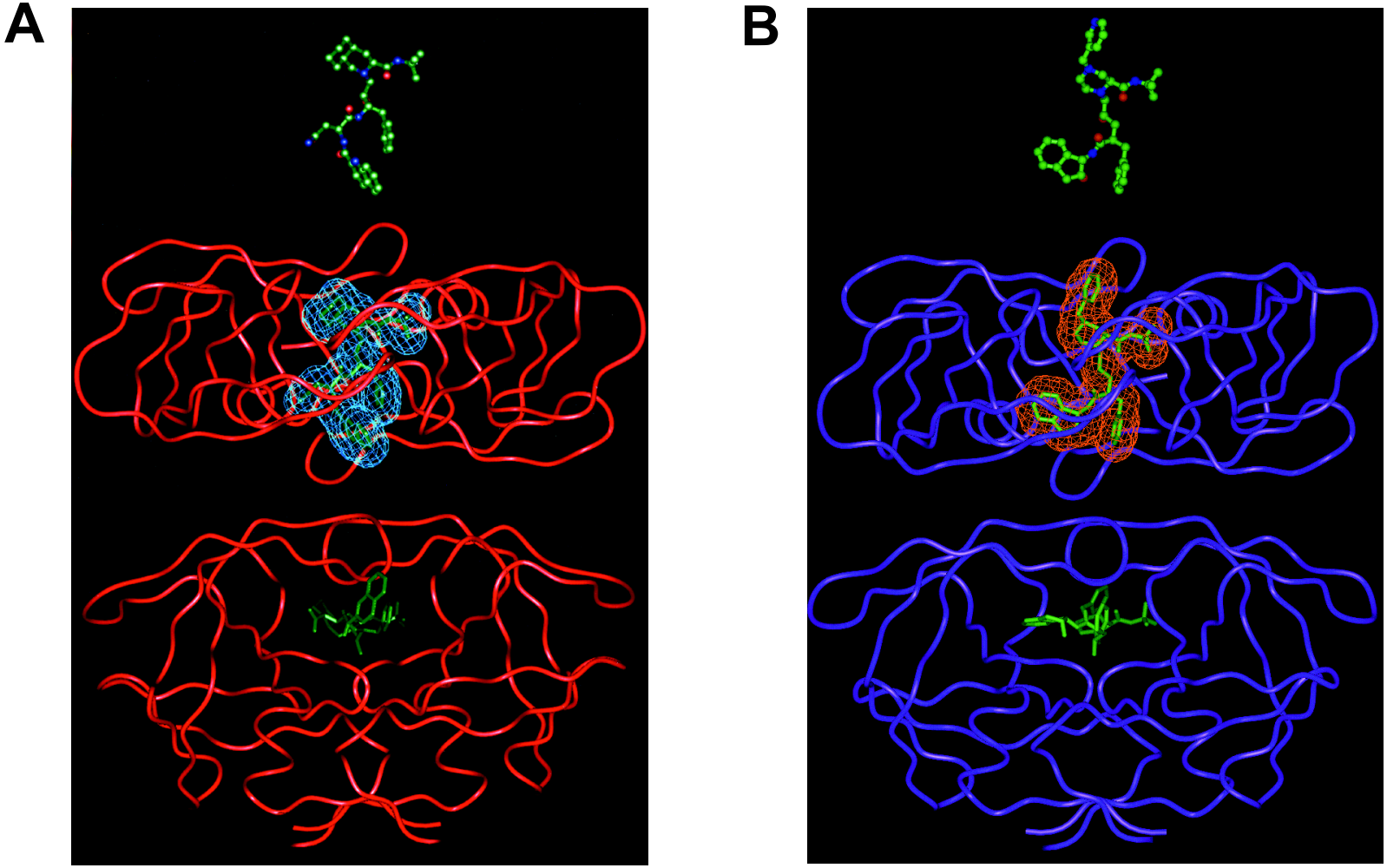
B Saquinavir



C Darunavir



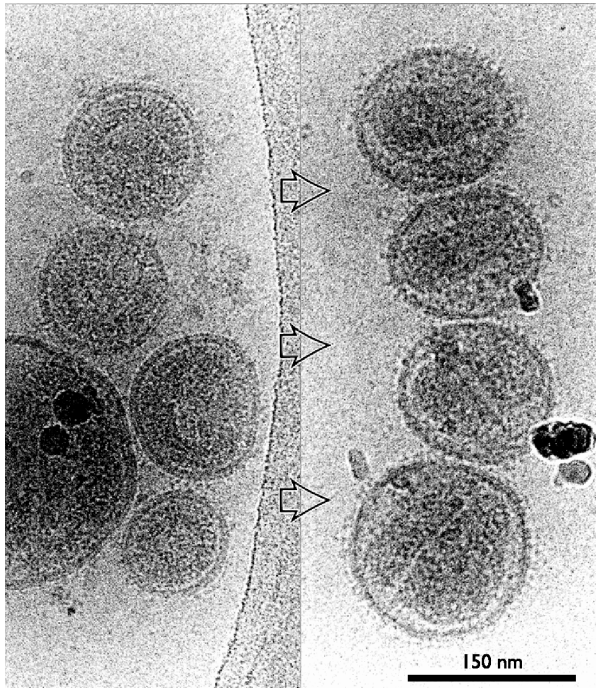
Development of HIV PR inhibitors



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

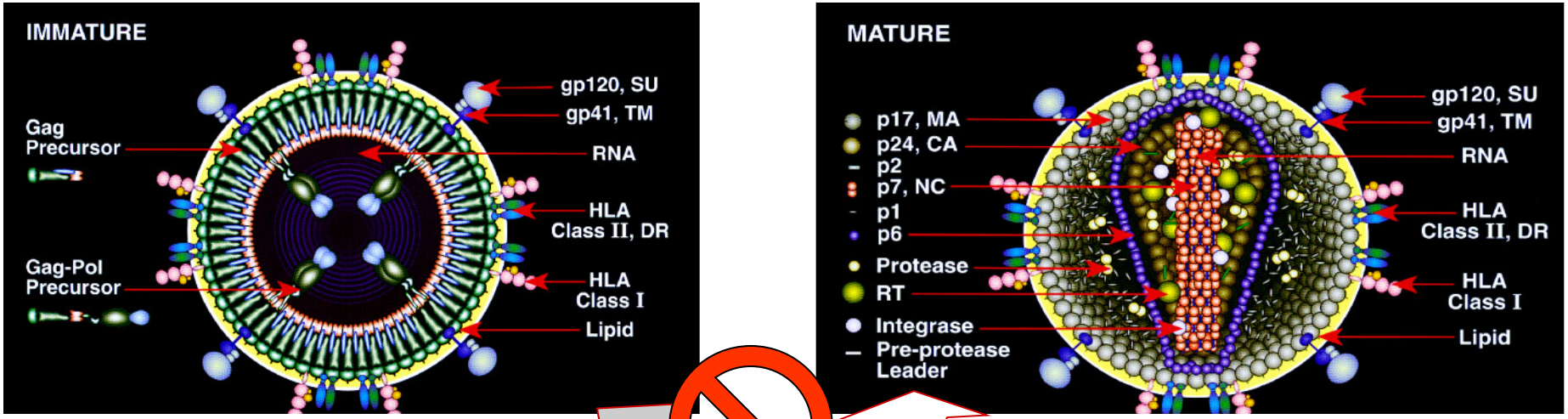
Vondrasek J. et al., *Nat. Struct. Biol.* 4:8, 1997

Effect of inhibition of HIV PR activity



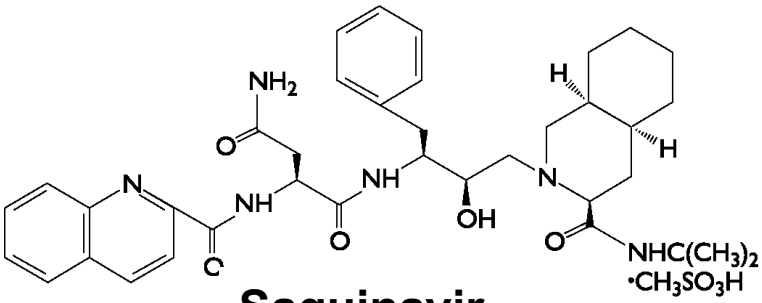
Immature virion

Mature virion

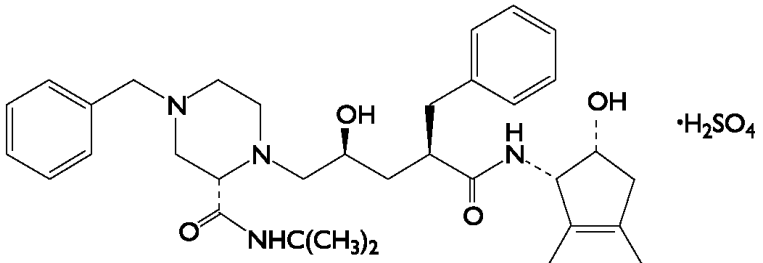


Viral protease

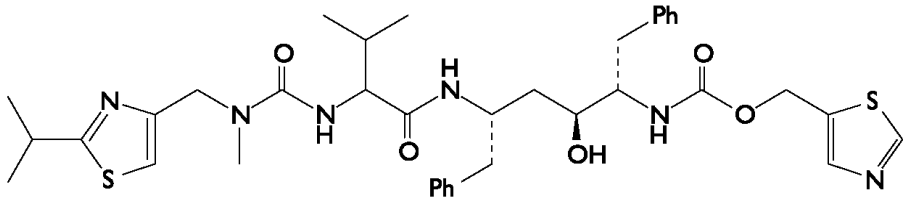
Inhibitors of HIV protease



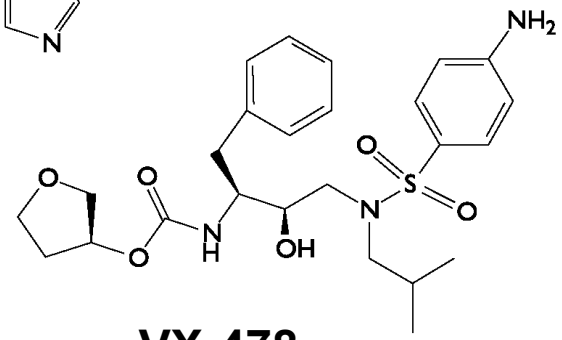
Saquinavir



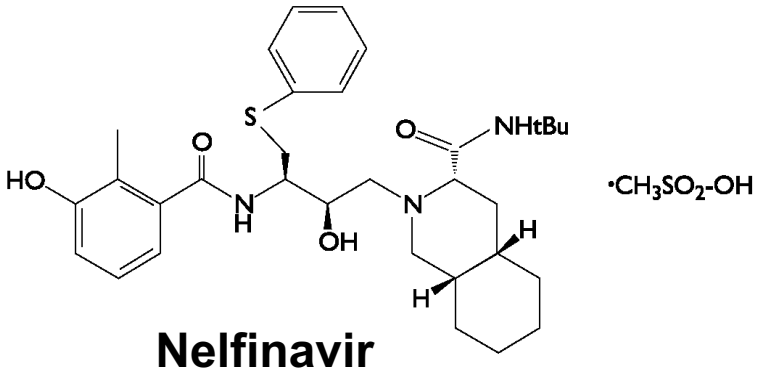
Indinavir



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

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

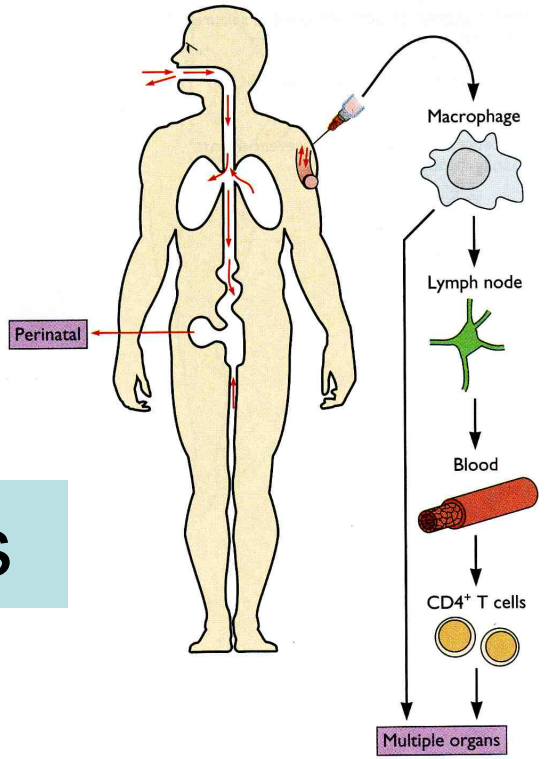
Infects mainly $CD4^+$ T cells and macrophages

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

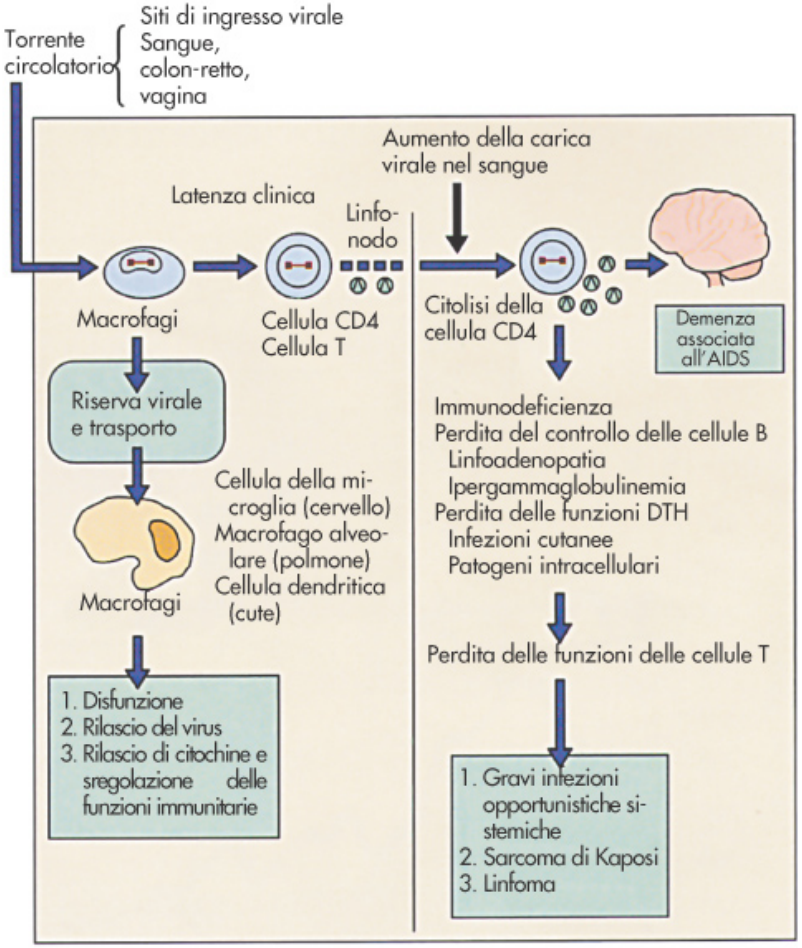
Infection alters T-cell and macrophage function; immunosuppression leads to secondary infection and death

Infects long-lived cells, establishing reservoir for persistent infection

Infected monocytes spread to brain, causing dementia



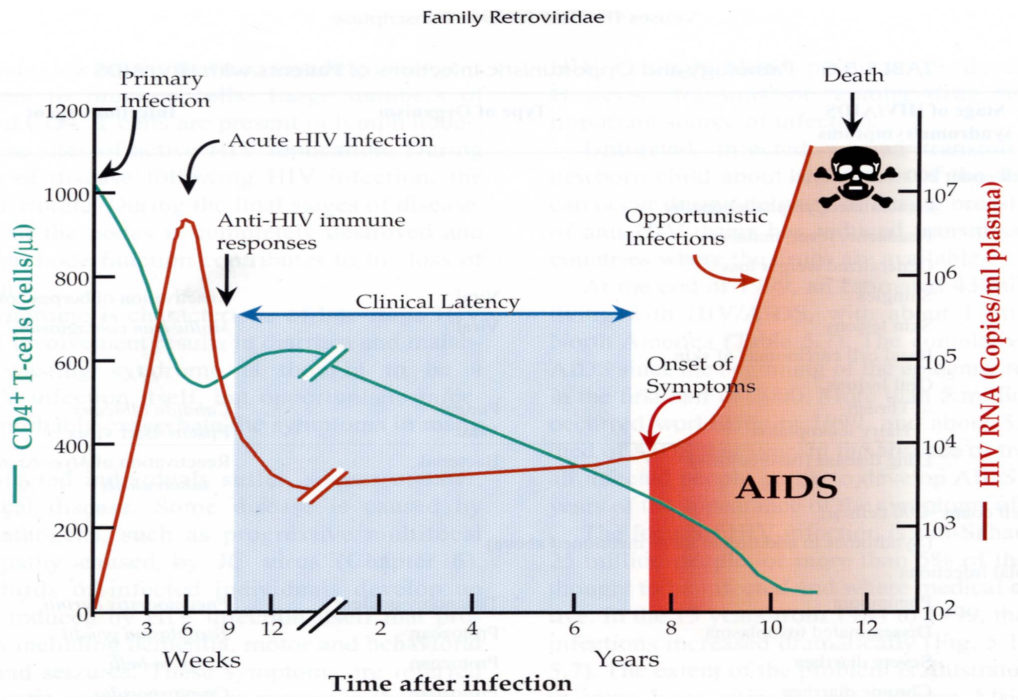
Summary of HIV pathogenesis

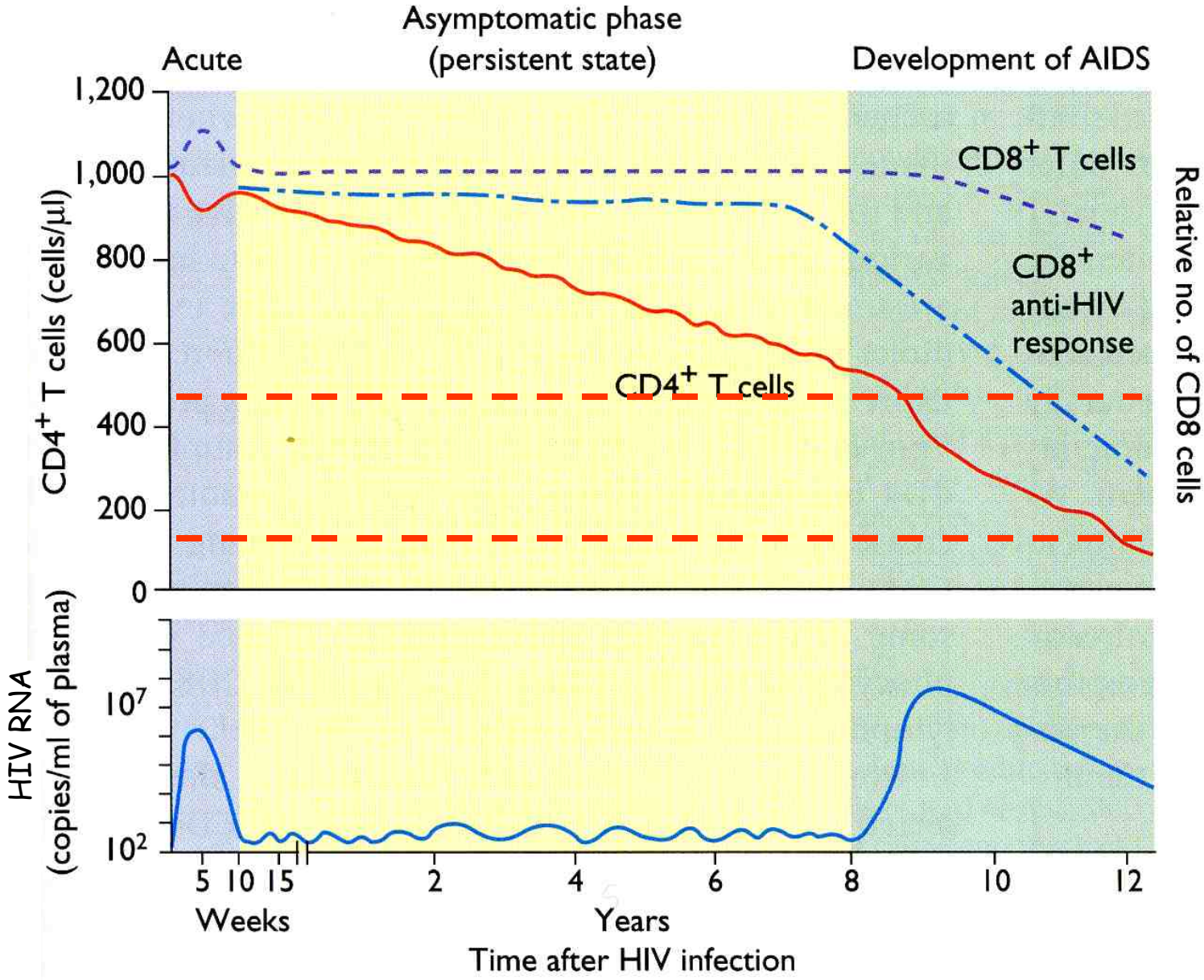


Summary of HIV pathogenesis

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 104 per template copied corresponds to an average of 1 mutatio in ***every*** replicated genome.

Math of HIV drug resistance

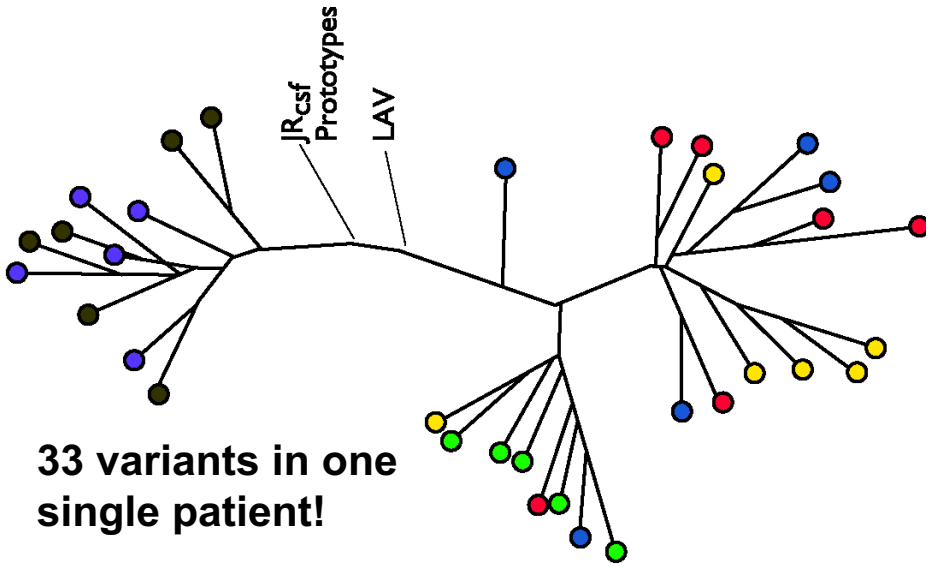
- Assume that one mutation is needed for drug resistance
- Mutation rate of 1 every 10^4 bases polymerized
- Each base is substituted in every 10^4 viruses
- Each HIV-infected individual makes 10^{10} new viruses/day
- $10^{10}/10^4 = 10^6$ viruses will be produced each day with resistance to one drug

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



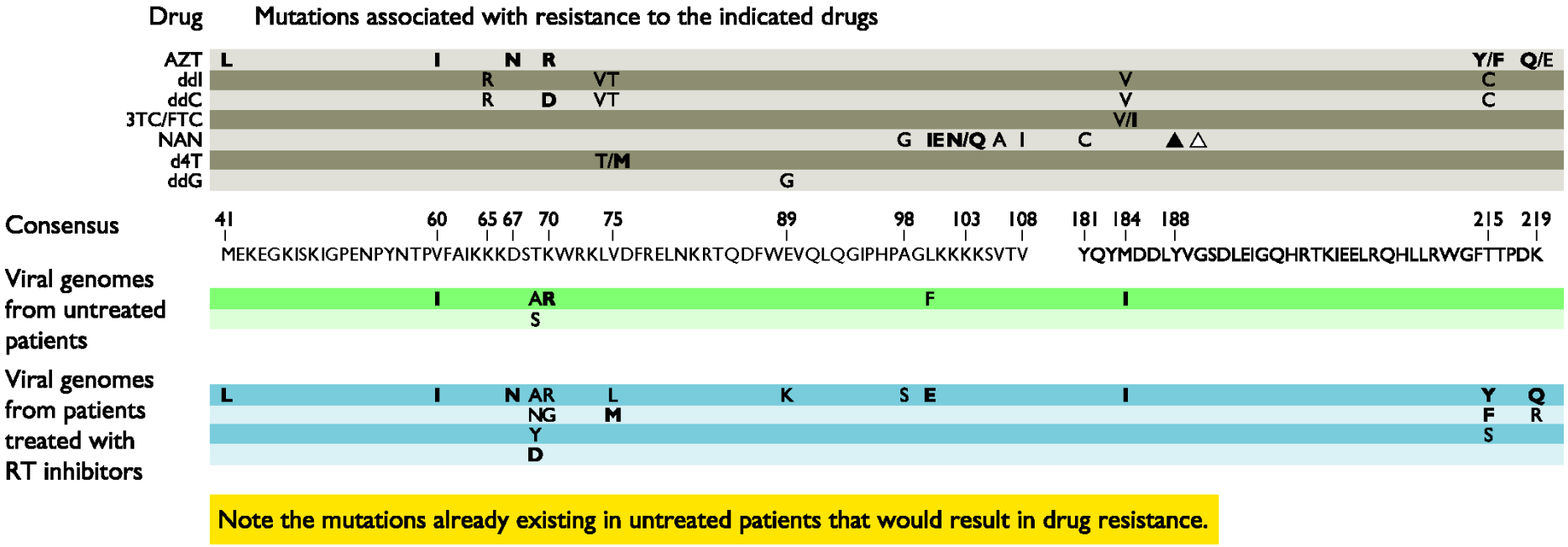
33 variants in one single patient!

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

The problem of viral resistances

REPLICATION=MUTATION

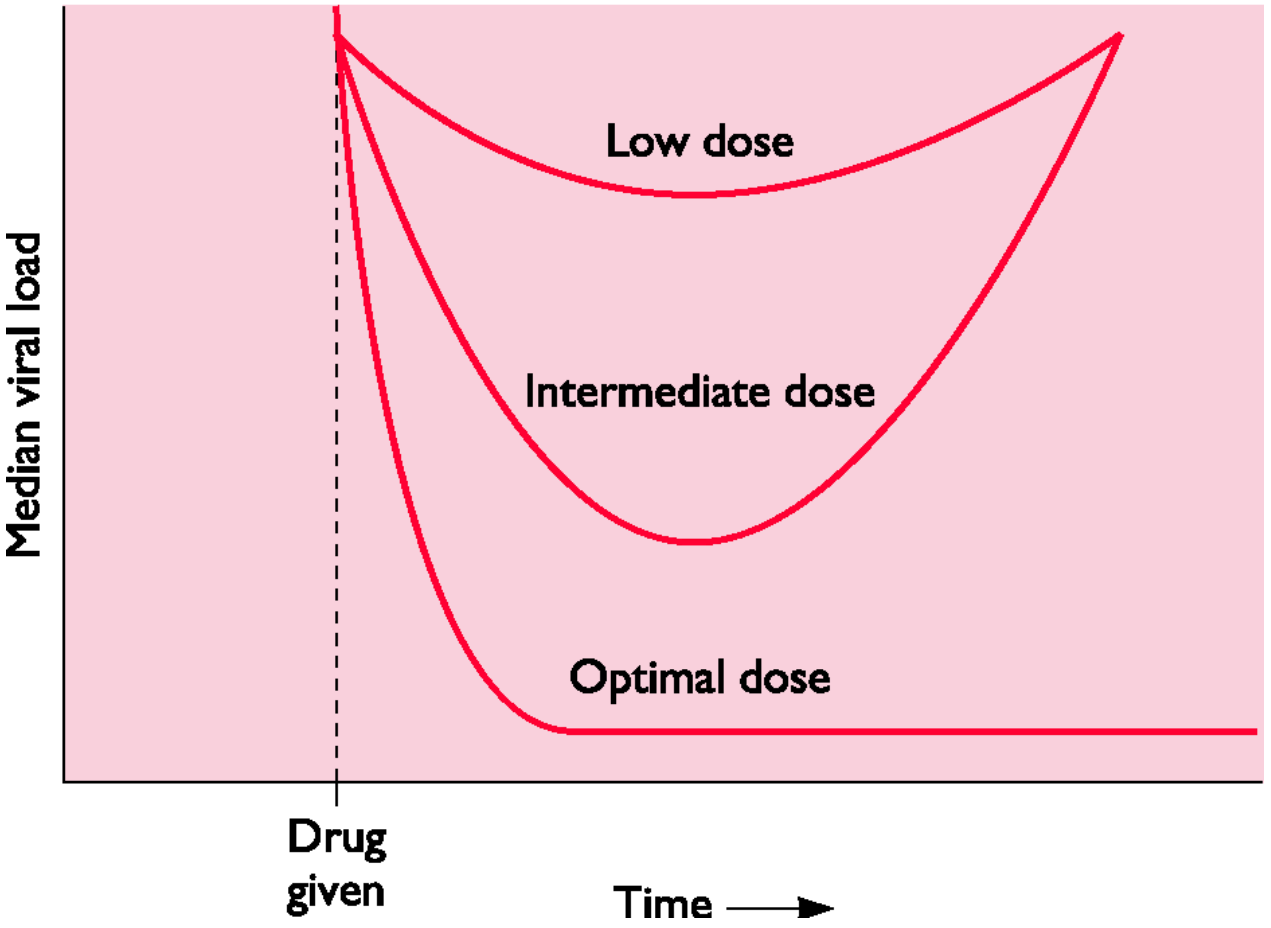


Note the mutations already existing in untreated patients that would result in drug resistance.

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)

http://aidsinfo.nih.gov/

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*

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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.

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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)

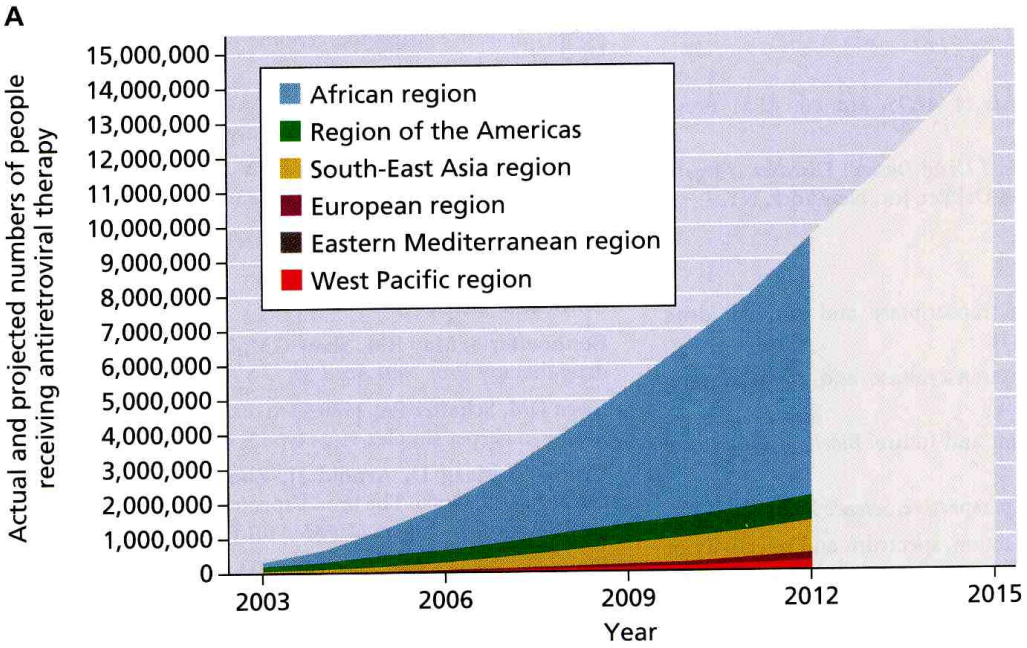
ARV Drug (Generic and Brand Names)	Strength Formulation	Dosing	Tablets, Capsules, or mLs per Month[a]	AWP[b] (Monthly)
Co-Formulated Combination Products as Single Tablet Regimens				
Dolutegravir/Abacavir/Lamivudine • Triumeq	50/600/300 mg tablet	1 tablet daily	30 tablets	$2,889.22
Efavirenz/Tenofovir 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

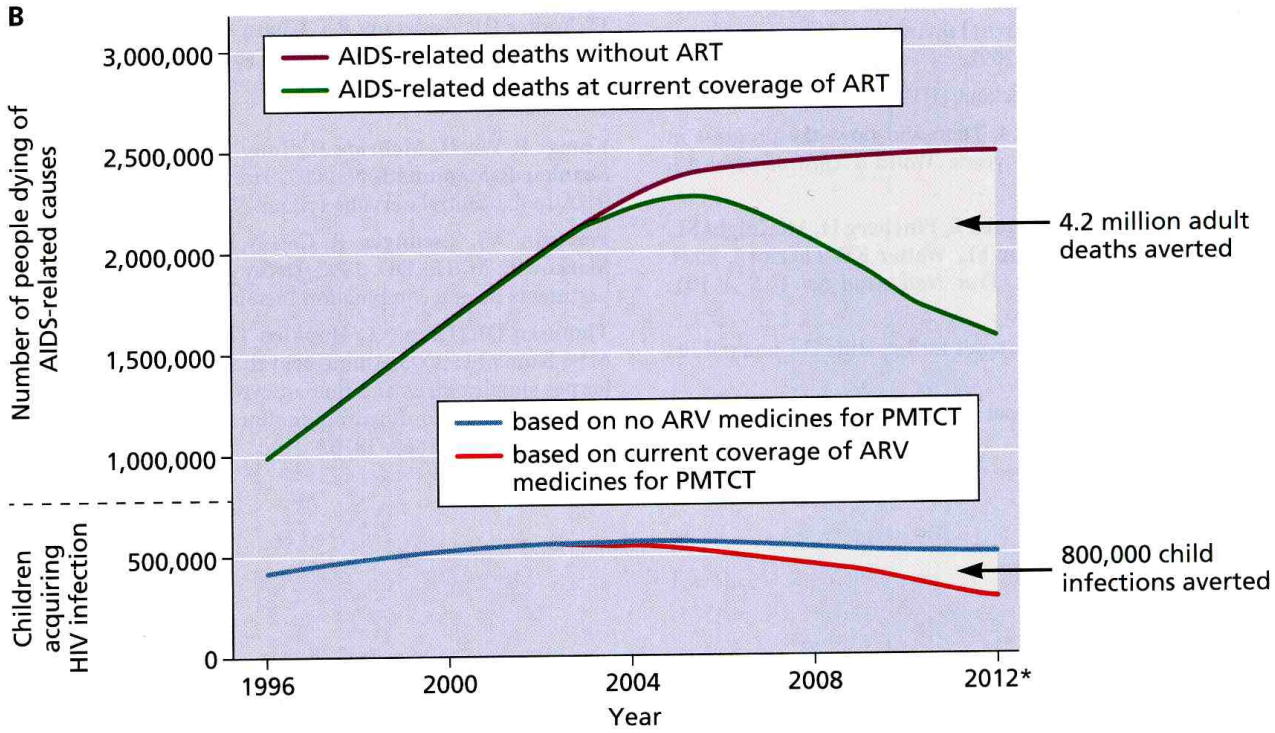
HAART: highly active antiretroviral therapy

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

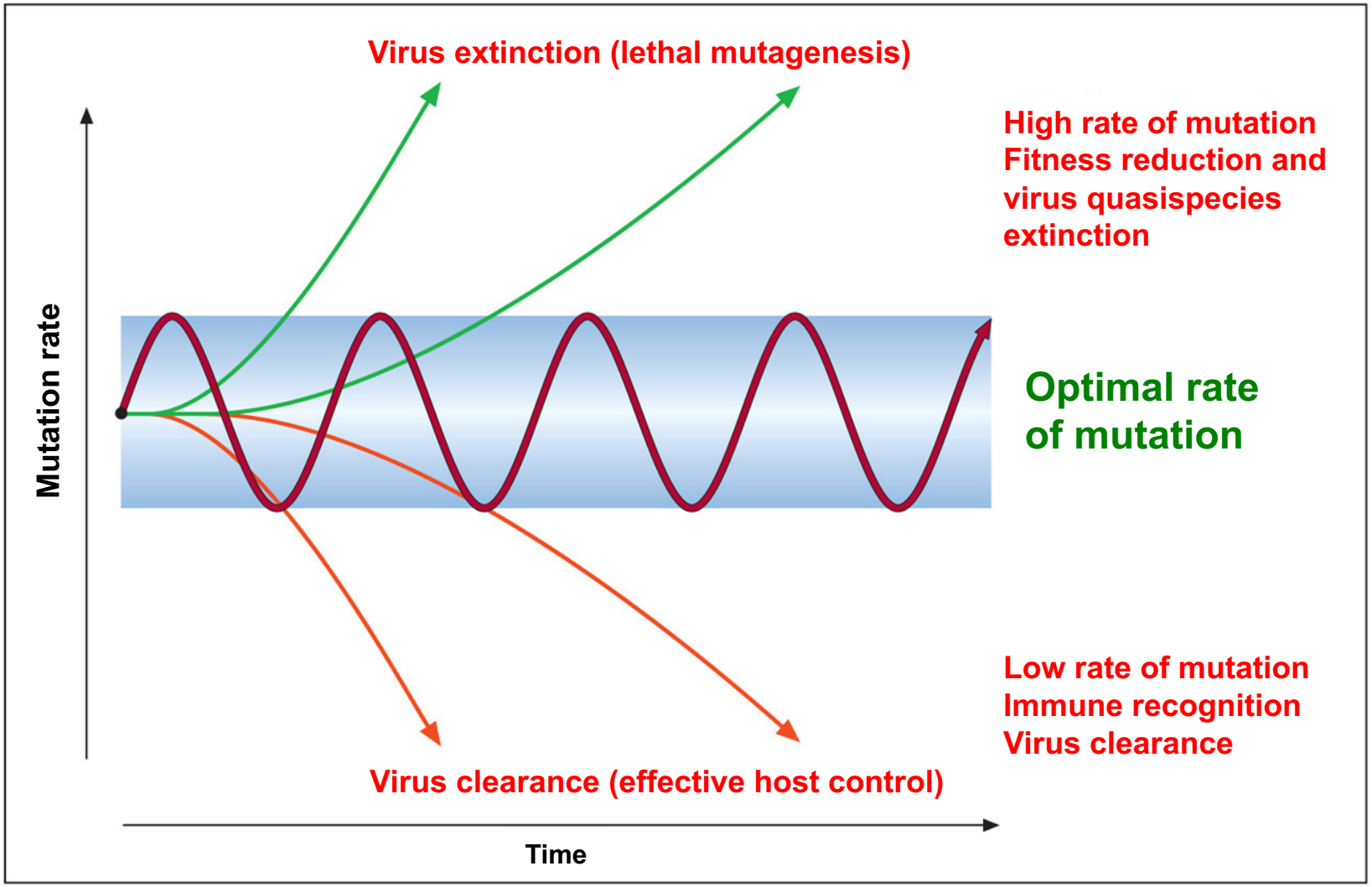
With this number of genomes, it is highly probable that HIV genomes exist that are resistant top every one of the antiviral drugs that we have now, or EVER WILL HAVE

Anti-HIV therapy saves million of lives





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**.