

# Antivirali

## Antiviral Characteristics

- **Antivirale di successo sarà in grado di:**
  1. Interferire con una funzione virale specifica

**oppure**

  2. Interferire con una funzione cellulare in modo da bloccare la replicazione del virus
- **Un farmaco ideale dovrebbe essere:**
  1. Solubile in acqua
  2. Stabile
  3. Facilmente incorporato dalle cellule
- **Un farmaco ideale non dovrebbe essere :**
  1. Tossico
  2. Tumorigenico
  3. Allergenico
  4. Mutagenico

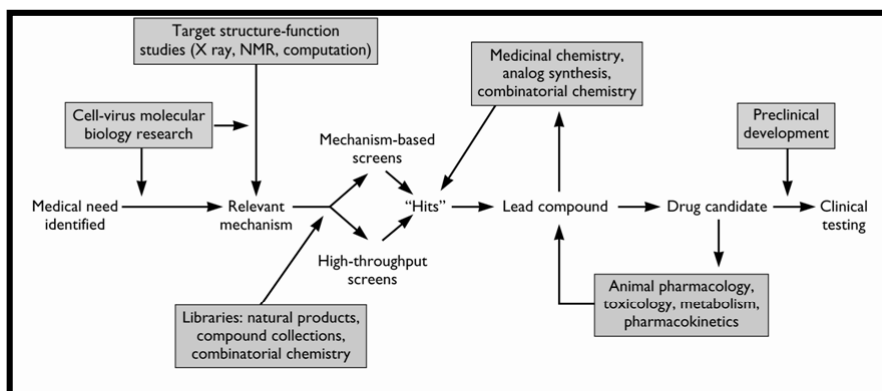
## Strumenti per lo sviluppo di farmaci antivirali

- Genomica
  - Microarrays
  - Determinazione della risposta dell'ospite all'infezione
- Proteomica
  - Struttura tridimensionale
  - Individuazione siti di legame
  - Protein Data Bank



HCV NS3 protein (helicase) bound to DNA

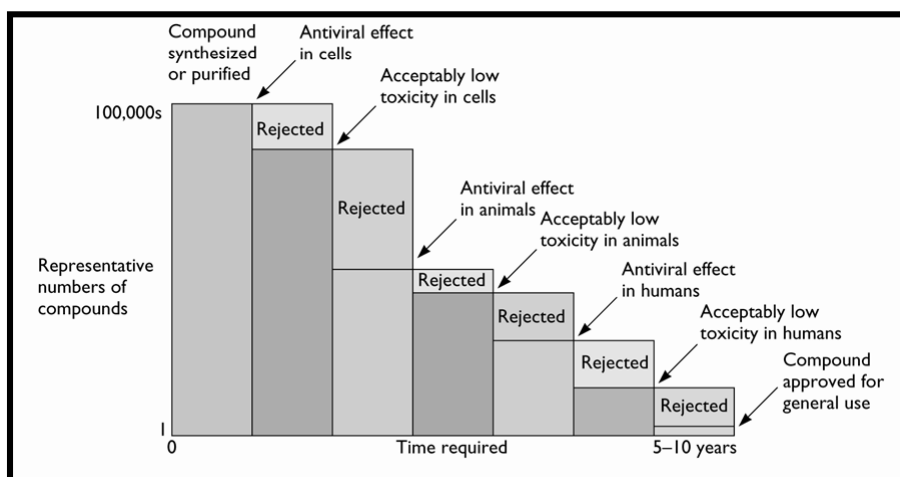
## Strumenti per lo sviluppo di farmaci antivirali



## Trials Clinici

- Phase I
  - Il farmaco viene usato nell'uomo per la prima volta
  - É sicuro e tollerato dall'uomo?
  - Tempi: mesi/un anno
  - Da 20 a 100 volontari sani
- Phase II
  - Usata per determinare l'effettività del farmaco
  - Centinaia di volontari
- Phase III
  - Confronto con trattamenti standard

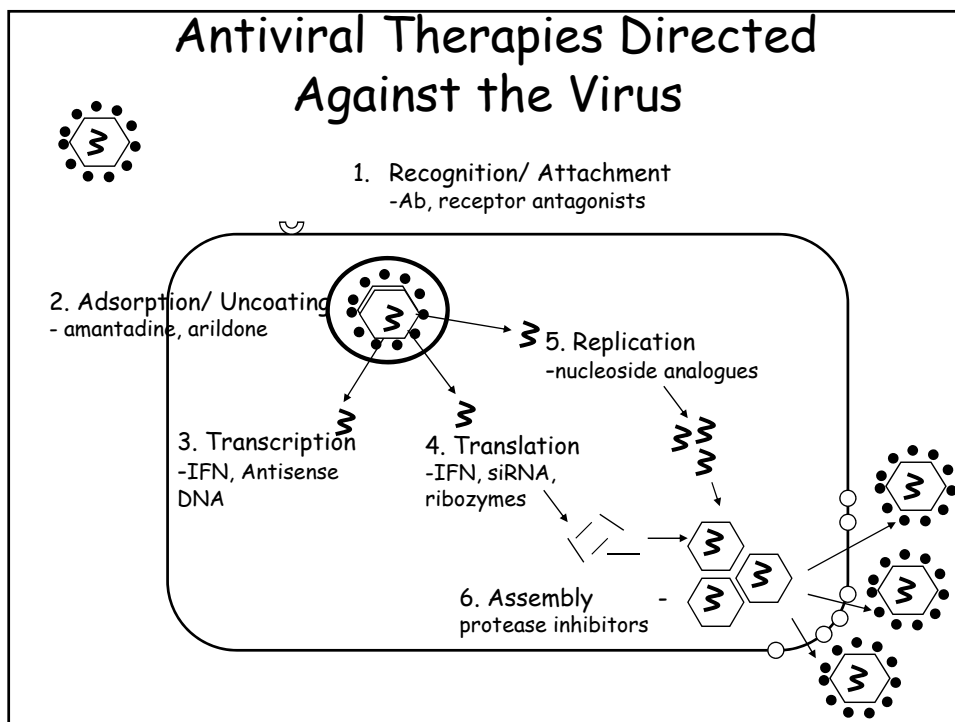
## Sviluppo di un nuovo farmaco antivirale





## Virus

- *Fasi della replicazione virale*
  - 1) adsorbimento e penetrazione nelle cellule
  - 2) uncoating dell'acido nucleico virale
  - 3) sintesi delle proteine regolatorie
  - 4) sintesi dell'RNA o del DNA
  - 5) sintesi delle proteine strutturali
  - 6) assemblaggio delle particelle virali
  - 7) rilascio dalla cellula ospite

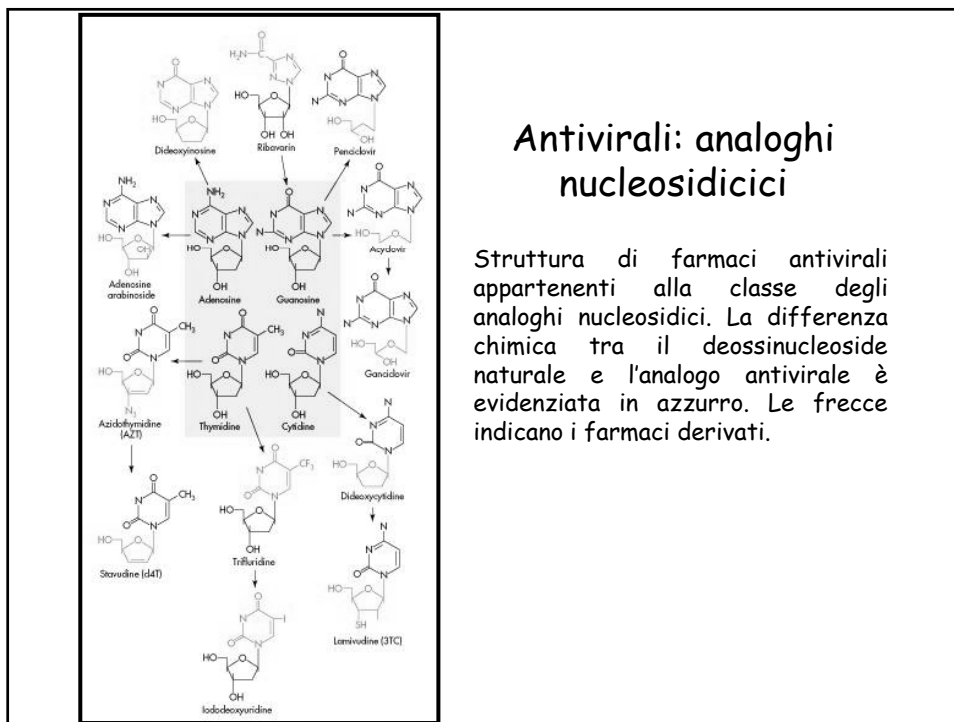


Some mechanisms targeted in antiviral drug discovery <sup>a</sup>		
Replication target	Selected compounds	Virus
<b>Attachment</b>	Peptide analogs of attachment protein	HIV
<b>Penetration and uncoating</b>	Dextran sulfate, heparin Amantadine, rimantadine Tromantadine Arildone, disoxaril, pleconaril	HIV, herpes simplex virus Influenza A virus Herpes simplex virus Picornaviruses
<b>mRNA synthesis</b>	Interferon Antisense oligonucleotides	Hepatitis A, B, and C viruses; papillomavirus Papillomavirus, human cytomegalovirus
<b>Protein synthesis</b>		
Initiation	Interferon	Hepatitis A, B, and C viruses; papillomavirus
IRES elements	Ribozymes; antisense oligonucleotides	Flavi- and picornaviruses
<b>DNA replication</b>		
Polymerase	Nucleoside and nonnucleoside analogs Phosphonoformate	Herpesviruses, HIV, hepatitis B virus Herpesviruses
Helicase/primase	Thiozole ureas	Herpes simplex virus
Processing/packaging	Benzimidazoles	Herpesviruses
<b>Nucleoside biosynthesis</b>		
Inosine monophosphate dehydrogenase	Ribavirin Levovirin, viramidine	Respiratory syncytial virus, Lassa fever virus Hepatitis C virus
<b>Nucleoside scavenging</b>		
Thymidine kinase	Nucleoside analog	Herpes simplex virus, varicella-zoster virus
Ribonucleotide reductase	Inhibitors of protein-protein interaction of large and small subunits	Herpes simplex virus
<b>Glycoprotein processing</b>		
Assembly	No lead compounds	Enveloped viruses
Protease	Peptidomimetics	HIV
<b>Virion integrity</b>		
Lipid raft disruption	Nonoxynol-9 β-Cyclodextrins	HIV, herpes simplex virus HIV, herpes simplex virus

The antiviral repertoire <sup>a,b</sup>		
Approach	Viruses	Compounds approved <sup>c</sup>
Virus adsorption inhibitors	HIV, HSV, CMV, RSV, and other enveloped viruses	None
Virus-cell fusion inhibitors	HIV, RSV, and other paramyxoviruses	Fuzeon
Virus-uncoating inhibitors	Influenza A virus	Amantadine, rimantadine
Viral DNA polymerase inhibitors	Herpesviruses (HSV-1, -2, VZV, CMV, EBV, HHV-6, -7, -8)	Acyclovir, valacyclovir, ganciclovir, valganciclovir, penciclovir, famciclovir, brivudin, foscarnet
Reverse transcriptase inhibitors	HIV	NRTIs: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir NNRTIs: nevirapine, delavirdine, efavirenz
Acyclic nucleoside phosphonates	DNA viruses (polyoma-, papilloma-, herpes-, adeno-, and poxviruses), HIV, HBV	CMV: cidofovir HIV: tenofovir
Inhibitors of viral RNA synthesis	HIV, HCV	None
Viral protease inhibitors	HIV, herpesviruses, rhinoviruses, HCV	HIV: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir
Viral neuraminidase inhibitors	Influenza A and B virus	Zanamivir, oseltamivir
Inosine monophosphate dehydrogenase inhibitors	HCV, RSV	Ribavirin
S-adenosylhomocysteine hydrolase inhibitors	(-) strand RNA hemorrhagic fever viruses, filoviruses	None

<sup>a</sup>Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; HSV, herpes simplex virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.  
<sup>b</sup>Brivudin is approved in some countries, e.g., Germany. Lamivudine is also approved for the treatment of HBV. Ribavirin is used in combination with IFN-α for HCV.

# Herpesviruses

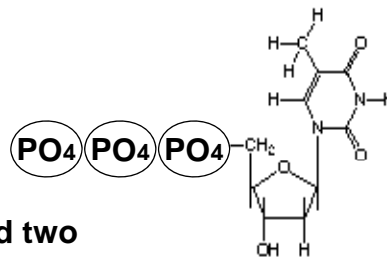


## Antivirali: analoghi nucleosidici

Struttura di farmaci antivirali appartenenti alla classe degli analoghi nucleosidici. La differenza chimica tra il deossinucleoside naturale e l'analogo antivirale è evidenziata in azzurro. Le frecce indicano i farmaci derivati.

## Thymidine Kinase

Viral or cellular thymidine kinase adds first phosphate



Cellular kinases add two more phosphates to form TTP

**Perchè l'herpes simplex codifica per la propria timidina chinasi?**

**Virus TK- non possono crescere nelle cellule neuronali perchè tali cellule non proliferano**

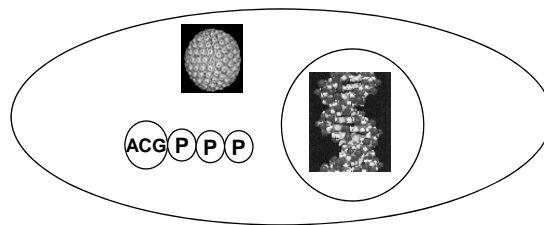
**Anche se purine e pirimidine sono presenti, i livelli di nucleosidi fosforilati sono bassi**

**La presenza di TK permette la replicazione di virus in cellule che non producono DNA**

**Thymidine kinase serve a fosforilare qualsiasi deossinucleoside, compreso il farmaco - come risultato della sua necessaria non-specificità**

**Tale farmaco attraversa la membrana**

- Permette selettività visto che solo le cellule infette contengono l'enzima per fosforilare il farmaco (prima fosforilazione) (es: HSV che contiene la propria TK)



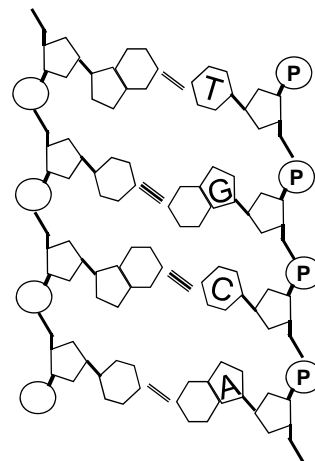
## Meccanismo d'azione *Acyclovir*

- Derivato aciclico della guanosina
- Fosforilato dalla *thymidine kinase virale*
- Di- e tri-fosforilato da enzimi cellulari
- Inibisce la sintesi del DNA virale:
  - 1) compete con dGTP per la DNA polimerasi virale
  - 2) terminatore di catena

***Acyclovir* : Meccanismo d'azione**

**ACV agisce in due modi:**

- **Compete con GTP**
- **Terminatore di catena**



Normal DNA synthesis

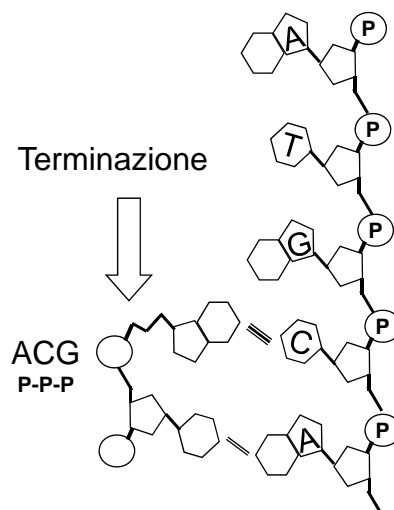
ACV agisce in due modi:

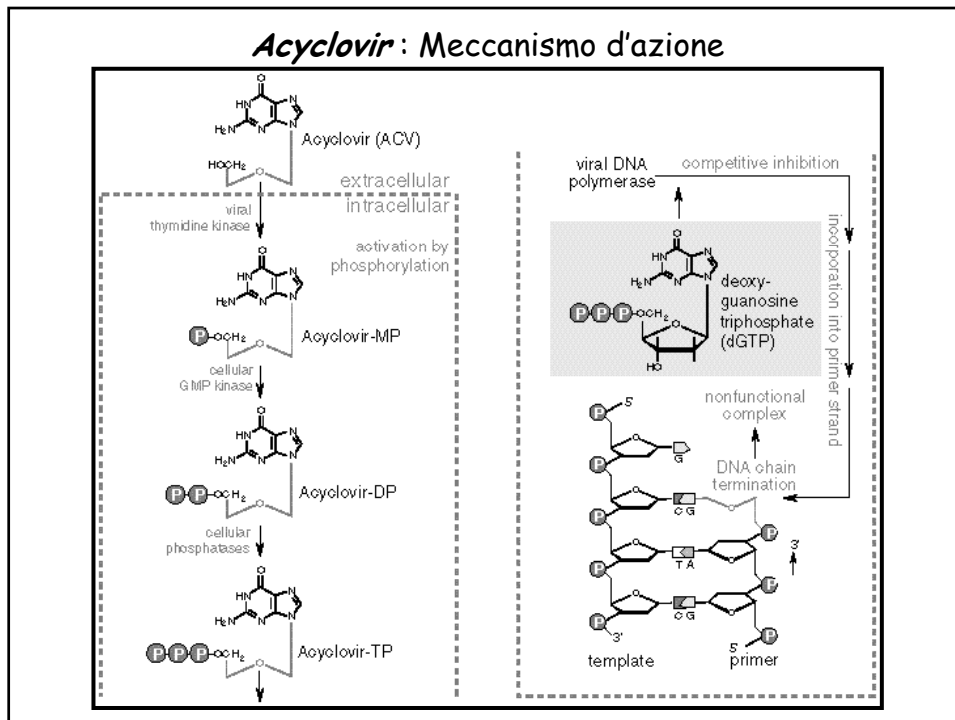
- **Compete con GTP**
- **Terminatore di catena**

Selettivo: TK virale

Inibisce anche:

- Epstein Barr
- Cytomegalovirus





## Chemotherapy

L'acyclovir è molto efficace contro:

- Herpes simplex keratitis (topical)
- Latent HSV (iv)
- Fever blisters – Herpes labialis (topical)
- Genital herpes (topical, oral, iv)

**Si possono sviluppare resistenze a carico della TK o della DNA polimerasi**

**Non sembra tossico o teratogeno**

**Il ganciclovir è molto efficace contro il citomegalovirus**

### Antivirali per trattare o prevenire le infezioni erpetiche

Agent	Route of Administration	Use	Recommended Adult Dosage and Regimen
Acyclovir	Oral	First episode genital herpes	400 mg tid or 200 mg five times daily
		Recurrent genital herpes	400 mg tid or 200 mg five times daily or 800 mg bid
		Genital herpes suppression	400 mg bid
		Herpes proctitis	400 mg five times daily
		Mucocutaneous herpes in the immunocompromised host	400 mg five times daily
		Varicella	20 mg/kg (maximum 800 mg) four times daily
		Zoster	800 mg five times daily
	Intravenous	Severe HSV infection	5 mg/kg q8h
		Herpes encephalitis	10–15 mg/kg q8h
		Neonatal HSV infection	20 mg/kg q8h
Famciclovir	Oral	First episode genital herpes	250 mg tid
		Recurrent genital herpes	125 mg bid
		Genital herpes suppression	250 mg bid
Valacyclovir	Oral	Zoster	500 mg tid
		First episode genital herpes	1 g bid
		Recurrent genital herpes	500 mg bid
		Genital herpes suppression	500 mg daily or twice daily
Foscarnet	Intravenous	Zoster	1 g tid
		Acyclovir-resistant HSV and VZV infections	40 mg/kg q8-12h
Docosanol	Topical	Recurrent herpes labialis	Thin film covering lesion every 2 hours

Table 49–2. Agents to treat cytomegalovirus (CMV) infection.

Agent	Route of Administration	Use	Recommended Adult Dosage
Ganciclovir	Intravenous	CMV retinitis treatment (induction or maintenance)	Induction: 5 mg/kg every 7 days Maintenance: 5 mg/kg every 14 days
	Intravitreal injection	CMV retinitis treatment (induction or maintenance)	Induction: 330 µg every 14 days Maintenance: 330 µg every 4 weeks
Cytosine arabinoside	Intravenous	CMV retinitis treatment (induction or maintenance)	Induction: 60 mg/kg q8h or 90 mg/kg q12h Maintenance: 90–120 mg/kg/d
		CMV retinitis treatment (induction or maintenance)	Induction: 5 mg/kg q12h Maintenance: 5 mg/kg/d or 6 mg/kg five times per week
Ganciclovir	Oral	CMV prophylaxis	1 g tid
		CMV retinitis treatment (maintenance only)	1 g tid
Acetazolamide	Intraocular implant	CMV retinitis treatment	4.5 mg every 6–8 months
Valacyclovir	Oral	CMV retinitis treatment (induction or maintenance)	Induction: 900 mg bid Maintenance: 900 mg qd The drug should be taken with food.
		CMV prophylaxis	900 mg qd

<sup>1</sup>Dosage must be reduced in patients with renal insufficiency.

## Transcription Inhibitors

- Antisense DNA
  - Fomivirsen
    - Antisense 21-mer phosphorothioate oligonucleotide
    - Complementary to the immediate-early gene of hCMV
    - Approved in 1998

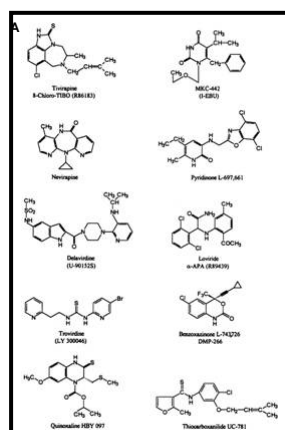


HIV

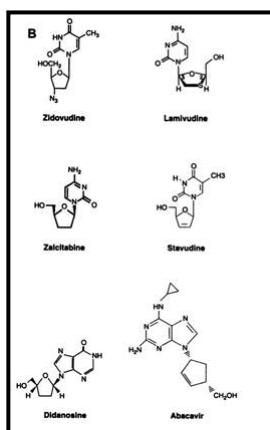
## Farmaci antiretrovirali

- 1) Inibitori **nucleosidici** della trascrittasi inversa (NRTIs)
- 2) Inibitori **non-nucleosidici** della trascrittasi inversa (NNRTIs)
- 3) Inibitori della proteasi

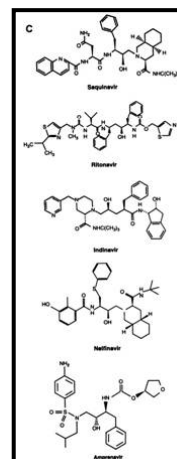
## Farmaci antiretrovirali



Struttura degli inibitori non-nucleosidici della trascrittasi inversa



Struttura degli inibitori nucleosidici della trascrittasi inversa



Struttura degli inibitori della proteasi

## **Meccanismo d'azione *Zidovudine (AZT)***

- Un'analogo della timidina
- Entra nelle cellule mediante diffusione passiva
- Deve essere convertito nella forma trifosfato dalla timidina chinasi cellulare
- Inibisce competitivamente la deossitimidina trifosfato per l'enzima della trascrittasi inversa
- Causa terminazione di catena

## **Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)**

- **Nevirapine**
- **Delavirdine**
- **Efavirenz**

## Meccanismo d'azione degli *NNRTIs*

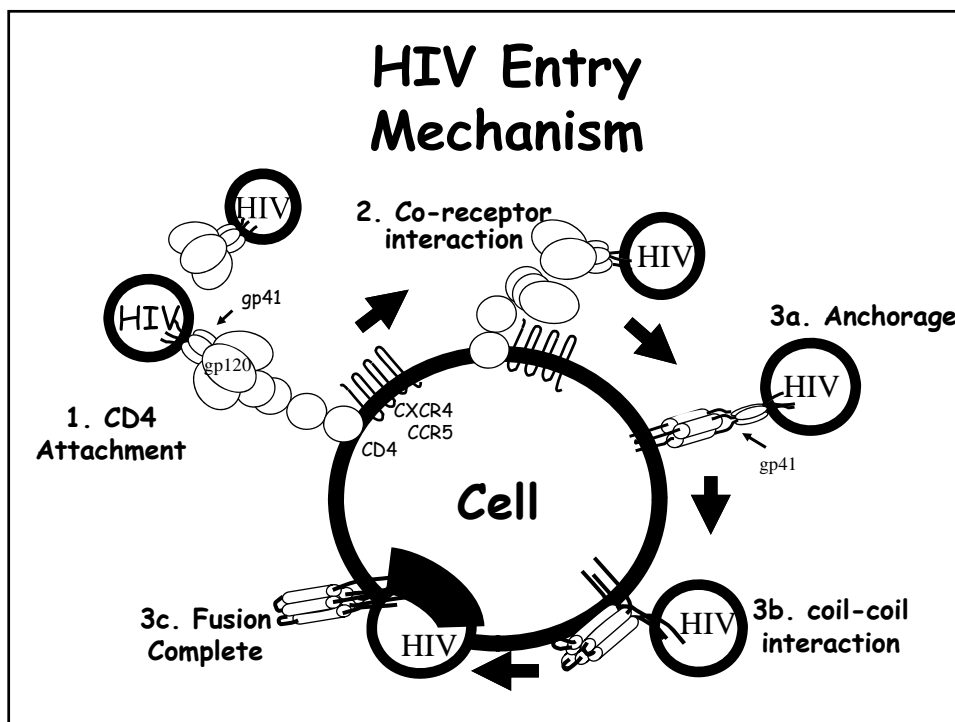
- Si legano a siti della trascrittasi inversa diversi da quelli degli analoghi nucleosidici
- Risulta in un blocco dell'attività della polimerasi
- Non compete con i nucleosidi trifosfati
- Non richiede fosforilazione
- Necessitano di altri farmaci

## Inibitori della Proteasi

- **Indinavir**
- **Ritonavir**
- **Saquinavir**
- **Nelfinavir**
- **Amprenavir**

## Inibitori della Proteasi

- La proteasi è un enzima che taglia le molecole nella loro forma di precursori per produrre virioni maturi infettivi
- Questi agenti inibiscono l'attività della proteasi impedendo la diffusione dell'infezione
- Effetti dannosi: sindrome di alterata distribuzione dei grassi corporeo, resistenza all'insulina, iperlipidemia

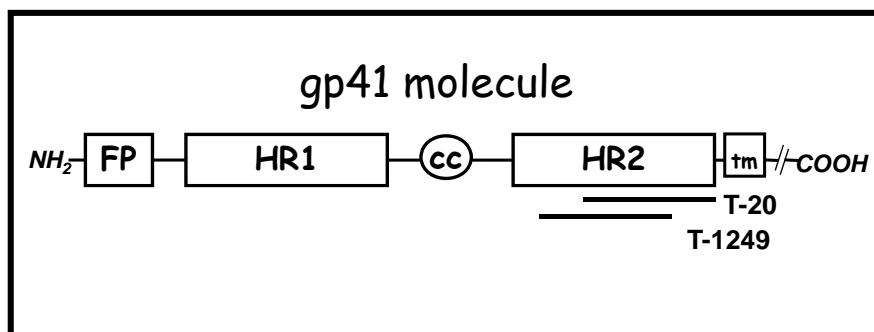


## AMD070- a CXCR4 Inhibitor

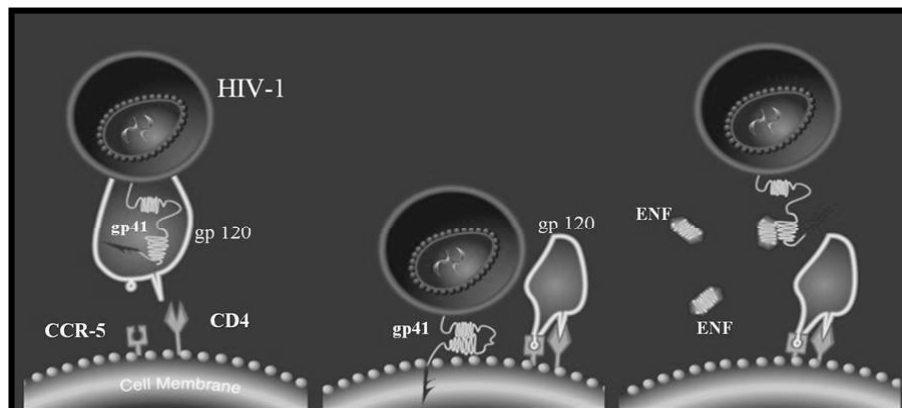


AMD070 impedisce l'adesione dell'HIV e la penetrazione nelle cellule bloccando l'uso del co-recettore CXCR-4 (phase I clinical trials)

## Anti-HIV Fusion Peptides



## Inibizione della Fusione



## Fusion Inhibitors

- **Enfuvirtide** (T-20)- si lega alla sub-unità gp41 dell'glicoproteina di membrana virale, prevenendo i cambiamenti di conformazione necessari per ottenere la fusione tra la membrana virale e quella cellulare
- Bloccando la fusione (penetrazione nelle cellule), FUZEON impedisce al virus HIV di penetrare nelle cellule CD4+

## Fusogenic Domains in Herpes Simplex Virus Type 1 Glycoprotein H\*

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 Published, JBC Papers in Press, June 2, 2005, DOI 10.1074/jbc.M505196200

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 Carlo Pedone<sup>§§§</sup>, and Massimiliano Galdiero<sup>§§§§</sup>

Infection of eukaryotic cells by enveloped viruses requires fusion between the viral envelope and the cellular plasma or endosomal membrane. The actual merging of the two membranes is mediated by viral envelope glycoproteins, which generally contain a highly hydrophobic region termed the fusion peptide. The entry of herpesviruses is mediated by three conserved proteins: glycoproteins B, H (gH), and L. However, how fusion is executed remains unknown. Herpes simplex virus type 1 gH exhibits features typical of viral fusion glycoproteins, and its ectodomain seems to contain a putative internal fusion peptide. Here, we have identified additional internal segments able to interact with membranes and to induce membrane fusion of large unilamellar vesicles. We have applied the hydrophobicity-at-interface scale proposed by Wimley and White (Wimley, W. C., and White, S. H. (1996) *Nat. Struct. Biol.* 3, 842-848) to identify six hydrophobic stretches within gH with a tendency to partition into the membrane interface, and four of them were able to induce membrane fusion. Experiments in which equimolar mixtures of gH peptides were used indicated that different fusogenic regions may act in a synergistic way. The functional and structural characterization of these segments suggests that herpes simplex virus type 1 gH possesses several fusogenic internal peptides that could participate in the actual fusion event.

*Journal of General Virology* (2006), 87

## Analysis of synthetic peptides from heptad-repeat domains of herpes simplex virus type 1 glycoproteins H and B

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 Annarita Falanga,<sup>4</sup> Craig Collins,<sup>5</sup> Katia Raieta,<sup>4</sup> Carlo Pedone,<sup>1,2,3</sup>  
 Helena Browne<sup>5</sup> and Massimiliano Galdiero<sup>2,4</sup>

Human herpesviruses enter cells by fusion of their own membrane with a cellular membrane through the concerted action of multiple viral proteins and cellular receptors. Two conserved viral glycoproteins, gB and gH, are required for herpes simplex virus type 1 (HSV-1)-mediated membrane fusion, but little is known of how these proteins cooperate during entry. Both glycoproteins were shown to contain heptad repeat (HR) sequences predicted to form  $\alpha$ -helical coiled coils, and the inhibitory activity against infection of four sets of synthetic peptides corresponding to HR1 and HR2 of gB and gH was tested. The interactions between these HR peptides were also investigated by circular dichroism, native polyacrylamide-gel electrophoresis and size exclusion high-performance liquid chromatography. gH coiled-coil peptides were more effective than gB coiled-coils peptides in inhibiting virus infectivity. The peptides did not impair fusion when added to cells immediately after infection. In contrast, inhibition of infection was observed, albeit to various extents, when peptides were added to virus before or during inoculation. The results of biophysical analyses were indicative of the existence of an interaction between HR1 and HR2 of gH and suggest that the HRs of gB and gH do not interact with each other.

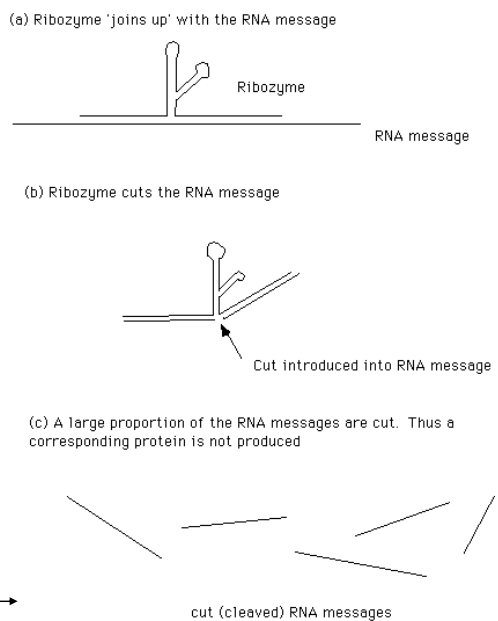
# Epatite C

## Translation Inhibitors

- IFN therapy
- siRNA (interference RNA)
- Ribozymes
  - Enzymes containing only RNA
  - Cut at a specific sequence
  - Heptazyme
    - Hepatitis C virus

Mechanism of Ribozyme action →

Mode of Action of A Ribozyme—the Molecular Scissors



## Anti-Hepatitis Agents

- **Lamivudine** -Nucleoside Reverse Transcriptase Inhibitor (NRTI)
- **Adefovir** -Nucleotide Inhibitor
- **Interferon Alfa**
- **Pegylated Interferon Alfa**
- **Ribavirin**

## *Interferons*

- *Interferon Alfa*
- Proteine endogene
- Inducono enzimi cellulari che inibiscono la traduzione dell'RNA virale e causano la degradazione dell'mRNA e dell'tRNA
- Si legano a recettori di membrana sulla superficie delle cellule
- Possono anche inibire la penetrazione virale, scapsulamento, sintesi dell'mRNA, traduzione, assemblaggio e rilascio

## *Ribavirina*

- Analogo della guanosina
- Viene fosforilato all'interno della cellula da enzimi cellulari
- Inibisce l'RNA polimerasi virale RNA-dipendente
- Inibisce la replicazione dei virus a DNA e RNA

**Table 49-5.** Drugs used to treat viral hepatitis.

Agent	Indication	Recommended Adult Dosage	Route of Administration
<b>Hepatitis B</b>			
[REDACTED]	Chronic hepatitis B	100 mg once daily	Oral
[REDACTED]	Chronic hepatitis B	10 mg once daily	Oral
[REDACTED]	Chronic hepatitis B	5 million units once daily or 10 million units three times weekly	Subcutaneous or intramuscular
<b>Hepatitis C</b>			
[REDACTED]	Acute hepatitis C	5 million units once daily for 3 weeks, then 5 million units three times weekly	Subcutaneous or intramuscular
[REDACTED]	Chronic hepatitis C <sup>2</sup>	3 million units three times weekly	Subcutaneous or intramuscular
[REDACTED]	Chronic hepatitis C <sup>2</sup>	3 million units three times weekly	Subcutaneous or intramuscular
[REDACTED]	Chronic hepatitis C <sup>2</sup>	9 µg three times weekly (consider 15 µg three times weekly if patient relapses or is unresponsive)	Subcutaneous
[REDACTED]	Chronic hepatitis C <sup>2</sup>	180 µg once weekly	Subcutaneous
[REDACTED]	Chronic hepatitis C <sup>2</sup>	40–150 µg once weekly, according to weight	Subcutaneous

<sup>1</sup>Dosage must be reduced in patients with renal insufficiency.

<sup>2</sup>For all agents, combination therapy with oral ribavirin is recommended if tolerated (dosage, 1000–1200 mg/d according to weight).

# Influenza

## Anti-Influenza Agents

- **Amantadine**
- **Rimantadine**
- **Zanamivir**

### ***Amantadine and Rimantadine***

- Ammine cicliche
- Inibiscono lo scapsulamento dell'RNA virale per cui inibiscono la replicazione
- Le resistenze emergono in seguito a mutazioni nella sequenza di RNA che codifica per la proteina strutturale M2
- Vengono usate nella prevenzione e nel trattamento dell'influenza A

### ***Zanamivir and Oseltamivir***

- Inibiscono l'enzima neuraminidasi
- Inibiscono la replicazione dell'influenza A e B

