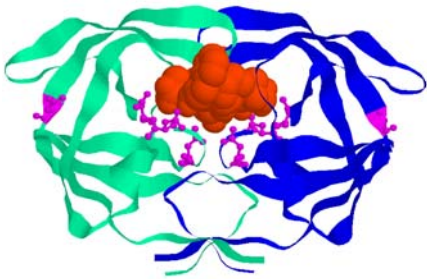


# MEDICAL MICROBIOLOGY PAMB 650/720

## LECTURE: 76

### ANTI-VIRAL CHEMOTHERAPY



Dr Richard Hunt

**OBJECTIVES:** To elucidate the drugs that are currently used as anti-viral agents and to determine why they are effective agents. The mode to action of these drugs will be discussed.

Anti-bacterial drugs such as penicillin antibiotics have proved very successful since they act specifically against a bacterial structure such as the cell wall that is not present in eucaryotic cells. In contrast, most anti-viral agents have proved of little use therapeutically since the virus uses host-cell metabolic reactions and thus, for the most part, anti-viral agents will also be anti-cell agents. Thus, the alternative approach of stimulating the host's immune responses using vaccines has been most often pursued. Nevertheless, there are activities (i.e. enzymes) that are virus-encoded and therefore offer potential *virus-specific* targets. This is particularly the case with the viruses that have large genomes and code for their own replication enzymes. Even so, unfortunately, many apparent anti-virals that are effective *in vitro* are ineffective *in vivo*.

A successful anti-viral will:

- interfere with a virus-specific function (either because the function is unique to the virus or the similar host function is much less susceptible to the drug) or
- interfere with a cellular function so that the virus cannot replicate--hopefully this will only kill virus-infected cells. This could be done by restricting drug activation to virus-infected cells.

An ideal drug will be:

- Water-soluble
- Stable
- Easily taken up by cells

An ideal drug should NOT be:

- Toxic
- Carcinogenic
- Allergenic
- Mutagenic
- Teratogenic

Toxicity may be acceptable if there is no alternative e.g. symptomatic rabies or hemorrhagic fever. Obviously, a good drug must show more toxicity to the virus than the host cell.

Therapeutic index (T.I.):  $\frac{\text{Minimum dose that is toxic to cell}}{\text{Minimum dose that is toxic to virus}}$

Effective drugs have a T.I. of 100-1000.

Just as with anti-bacterials, we must find a virus Achilles' heel. This could be an enzyme that is unique to the virus so that the drug is not toxic to the host cell.

The following is a list of viruses that are known to code for their own enzymes. Among the *other* enzymes are: proteases, mRNA capping enzymes, neuraminidases, ribonucleases, kinases and uncoating enzymes.

Virus	RNA/DNA polymerase	Other
Picorna	+	+
Reo	+	+
Toga	+	+
Orthomyxo	+	+
Paramyxo	+	+
Rhabdo	+	+
Arena	+	?
Corona	+	+
Bunya	+	?
Parvo	-	+
Adeno	+	+
Herpes	+	+
Irido	+	+
Pox	+	+
Hepatitis B	+	+

The very first licensed anti-viral drug was **idoxuridine** (1963), a pyrimidine analog that acts against viral DNA synthesis. It can still be used topically for epithelial herpetic keratitis but has largely been replaced because other drugs are less toxic. It is toxic because it lacks specificity, i.e. the drug inhibits the host DNA polymerase as well as the viral enzyme.

One of the better anti-viral drugs that we have dates from 1983: **Acyclovir (acycloguanosine)** which is a purine analog. It inhibits herpes DNA replication. It is also a nucleoside analog but, in contrast to idoxuridine, is highly specific and does not show very great toxic side effects. For the reason for this, see below.

## POSSIBLE PHASES OF LIFE CYCLE ON WHICH ANTI-VIRAL ATTACK MIGHT BE LAUNCHED

- 1) Attachment to the cell surface, perhaps competition with a specific viral receptor
- 2) Uptake into intracellular vesicles (endosomes)
- 3) Uncoating of virus (loss of protein coat, fusion of lipid membrane with endosome/lysosome). Note: the endosome/lysosome compartment is acidic and inhibition of acidification of this compartment might be a good target
- 4) Transcription of genome to new RNA or DNA (polymerases are the target)
- 5) mRNA transcription
- 6) mRNA processing (poly A, methylation, capping, splicing)
- 7) Translation to protein
- 8) Post-translational modification of proteins (glycosylation, phosphorylation, fatty acylation, proteolysis).  
Some of these are essential for functional, infective viral progeny.
- 9) Assembly of the components into the whole virus

### BINDING TO RECEPTOR OR UPTAKE INTO INTRACELLULAR VESICLES

There, were until recently, no good drugs that stop this for any virus (but see influenza sialidase inhibitor below). We could use a peptide that mimics the receptor such as **soluble CD4 protein**, which would bind HIV gp120 and stop it binding to the receptor on the cell surface but there is a stability problem *in vivo*. The soluble protein is rapidly broken down and cleared from the circulation, i.e. an efficacious concentration is not achieved for a useful period. Attempts have been made to stabilize proteins but little success has been achieved. There have been attempts to couple soluble CD4 to toxins to kill infected cells, again with little success.

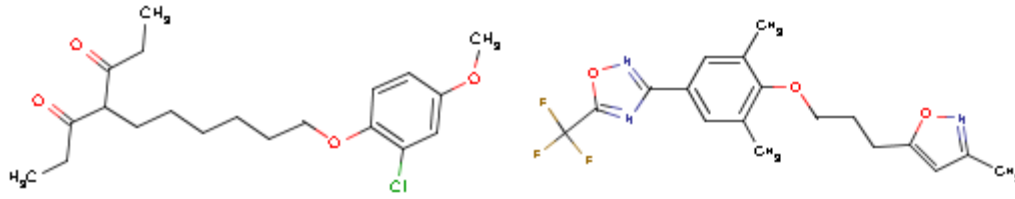
Small molecule HIV co-receptor antagonists have been developed. These are often highly negatively charged molecules. Because of their charge, small molecule antagonists of the co-receptors have very low oral bioavailability but AMD3100 is undergoing clinical trials in which it is administered by injection. It appears to bind to CXCR4 (fusin).

Peptides derived from gp41 can inhibit infection, probably by blocking the interaction of gp41 with cell membrane proteins during fusion or by stopping the conformational change that results from the association of two gp41 molecules and which is necessary for fusion. T-20 (Fuzeon) blocks this conformational change. In clinical trials, a nearly two log reduction in plasma viral levels was achieved. This drug was approved in 2003 but recent reports suggest low bioavailability and the emergence of resistant mutants.

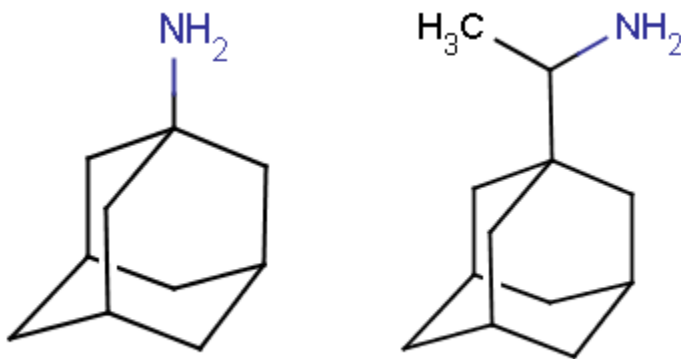
RFI-641 (biphenyl triazine) inhibits fusion of the membrane of respiratory syncytial virus (RSV) with the cell membrane. It seems to alter the conformation of the fusion (F) protein of the virus and is active *in vivo* in several animal models. It is active against RSV A and B strains but has been abandoned because of toxicity concerns. Derivatives are being developed.

### UNCOATING

Uncoating of the virus (i.e. the loss of the lipid envelope in membrane-coated viruses) often occurs in low pH endosome or lysosomes, as the result of a pH-dependent fusogen. Note: Some viruses fuse with the plasma membrane (non-pH dependent); this is the case with herpes viruses and HIV. Other viruses (e.g picorna viruses) do not have a lipid membrane but must lose their nucleocapsid proteins before they can replicate.



**Arildone** (above left) and the **WIN compounds** inhibit uncoating of picornaviruses (which have no lipid membrane). The drug inserts into a canyon in VPI protein of virus. When the virus binds to its receptor, a pore cannot be formed for entry of the RNA into the cell because of the presence of the drug. A derivative of the WIN drugs, **Pleconaril** (above right), acts like a WIN compound in that it fits into a hydrophobic pocket in the nucleocapsid and interrupts the replication of the virus by stopping the shedding of nucleocapsid proteins from the RNA. This orally taken compound is broadly active against a variety of entero- and rhinoviruses (picornaviruses). An intranasal formulation of pleconaril represents an optimized delivery approach, as compared to the earlier oral formulation



**Amantadine** and **Rimantadine** (left)

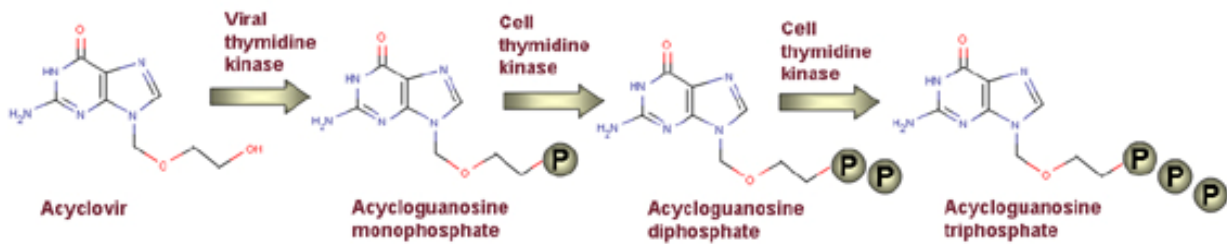
These were originally thought to be lysosomotropic, that is they were thought to stop acidification of the endocytic vesicles and lysosomes. However, they are now known to act on the M2 ion channel which is necessary for the acidification that must occur before uncoating of the virus. These drugs act on maturation of influenza HA glycoprotein so that progeny virions are

poorly infective.

These drugs are good for oral prophylaxis against influenza A (but not influenza B). They are a good alternative to the vaccine in immunocompromised patients and the elderly. Other than this, they are not used much in western countries. Prophylactic rimantadine has been used a lot in countries of the former USSR. Both of these drugs are licensed for use in US. Interest in these drugs has risen because of the possibility of an avian flu pandemic since currently there is no vaccine for this type of influenza virus (H5N1) and it will take several months to develop a vaccine after the pandemic strain is identified.

Because of circulating resistant influenza virus strains, the use of Amantadine and Rimantadine is currently (2008) not recommended.

## NUCLEIC ACID SYNTHESIS:



The best anti-viral drugs that we have are of this type.

They are selective because the virus can:

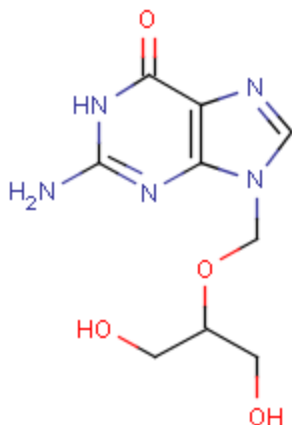
- use its own enzyme to activate the drug and/or
- the viral polymerases are much more sensitive to the drug than the corresponding host enzymes

Selective sensitivity to the best drugs that interfere with nucleic acid synthesis results from the virus activating the inactive drug to a toxic form while the uninfected cell does not. This occurs with herpes viruses and acyclovir.

The thymidine kinase of herpes simplex (and other) viruses allows the virus to grow in cells that do not have a high concentration of the phosphorylated nucleic acid precursors (e.g. nerve cells that are not replicating). Resting cells do have unphosphorylated nucleosides. By bringing in its own kinase the virus can grow in non-dividing cells.

The name of the enzyme is a bit of a misnomer since it can work on other nucleosides than thymidine (thymidine happens to be the best substrate) i.e. the enzyme is non-specific as to substrate. This is in contrast to the host cell thymidine kinase which is very specific to thymidine (since there are other enzymes to phosphorylate the other nucleosides). This lack of specificity of the viral enzyme allows it also to work on nucleoside-analog drugs and phosphorylate them. The host enzyme, because of its greater specificity, is much less good at this (and often does not phosphorylate the drug at all).

The fact that the viral enzyme is good at phosphorylating the drug has another advantage. We can administer the nucleoside-analog in a non-phosphorylated form. This is useful as it is difficult to get phosphorylated drug into the cell because the plasma membrane is poorly permeable to phosphorylated compounds in the absence of a specific transport protein.



Thus, the need for activation restricts use of drug to viruses with their own activating enzyme or that cause the infected cell to overproduce the endogenous enzyme.

Most nucleic acid synthesis inhibiting drugs are **nucleoside analogs** with altered sugar, base or both.

**Acyclovir (acycloguanosine)** (left) is the best example of such a drug. It is phosphorylated specifically by herpes simplex virus thymidine kinase to an active form and then it blocks DNA synthesis by inhibiting the polymerase competitively (binds to the active site of the enzyme) and terminating DNA

chain elongation.

## DNA POLYMERASE INHIBITORS

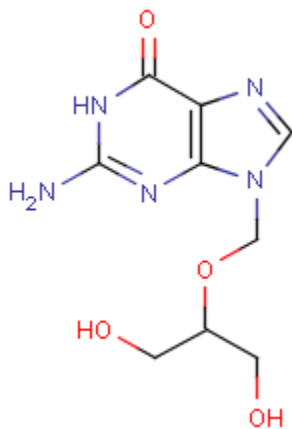
### (1) Sugar modifications

#### ACYCLOVIR / ACYCLOGUANOSINE (Zovirax).

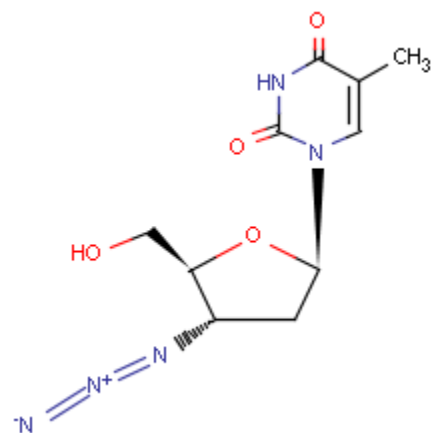
As noted above, this drug is very selective and one of the better anti-virals. It is non-toxic to uninfected cells (except some renal dysfunction) because it is not activated by uninfected cells. It is a poor substrate for the endogenous cell kinase. Moreover, the viral polymerase of HSV is 10 times more sensitive than cellular DNA polymerase. Acyclovir is a competitive inhibitor - it competes with dGTP. When it gets incorporated into DNA, it acts as a chain terminator. HSV-1, HSV-2 and VZV are susceptible to acyclovir.

This drug also inhibits Epstein-Barr virus and Cytomegalovirus, which do not have their own thymidine kinase. In this case, the specificity results from the viral DNA polymerase being very sensitive to the small amounts of drug that are activated by the host cell enzyme.

Acyclovir is effective against HS keratitis, latent HSV, fever blisters (H. labialis), genital herpes. Acyclovir-resistant mutants are a problem after long term use and have been shown to result from changes in the kinase or polymerase gene.



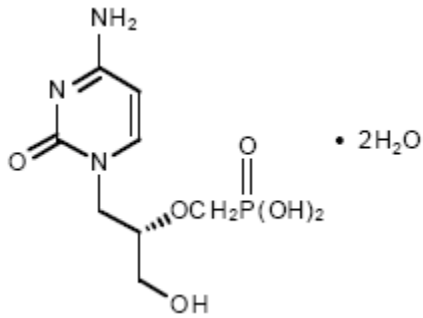
**GANCICLOVIR (Cytovine)** (left). This drug is very similar to Acyclovir; it just has an extra -OH. This makes it active against CMV (which does not encode a thymidine kinase). The reason that it is active against CMV is that it is a good substrate of host cell thymidine kinase. Selectivity is achieved because the viral polymerase has 30 times greater affinity for Ganciclovir than the host enzyme. But it is still toxic and it is difficult to achieve therapeutic dose orally. Ganciclovir is used mostly for CMV retinitis in AIDS patients.



**ADENOSINE ARABINOSIDE. (Ara-A)** This has severe side effects and is only used in potentially lethal disease. It is also easily deaminated in the bloodstream to a less effective form, ara-hypoxanthine.

**AZIDOTHYMININE. (AZT, Retrovir, Zidovudine, left)** This drug is also a chain terminator. It is phosphorylated by a cell kinase so it can be used against viruses without their own kinase. Reverse transcriptase (RNA-dependent DNA polymerase) is more sensitive to the drug than human DNA-dependent DNA polymerase accounting for the specificity but there are severe toxicity effects. It is used as an anti-HIV drug (see HIV lectures). Because of the use of RNA polymerase II in the synthesis of the viral genome and the consequent high rate of mutation of the virus, the selective pressure of the presence of the drug rapidly leads to the emergence of resistant viral mutants. All of these have changes in reverse transcriptase.

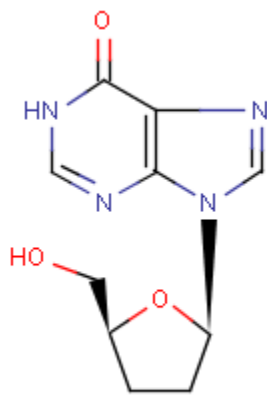
**CIDOFOVIR.** Cidofovir is both a DNA chain terminator and DNA polymerase inhibitor. It is an acyclic nucleoside phosphonate (not a phosphate) in which the C-O-P bond in a nucleoside monophosphate has been replaced by a phosphonate (C-P) bond that provides an enzymatically stable derivative with a long half life. The drug is administered in the phosphonmethoxy-nucleoside form and is phosphorylated twice intracellularly to the active diphosphate form using two cellular kinases (pyrimidine nucleoside monophosphate kinase and pyrimidine nucleoside diphosphate kinase). A viral kinase is not involved. Compare with acyclovir, which is administered as the nucleoside form and the first phosphate, is added by viral thymidine kinase.



Cidofovir is particularly useful in the treatment of cytomegalovirus and is indicated for the treatment of CMV retinitis in patients with AIDS. It may be useful for treatment of acyclovir-resistant herpes infections. It is also active against pox viruses, including the *molluscum contagiosum virus*, BK virus, which is a polyoma virus, and adenoviruses. It is promising for the treatment of immunocompromised patients for gastroenteritis caused by adenovirus, although no control studies have been carried out, and has been used as an adjunctive treatment in addition to HAART in

the treatment of AIDS patients with progressive multifocal leukoencephalopathy (PML). The latter is caused by JC, another human polyoma virus.

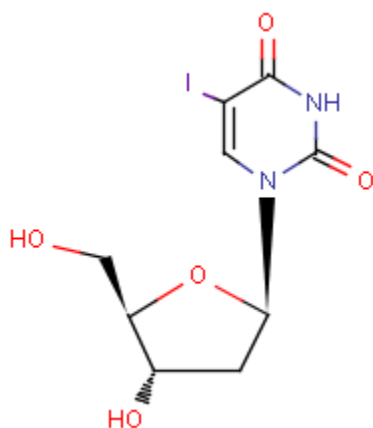
**Other sugar modifications:**



**DIDEOXYINOSINE (DDI, Didanosine, left).** This is licensed for use with HIV in AZT-resistant patients and in combination drug treatments along with AZT.

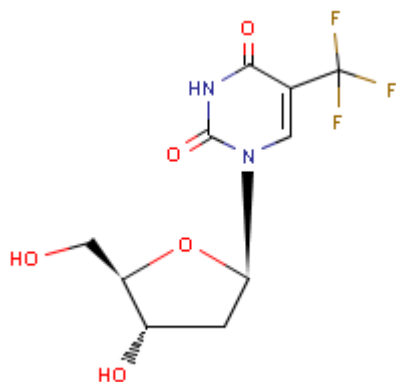
**DIDEOXYCYTOSINE (DDC).** This is also licensed for use with AZT in HIV patients. Both of these drugs exhibit the same problems as AZT: pronounced toxicity and the rapid emergence of resistant HIV mutant strains.

**(2) BASE MODIFICATIONS.**



**BROMOVINYL DEOXYURIDINE (BVDU)**  
**IDO-DEOXYURIDINE (IDU - shown at left).**

These are pyrimidine analogs that are incorporated into DNA. They form unstable base pairs and mutant proteins result.



**TRIFLUOROTHYMININE** (shown at left). This is similar to BVDU. It also is activated by a cellular enzyme

#### PRODRUG

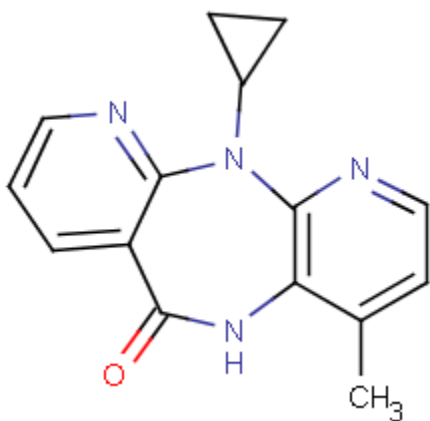
Famciclovir is converted to Penciclovir by the host and then phosphorylated by HSV thymidine kinase and host kinases. Penciclovir itself is too insoluble to be used other than in a topical cream.

### (3) NON-NUCLEOSIDE INHIBITORS OF REVERSE TRANSCRIPTASE.

Because of the problems with AZT and the other nucleoside analogs in the treatment of HIV, interest grew in another approach to inhibiting the same enzyme, reverse transcriptase. Alternative drugs might be useful in combination therapy since there is a limit to the number of mutations that reverse transcriptase can bear without losing function. Clearly, mutations resistant to non-nucleoside non-competitive inhibitors of reverse transcriptase would be at a different site in the enzyme from the mutation that makes the enzyme resistant to a competitive nucleoside analog.

Non-nucleoside inhibitors are the most potent and selective RT inhibitors that we have, working at nanomolar concentrations. They have minimal toxicity in tests with cultured cells (anti-viral activity at 10,000 to 100,000-fold lower concentration than cytotoxic concentration) and have been shown to work synergistically with nucleoside analogs such as AZT. Moreover, they work against nucleoside-analog resistant HIV. Thus, these drugs have high therapeutic index and also show good bioavailability so that anti-viral concentrations are readily achievable. These drugs are non-competitive RT inhibitors.

Not surprisingly, since these drugs target reverse transcriptase, resistant mutants rapidly emerge, even after only a few passages in cultured cells. In clinical trials, resistant mutants also arose rapidly. They are usually therefore of little use in monotherapy; however, although resistant virus strains are often cross-resistant to other non-nucleoside RT inhibitors, they are not to nucleoside analog inhibitors. There is also some evidence that the drugs may be able to overcome resistance at the high concentrations that seem to be achievable.

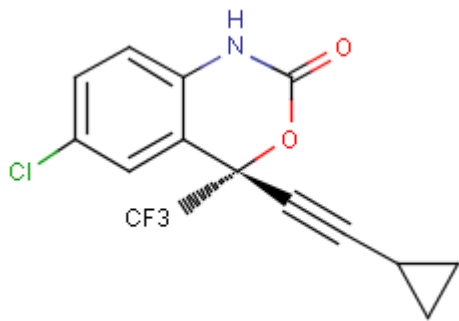


**NEVIRAPINE (NVP OR BIRG-587)** (left). In monotherapy, this drug gives an initial fall in HIV but resistance sets in and virus titers rise again to a high level. It is approved as anti-HIV drug in combination therapy. This drug has found particular use in preventing mother to child transmission. It has a long half life, is lipophilic and present in breast milk. In the standard

treatment, the mother receives the drug at the time of labor and the infant at 72 hours. This can cut transmission by 50%. Coupled with pre-labor therapies, transmission can be almost prevented.

#### **BIS (HETEROARYL) PIPERAZINE COMPOUNDS** (e.g. *atavirdine* and *delavirdine* (DLV)).

Considerable increases are observed in CD4<sup>+</sup> cells in combination therapy (with AZT and 3TC). In combination with AZT and 3TC, DLV may delay emergence of resistance to AZT. The drug is absorbed rapidly.



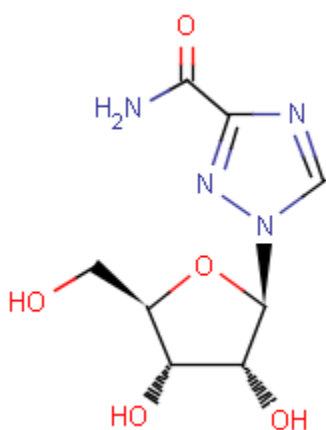
**EFAVIRENZ** (*Sustiva*) (left). *Sustiva* (formerly known as DMP-266) is used in combination with other drugs. It can suppress viral load at least as well as the protease inhibitors in the equivalent combination with nucleoside reverse transcriptase inhibitors. The most noteworthy result was a comparison of viral load reduction with Efavirenz plus AZT plus 3TC, vs. a standard-of-care control group treated with Indinavir plus AZT plus 3TC. The Efavirenz combination suppressed viral load to below 400 copies in a significantly higher proportion of the volunteers than the control arm, at all time points between week 2 and week 24.

#### **4) OTHER NON-NUCLEOSIDE POLYMERASE INHIBITORS**

##### **FOSCARNET** (PFA, phosphono formic acid).

This is a competitive inhibitor of DNA polymerase - It binds to the pyrophosphate site of the enzyme. Herpes DNA polymerase is inhibited at 10-100x lower concentration than cell DNA polymerases giving *some* selectivity.

#### **RNA SYNTHESIS INHIBITORS**



##### **RIBAVIRIN** (left).

This drug is not a pyrimidine or purine. It inhibits influenza RNA polymerase non-competitively *in vitro* but poorly *in vivo*. It may act as guanosine analog and inhibit 5' cap formation on mRNA. The cap normally contains methyl guanosine. It is also incorporated into the RNA of some viruses (e.g. picorna viruses). It appears to cause multiple mutations leading to non-infectious virus particles.

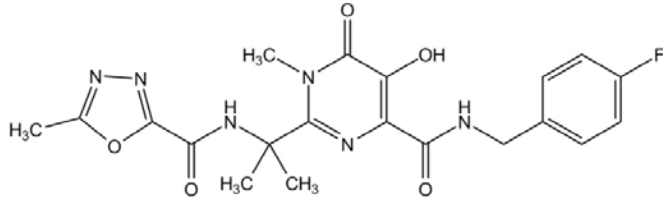
An aerosol form is used against respiratory syncytial virus and the drug is used intra-venously against Lassa fever. N.B. Ribavirin can antagonize the effect of AZT as was found in some initial combination therapy trials against HIV.

##### **NEOPLANOCIN A** (dihydropropyl adenine).

This also inhibits capping of mRNA.

## DRUGS THAT INHIBIT INTEGRASE ACTIVITY

Integration of HIV DNA into the host cell chromosome is essential for the production of new virus particles and this would be an excellent target for a specific anti-viral agent since there is no homologous enzyme in humans. A new class of anti-retroviral drugs targets the integration enzyme, the integrase.



### ISENTRESS® (Raltegravir, MK-0518)

Isentress was approved for use in adults by the USFDA in October, 2007. It can be used as part of a HAART regimen when the patient is resistant to other drugs such as protease inhibitors. It was comparable to Sustiva (standard of care) in HAART over a period of 24 weeks. More than 80 percent of those who took the drug showed a drop in the blood level of virus to barely detectable levels. It is not approved for HIV-infected children.

## PROTEIN SYNTHESIS INHIBITORS

No *specific* inhibitors are available at this time

## PROTEIN PROCESSING INHIBITORS

### A) PROTEASE INHIBITORS

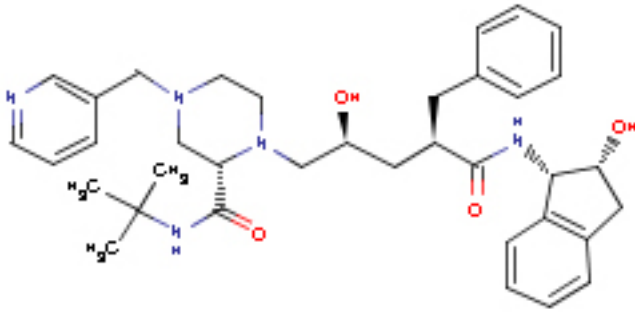
Many viruses must cleave the proteins that they make. In the case of surface glycoproteins, this is usually carried out by a host protease in the secretory pathway (e.g. in Golgi Body). In the case of internal proteins, such as the polymerase or the group-specific antigens (GAGs), there is a viral protease that is encoded in the POL gene of retroviruses and by some other viruses.

Active site-directed inhibitors of the HIV aspartyl protease have been developed since this enzyme is not similar to known host proteolytic enzymes. The action of the HIV protease is crucial to viral infectivity. Spectacular results have been achieved in terms of lowering of viral load with these drugs. They are one of our better hopes for an anti-HIV chemotherapy regimen that could make AIDS a tractable disease. Now we have the promise of a drug regimen that can suppress indefinitely the progress of disease.

Many aspartyl protease inhibitors are being developed and several are approved.

Drug	Brand Name
Saquinavir	Fortonase
Saquinavir mesylate, SQV	Invirase
Ritonavir, ABT-538	Norvir

Lopinavir and Ritonavir	Kaletra
Indinavir, IDV, MK-639	Crixivan
Nelfinavir mesylate, NFV	Viracept
Amprenavir	Agenerase
Fosamprenavir Calcium	Lexiva
Tipranavir	Aptivus
Atazanavir sulfate	Reyataz
Darunavir	Prezista
Atazanavir	Reyataz



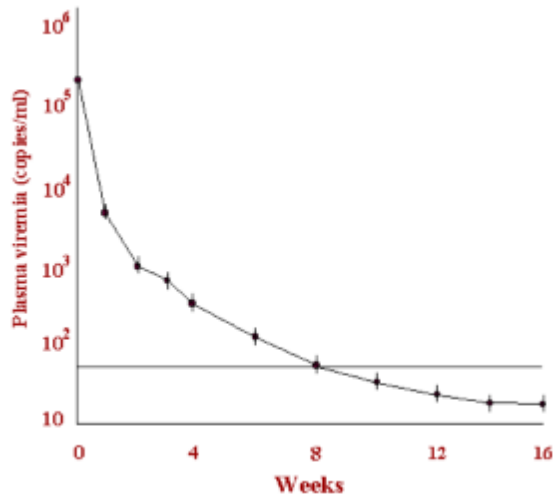
Indinavir is shown at the left. The protease inhibitors are substrate analogs. When used individually, they can drive down viral load to between one 30<sup>th</sup> and one 100<sup>th</sup> of the initial value but sub-optimal doses of these inhibitors, when used alone, can result in loss of suppression after several months and an accumulation of multiple mutations in the protease gene giving a high level of drug resistance. Note: patients with sustained

suppression do not develop the resistant mutations. This is because replication must be maintained for the development of such mutations under the selective pressure of the drug. This is very encouraging.

## HIGHLY ACTIVE ANTI-RETROVIRAL THERAPIES (HAART)

A very effective combination therapy consists of zidovudine (AZT), lamivudine (3TC) and protease inhibitor (such as Indinavir). Viral RNA levels before treatment start as high as 11 million copies per ml. and are reduced to undetectable levels in few weeks (we can measure as low as 20 copies /ml). The evidence suggests that there is NO replicating virus in these patients. This has been sustained over several years.

Another triple drug combination consists of two nucleoside analog reverse transcriptase inhibitors (Tenofovir, (R)-9-(2-Phosphonylmethoxypropyl)adenine) and Emtricitabine (2',3'-Dideoxy-5-fluoro-3'-thiacytidine) plus the non-nucleoside inhibitors of reverse transcriptase, Efavirenz (Sustiva).



*Level of HIV RNA in serum as measured by PCR after treatment with HAART*

One problem with all of these complicated drug regimens is compliance. Also the combination of drugs must be taken at certain times. For example, failure to take Saquinavir within 2 hours of high fat meal leads to no absorption of drug. On the other hand, Indinavir must be ingested with minimal food intake. There is now an approved combination drug. Atripla (Gilead) consists of Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate and is taken orally on an empty stomach.

Total cost of zidovudine, lamivudine and protease inhibitors: \$12,000 per year although in third world countries the cost has been reduced to \$300. The combination drug, Atripla, also costs about \$12,000 per year.

**Can we cure an HIV infection with drug therapy?** The original euphoria concerning HAART as a curative drug regimen has eroded as it has become clear that there are long-lived infected cells even when plasma virion levels become undetectable. Some years ago, available drugs reduced viral load only to small extent and a double drug combination was thought to be acting well if it led to rise in CD4 cells of 50/cu mm and viral load was down 1.5 logs. Now these are considered to be infinitesimally small changes. As shown above, the combination of three drugs including a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor can reduce the number of HIV particles to below detectable levels. This gave hope that, if HIV were not replicating in infected cells and therefore no more cells became infected, the infection might die out as the chronically-infected CD4+ T cells and macrophages turned over. In the blood, infected cells seemed to have a half life of about two weeks which would mean that all of these cells would disappear within 3 to 4 years provided that no further rounds of virus replication and infection occurred. Provided that the patient adhered strictly to the HAART regimen, this would seem to be achievable with the triple drug therapy. However, it soon became clear that the short half-life cells in the circulation were not the only infected ones in the body and other cells remained infected for much longer. Memory CD4+ T cells were found to be infected at a rate of 0.1 to 1 per million and this population of cells showed an extremely slow rate of decay. The half-life of these cells has been calculated at 43.9 months. This means that current therapy is very unlikely to have a significant effect on this cell population. It seems that it would take from 10 to 60 years to eliminate HIV from the infected patient, even assuming that HAART was effective a prohibiting infection of new cells.

It should be noted that despite the spectacular results achieved using HAART, the drugs are not without side effects. The protease inhibitors, for example, can lead to abnormal redistribution of body fat, called lipodystrophy (40-60% of patients) which may be quite disfiguring. Lipodystrophy results in loss of subcutaneous fat. There is increased abdominal girth, enlargement of the dorsocervical fat pad ("buffalo hump"), enlargement of the breasts and fat accumulation around various organs (visceral fat). Some protease inhibitors also lead to red blood cell destruction (hemolytic anemia) and hemorrhaging.

## B) PROTEIN MODIFICATION INHIBITORS

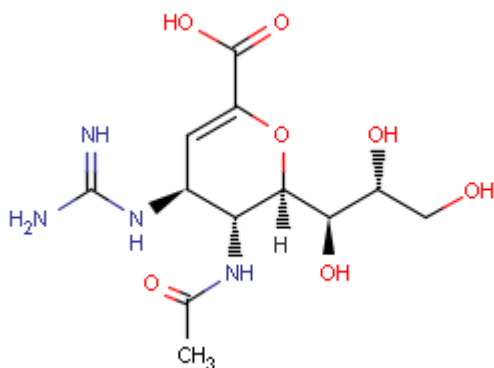
(i) **Glycosylation.** 2-deoxyglucose and D-glucosamine interfere with glycosylation *in vitro* but, not surprisingly, have little effect *in vivo*. Castanospermine (from an Australian chestnut) interferes with glycosylation of HIV and other retroviruses. It leads to a dramatic decrease in syncytia. Interest in this drug as an anti-HIV agent has waned.

(ii) **Phosphorylation.** There are no good drugs in this category

(iii) **Myristoylation.** An anti-HIV drug of this type is being tested. Fatty acylation is necessary for viable virus.

(iv) **Sialidation.** Two glycoproteins are found on the surface of influenza viruses; the hemagglutinin and the neuraminidase (sialidase). The latter has several functions.

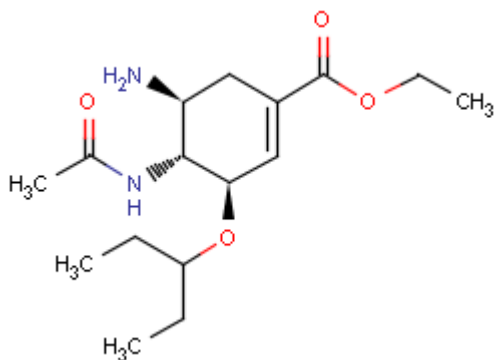
- It allows the virus to move through mucous secretions in the respiratory tract so that it may infect new cells.
- Since sialic acid is the influenza receptor, it is necessary to remove sialic acid from the surface of the infected cell and of the virus so that viral particles may escape.



The neuraminidase is therefore very important for the spread of the virus from cell to cell.

**ZANAMIVIR** (Relenza, left), an antiviral agent against influenza introduced in 1997, is a potent inhibitor of the viral neuraminidase of types A and B influenza viruses. (Note this is important, as the previously available drugs such as rimantadine are ineffective against influenza type B). Treatment of community-acquired type A and B influenza with Zanamivir shortens the duration of major symptoms on average by about one day and about three days in the sicker

patients if the drug was started early.



A more recent neuraminidase inhibitor (Tamiflu - generically called Oseltamivir, left), is also active against influenza A and B and can be given orally.

CDC states:

- Oseltamivir is approved for treatment of persons aged >1 year, and Zanamivir is approved for treatment of persons aged >7 years.
- Oseltamivir and Zanamivir can be used for chemoprophylaxis of influenza; Oseltamivir is licensed for use in persons aged >1 year, and Zanamivir is licensed for use in persons aged >5 years.

### Resistance of neuraminidase inhibitors

Development of viral resistance to Zanamivir and Oseltamivir during treatment has been identified. No transmission of neuraminidase inhibitor-resistant viruses in humans has been documented to date. Data are limited concerning the effectiveness of Zanamivir and Oseltamivir for treatment of influenza among persons at high risk for serious complications of influenza.

Among influenza virus-infected participants in 10 clinical trials, the risk for pneumonia among those participants receiving Oseltamivir was approximately 50% lower than among those persons receiving a placebo.

Chemoprophylactic drugs are not a substitute for vaccination