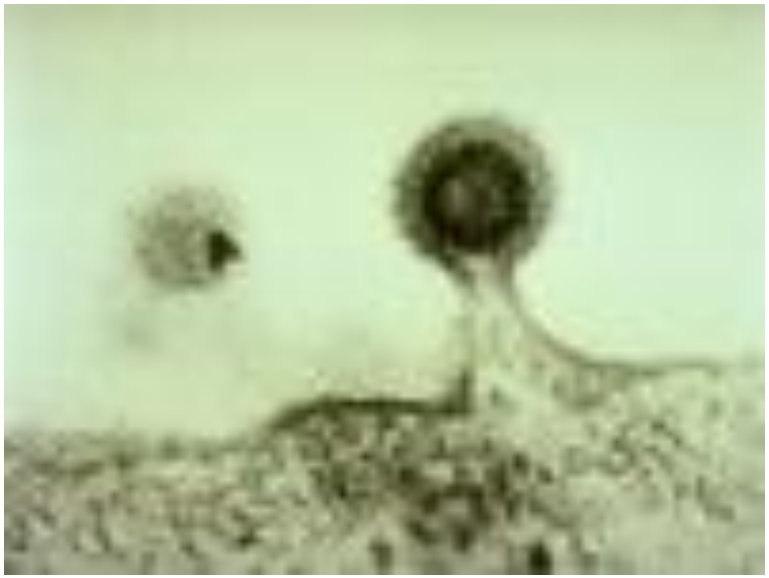
Inhibitors of purine and pyrimidine biosynthesis:

Application to disease



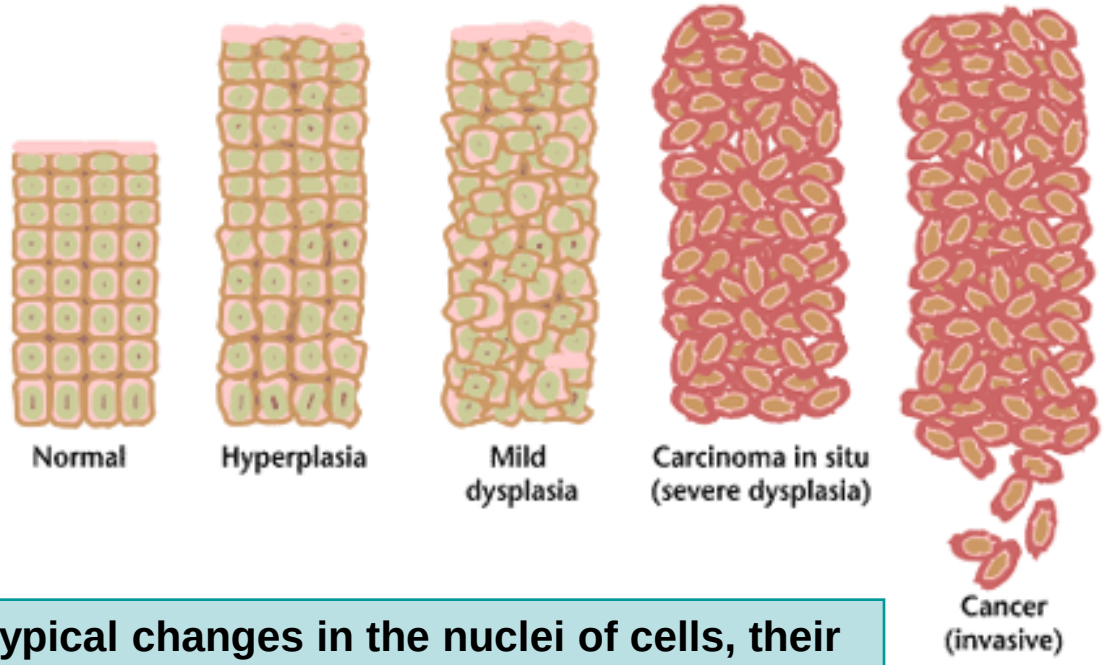
electronmicrograph of an HIV particle budding from a cell.

Cancer

HIV

Cancer

- Cancer cells grow faster than normal cells
- Therefore require more nucleotides for RNA and DNA synthesis
- Cancer cells are therefore also more sensitive to inhibitors of nucleotide synthesis than normal cells



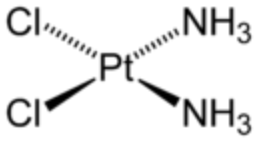
Dysplasia: atypical changes in the nuclei of cells, their cytoplasm or in the growth pattern of cells.

TISSUE-based classification

- **Carcinoma** - malignant tumor **of epithelial origin**, including internal epithelium (bladder lining, pancreatic duct lining, prostate lining etc….)
- **Sarcoma** - cancer that originates **in supportive and connective tissues** such as bones, tendons, cartilage, muscle, and fat
- **Leukemia** - **cancers of the bone marrow (liquid phase)**
- **Lymphoma** - **tumors from the lymphocytes from lymph nodes (solid tumors)**
- **Teratoma** - mixed type of tumor, often it is derived from **embryonic stem cells**

Tumor: an abnormal mass of tissue that results when cells divide more than they should or do not die when they should. May be benign (not cancerous), or malignant (cancerous). Also called neoplasm.

Traditional chemotherapeutic drugs



- Alkylating (-like) agents (e.g. cisplatin)
- Antimetabolites (e.g. Folate, purine or pyrimidine analogs)
- Plant derivates (vinca alcaloids, taxanes, etoposide)

Tumor could become resistant
to any of this compounds or their combinations

Alkylating agents:

Nitrogen mustards, **Nitrosoureas,**
Platinum agents (Cisplatin), **Cyclophosphamides**

The alkylating agents impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules

The electron-rich nitrogen at the **N7 position of guanine** is particularly susceptible to alkylation.

-CH_3 = type of alkyl group

Antimetabolite agents:

Antimetabolites are **structural analogs** of the naturally occurring metabolites involved in DNA and RNA synthesis.

Antimetabolites exert their cytotoxic activity either by **competing with normal metabolites** for the catalytic or regulatory site of a key enzyme or **by direct substituting for a nucleotide** that is normally incorporated into DNA and RNA.

Antimetabolites are **most active** in **S phase** cells and have little effect on cells in G0. Consequently, these drugs are **most effective in rapidly dividing tumors**

Antimetabolites:

Folate analogs -- Methotrexate (MTX)
(bind to catalytic site of dihydrofolate reductase DHFR)

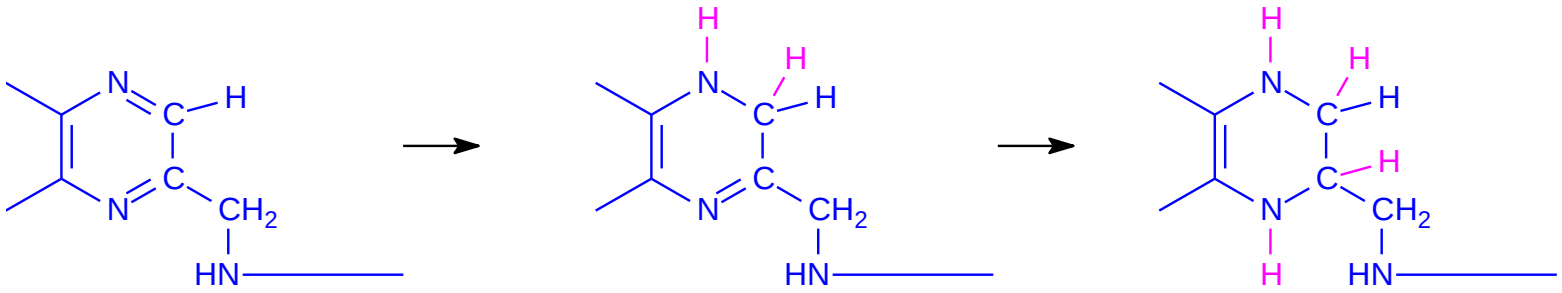
Purine analogs -- Mercaptopurine,Fludarabine
(inhibit enzymes involved in purine metabolism)

Pyrimidine analogs -- Flurouracil (5-FU)
(inhibit enzymes involved in pyrimidine metabolism)

Folate analogues

Higher organisms cannot synthesize THF and must obtain folate in their diet (THF required for purine and pyrimidine synthesis)

Folate undergoes 2 reductions to form the active coenzyme THF.



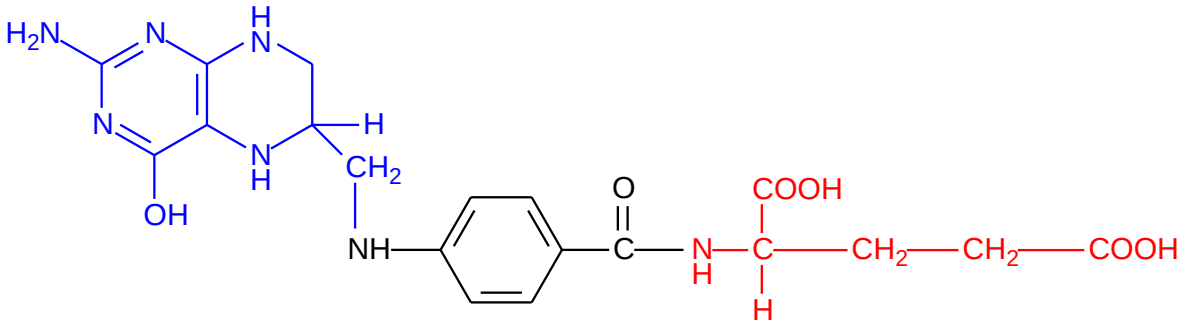
Folate Dihydrofolate Tetrahydrofolate

Both reductions require NADPH.
Both are catalysed by dihydrofolate reductase (DHFR).

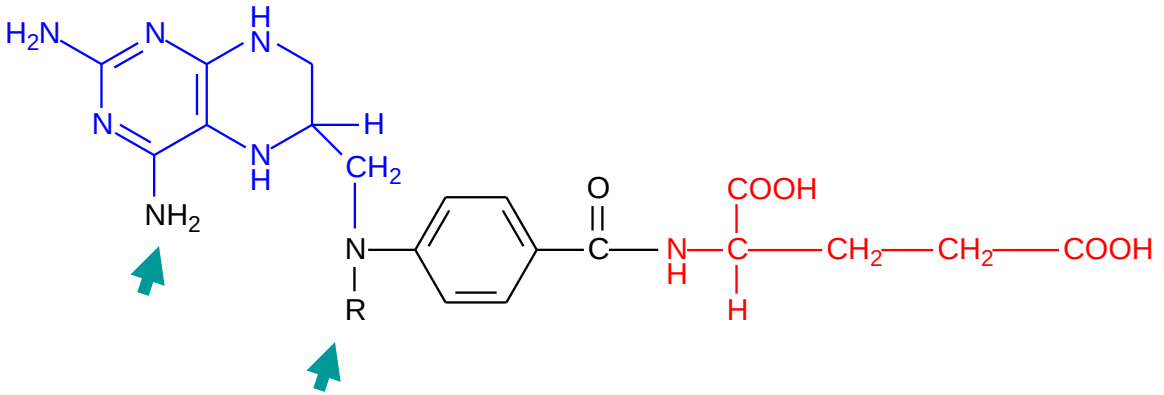
DHFR is therefore a good target for anti-cancer drugs.

If DHFR is inhibited, THF runs out
and all THF-dependent reactions are blocked.

Tetrahydrofolate

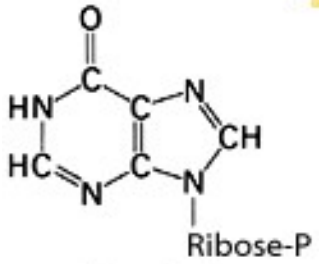


R = H, aminopterin
R = CH_3, methotrexate



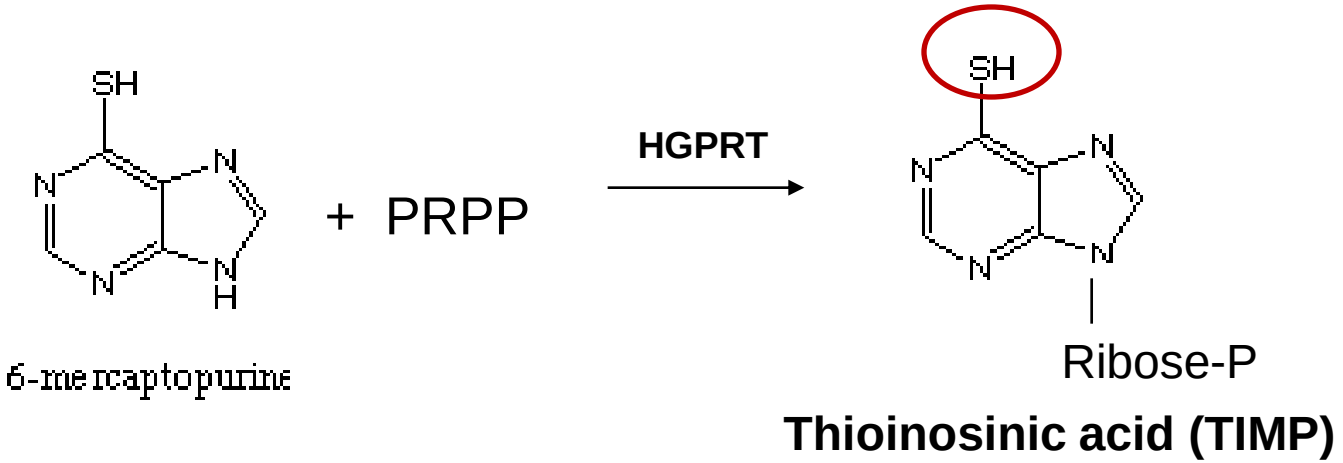
-Aminopterin and methotrexate structurally similar to folate
-Inhibit DHFR (competitive)
-Have 1000 fold higher affinity for DHFR than DHF

Purine analogue



Inosinate (IMP)

- Mercaptopurine
  - Requires conversion by HGPRT to be cytotoxic (competes with hypoxanthine and guanine)



6-mercaptopurine

Thioinosinic acid (TIMP)

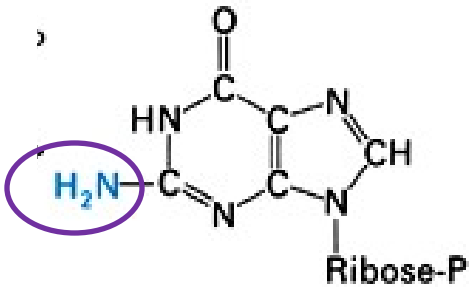
  - Mercaptopurine converted to TIMP
    - inhibits IMP → AMP (via adenylosuccinate) – SH group
    - inhibits IMP → XMP (GMP synthesis) – competes with IMP

- TIMP can be further catalysed via IMP dehydrogenase and then GMP synthetase

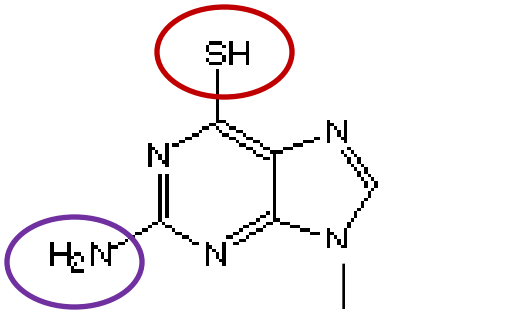
TIMP ⟶ TGMP

Thioinosinic acid — Thioguanylic acid

- TGMP incorporated into nucleic acids, rendering the resulting nucleic acids (DNA, RNA) unable to direct proper protein synthesis.

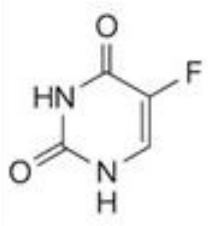


Guanylate (GMP)



TGMP

Pyrimidine analogue



- 5-Fluorodeoxyuridylate (F-dUMP)
- 5-Fluoruracil
  - Suicide inhibitors

5-Fluorocytosine

Cytosine deaminase

Fluorouracil

Fluorodeoxyuridylate (Suicide inhibitor)

Conversion via Thymidine Phosphorylase & kinase

dUMP

Thymidylate synthase

dTMP

N^5,N^{10}-methylene-tetrahydrofolate

Dihydrofolate

Glycine

Serine

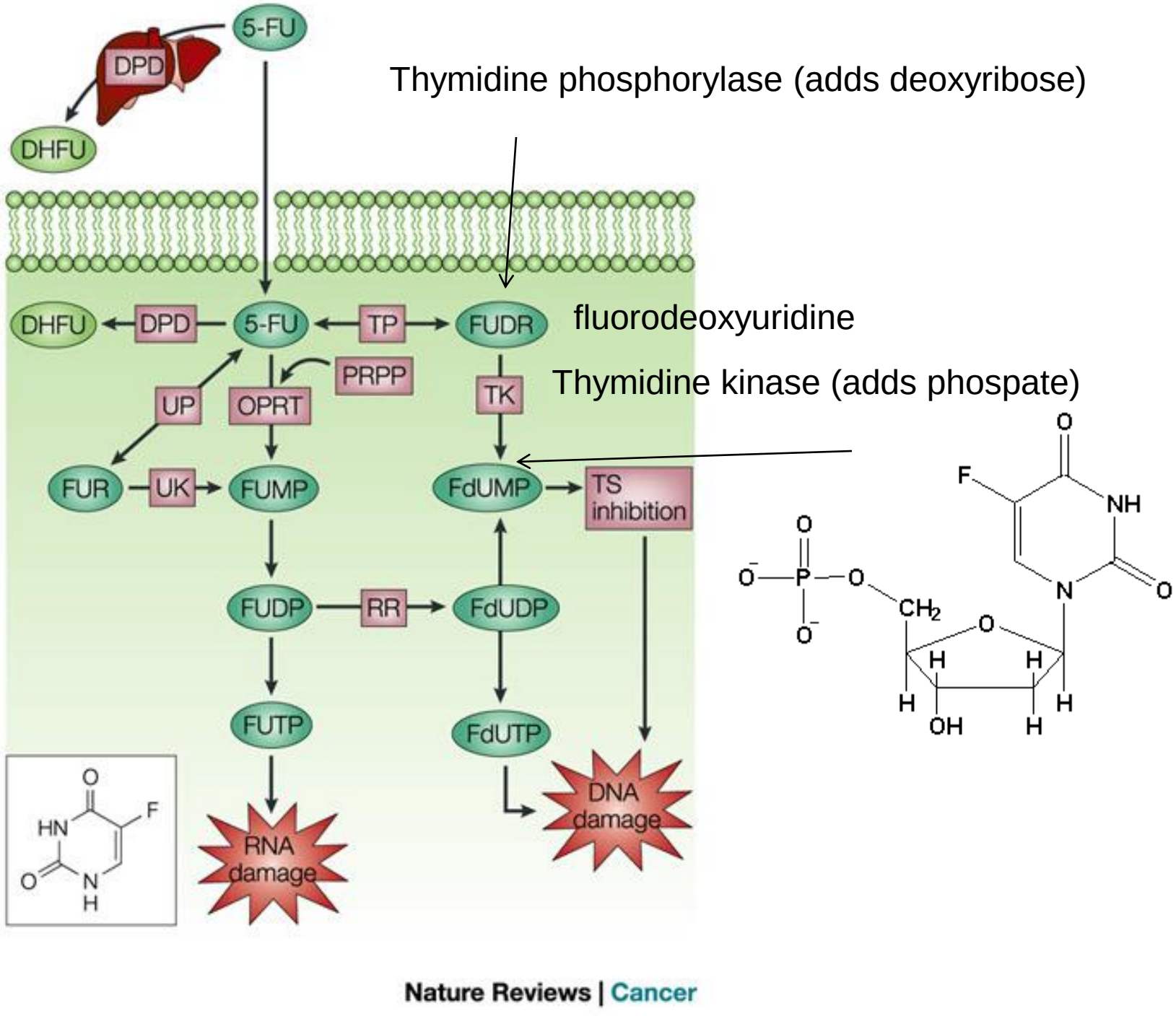
NADPH + H^+

Dihydrofolate reductase

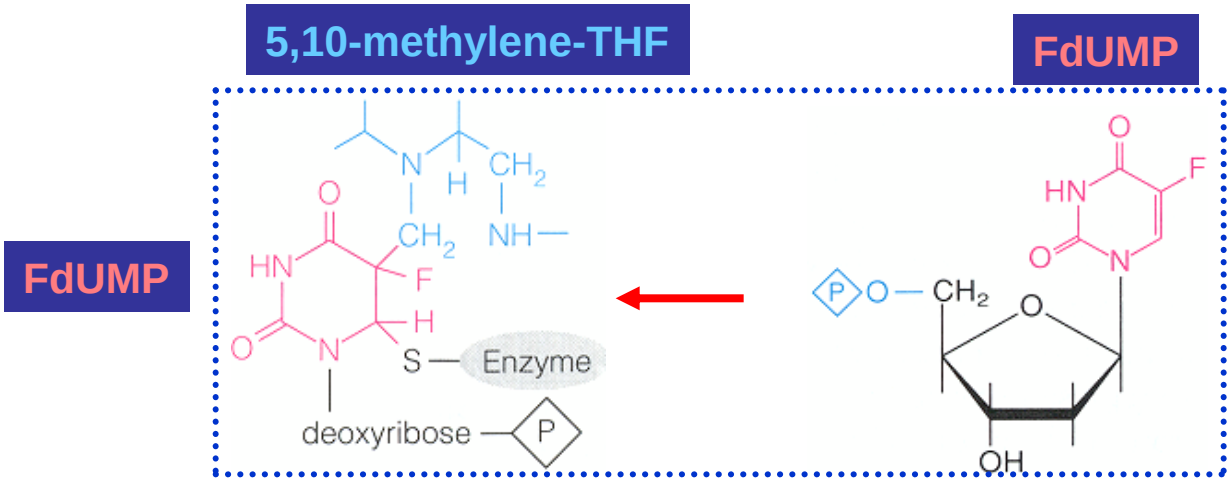
NADP

Tetrahydrofolate

Aminopterin and methotrexate (amethopterin)


5-FU
DPD
DHFU
Thymidine phosphorylase (adds deoxyribose)
DHFU
DPD
5-FU
TP
FUDR
fluorodeoxyuridine
PRPP
UP
OPRT
TK
Thymidine kinase (adds phospate)
FUR
UK
FUMP
FdUMP
TS inhibition
FUDP
RR
FdUDP
FUTP
FdUTP
RNA damage
DNA damage
Nature Reviews | Cancer

FdUMP Inhibits Thymidylate Synthase

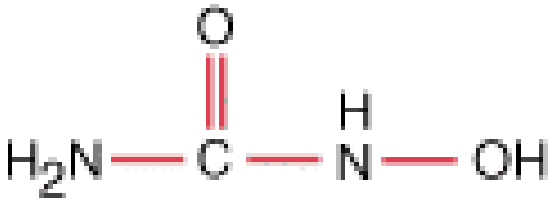


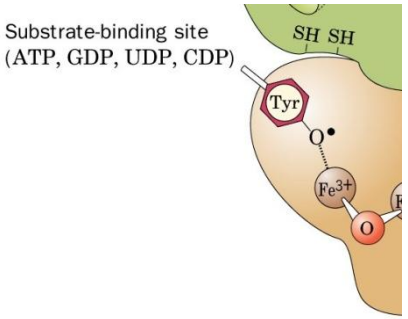
FdUMP irreversibly binds to the enzyme by forming a covalent bond with the methylene group of 5,10-methylene-THF

animation

Other cancer drugs related to nucleotide synthesis

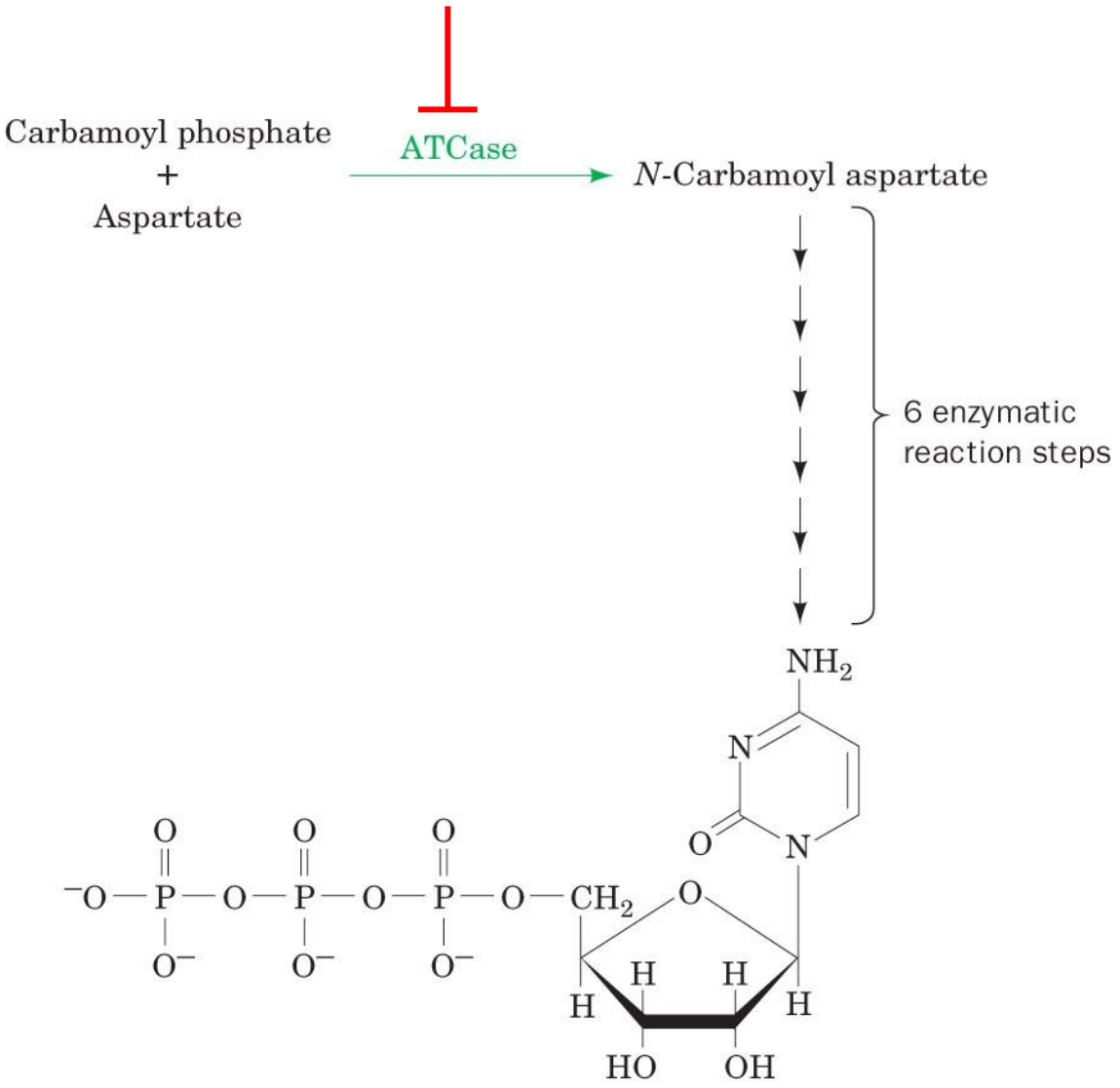
- Hydroxyurea
  - Urea analogue
  - Inhibits ribonucleotide reductase (via inactivation of tyrosine free radical)
  - Tested anti-cancer, but rapidly cleared
  - Inhibits NDP → dNDP





- Aspartate analogue
  - N-Phosphonacetyl-L-aspartate (PALA)
  - Inhibits aspartate transcarbomylase (ATCase)
  - Allosteric – forms stable complex
  - Inhibits pyrimidine synthesis

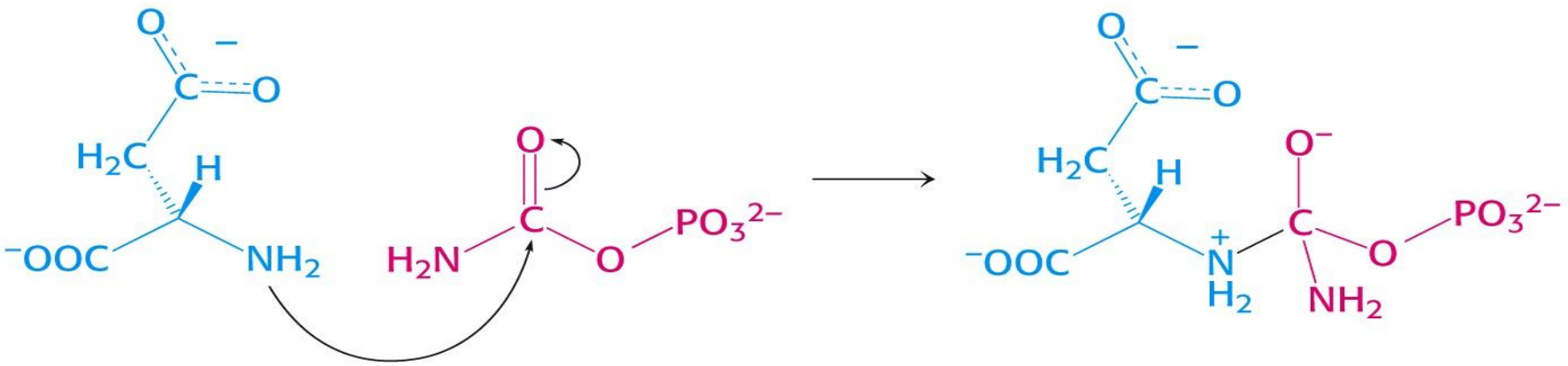
N-Phosphonacetyl-L-aspartate (PALA)

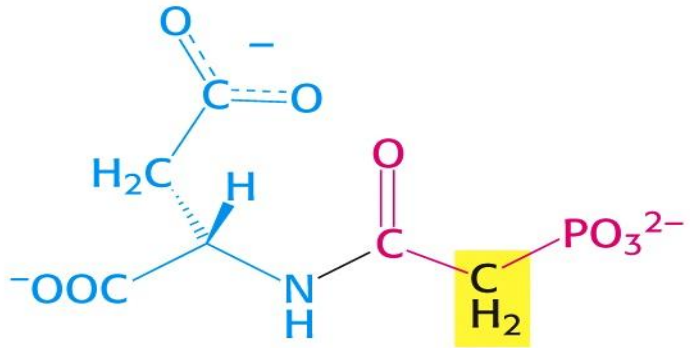


Cytidine triphosphate (CTP)

PALA is a bisubstrate analog that mimics the reaction intermediate on the way to carbamoyl aspartate

Aspartate **Carbamoyl phophate**



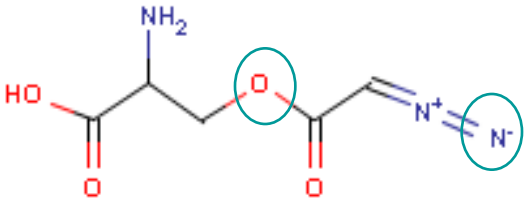


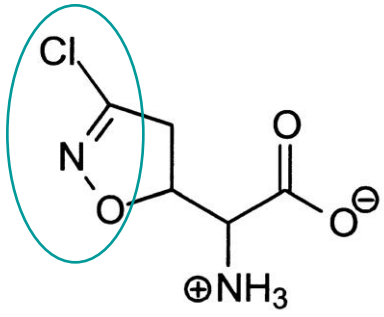
***N*-(Phosphonacetyl)-L-aspartate (PALA)**

Bisubstrate analog

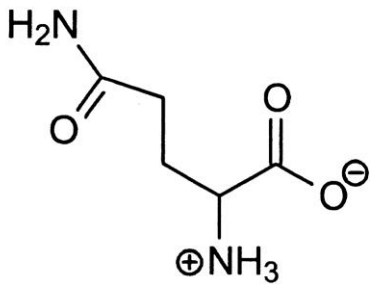
- Azaserine and Acivicin
  - Glutamine analogues
  - Inhibit reactions in purine/pyrimidine synthesis where glutamine donates the NH_2 group
  - Which reactions?

Azaserine





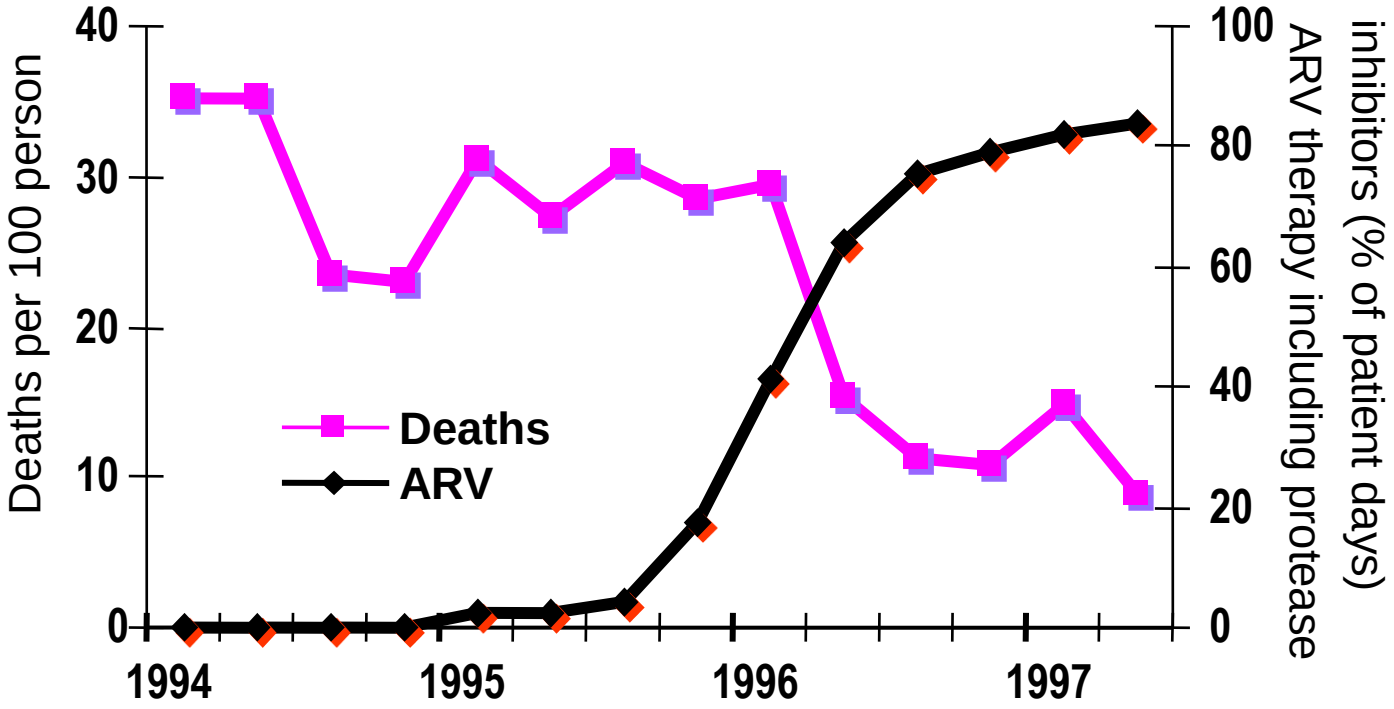
Acivicin



Glutamine

HIV

The importance of anti-viral treatment



Source: Palella *et al., New England Journal of Medicine,* 1998 Mar, **26:**338–60

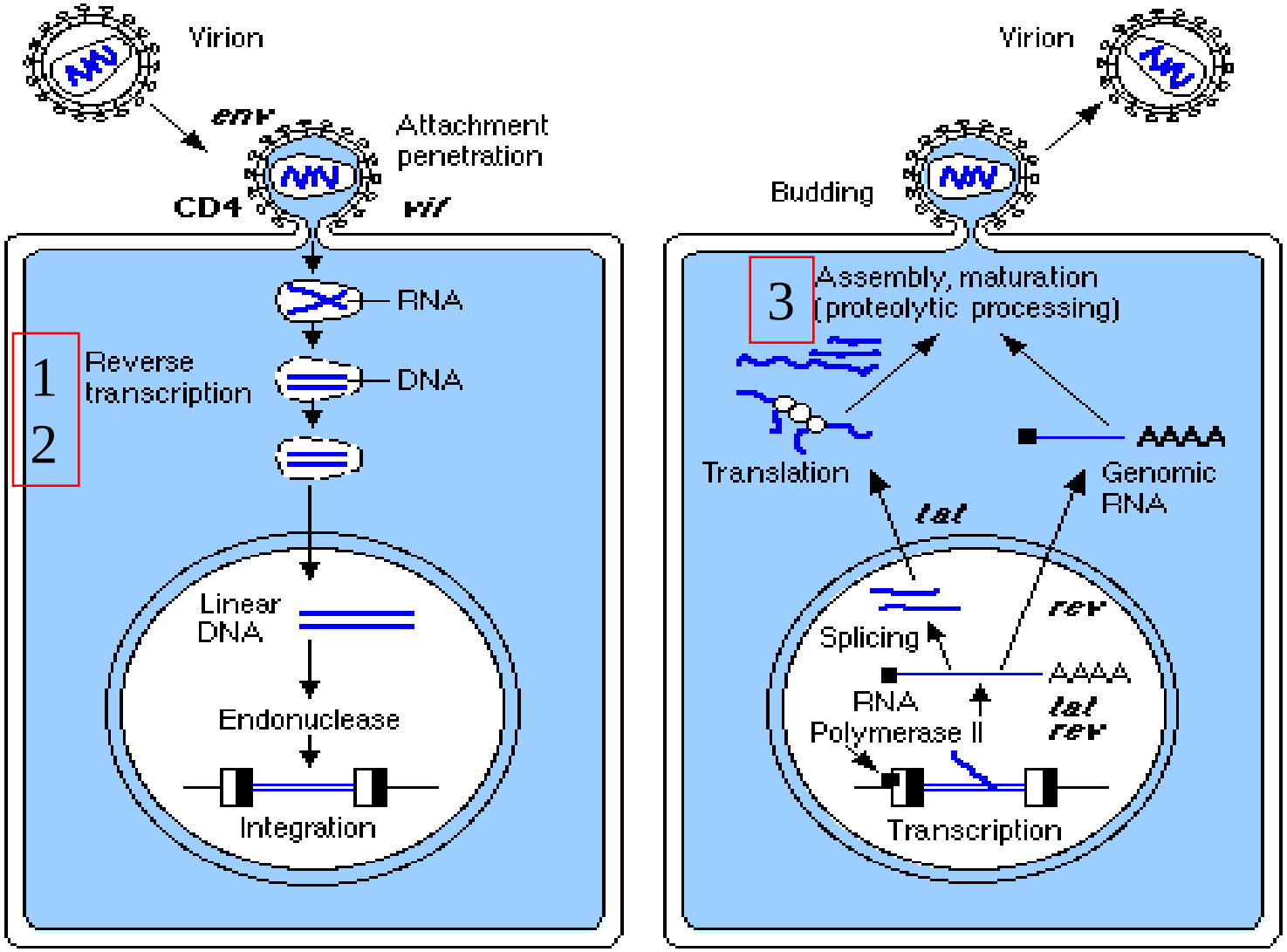
98036-E-35 – 15 July 1998

Mortality in patients with CD4<100; effect of antiretroviral (ARV) therapy including a protease inhibitor among those patients, USA, 1994–1997

Antiretroviral therapy

- **NRTI (nucleoside RTI)**
  - competitive inhibitors of reverse transcriptase by competing with natural pyrimidines or purines and inhibiting chain elongation
- **NNRTI (non-nucleoside RTI)**
  - noncompetitive inhibitors of RT by binding to the enzyme and changing its conformation
- **Protease inhibitor**
  - prevents proteolytic cleavage of final translation product necessary for assembly and release of virus
- **Entry inhibitor**
- **Integrase inhibitor**

Lifecycle of HIV infection



Currently available antiretroviral agents

NRTI 1	**NNRTI** 2	**Protease inhibitors** 3
Abacavir (ABC)	Delavridine (DLV)	Amprenavir
Didanosine (ddI)	Efavrinez (EFV)	Ritonavir
Lamivudine (3TC)	Nevirapine (NVP)	Saquinavir
Stavudine (d4T)		Indinavir
Zidovudine (AZT/ZDV)		Nelfinavir
Zalcitabine (ddC)		

October, 1999

Tenofovir

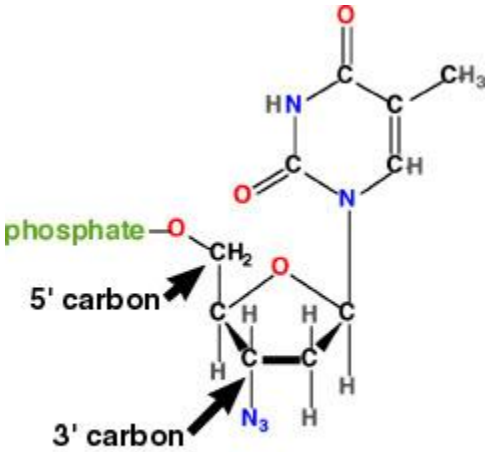
Nucleoside analogs

- Purine or pyrimidine analogs
- Inhibit viral replication by competing with host purine and pyrimidine
- This results in premature termination of viral replication
- **HIV nucleoside reverse transcriptase inhibitors**
  - **AZT**
  - **Didanosine**

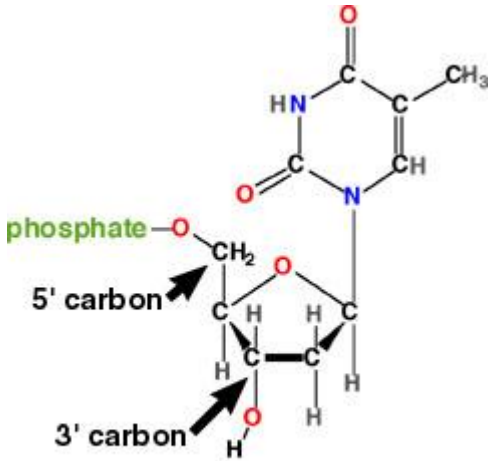
AZT (azidothymidine)

Nucleoside reverse transcriptase inhibitor

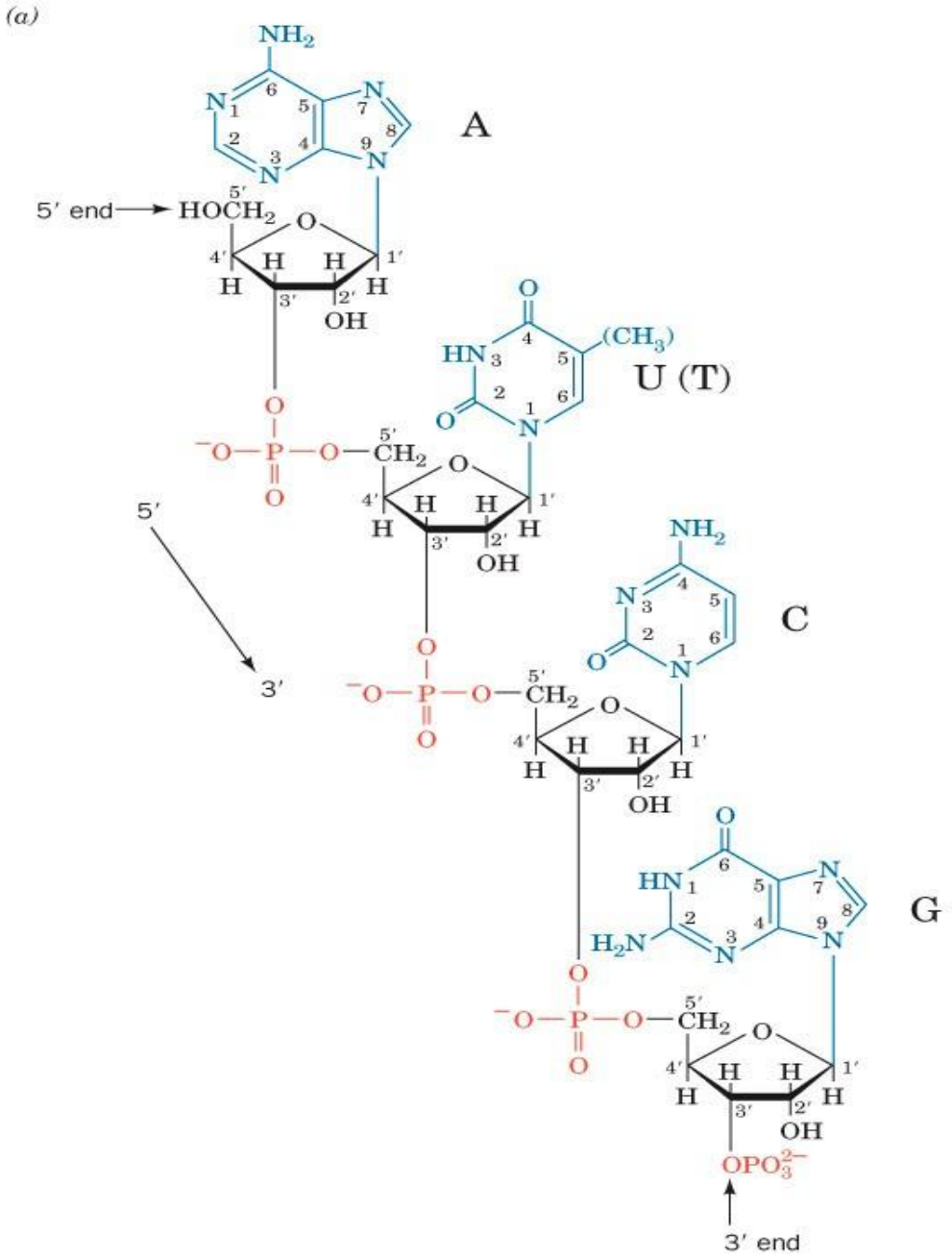
- Analogue of thymidine
- Is one of few drugs that can cross blood-brain barrier (HIV in CNS)
- Converted to AZTMP by thymidine kinase (salvage pathway)
- Then converted to AZTTP by thymidylate kinase and nucleoside diphosphate kinase
- AZTTP inhibits viral reverse transcriptase
  - AZTTP is incorporated into the growing DNA chain
  - But it has no 3'OH group for attaching next nucleotide
  - Therefore chain termination
- 1st anti-HIV drug approved for treatment



Zidovudine (AZT)



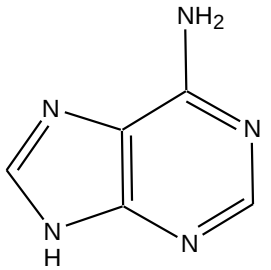
Thymidine



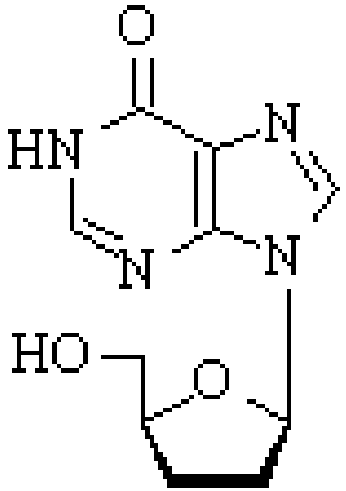
Didanosine

Nucleoside reverse transcriptase inhibitor

- Analogue adenosine
- Competes with natural dAMP
  - Inhibits reverse transcriptase
  - Results in chain termination
  - Used as part of HAART (highly active anti-retroviral therapy)



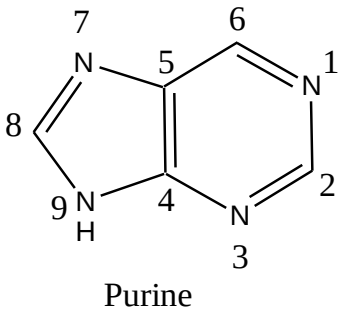
Adenine (A)



Didanosine

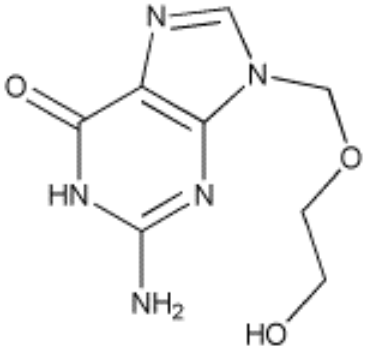
Nucleoside analogs for other viral infections

- Many STD's (sexually transmitted diseases)
- Herpes Simplex, Varicella Zoster, Hep B
  - Acyclovir
  - Valaciclovir
  - Famciclovir



Purine

Acyclovir (ACV)



- Prototype antiviral drug
- Purine analog
- Requires activation by viral thymidine kinase (salvage pathway) to inhibit DNA polymerase and block replication
- Open chain structure instead of ribose

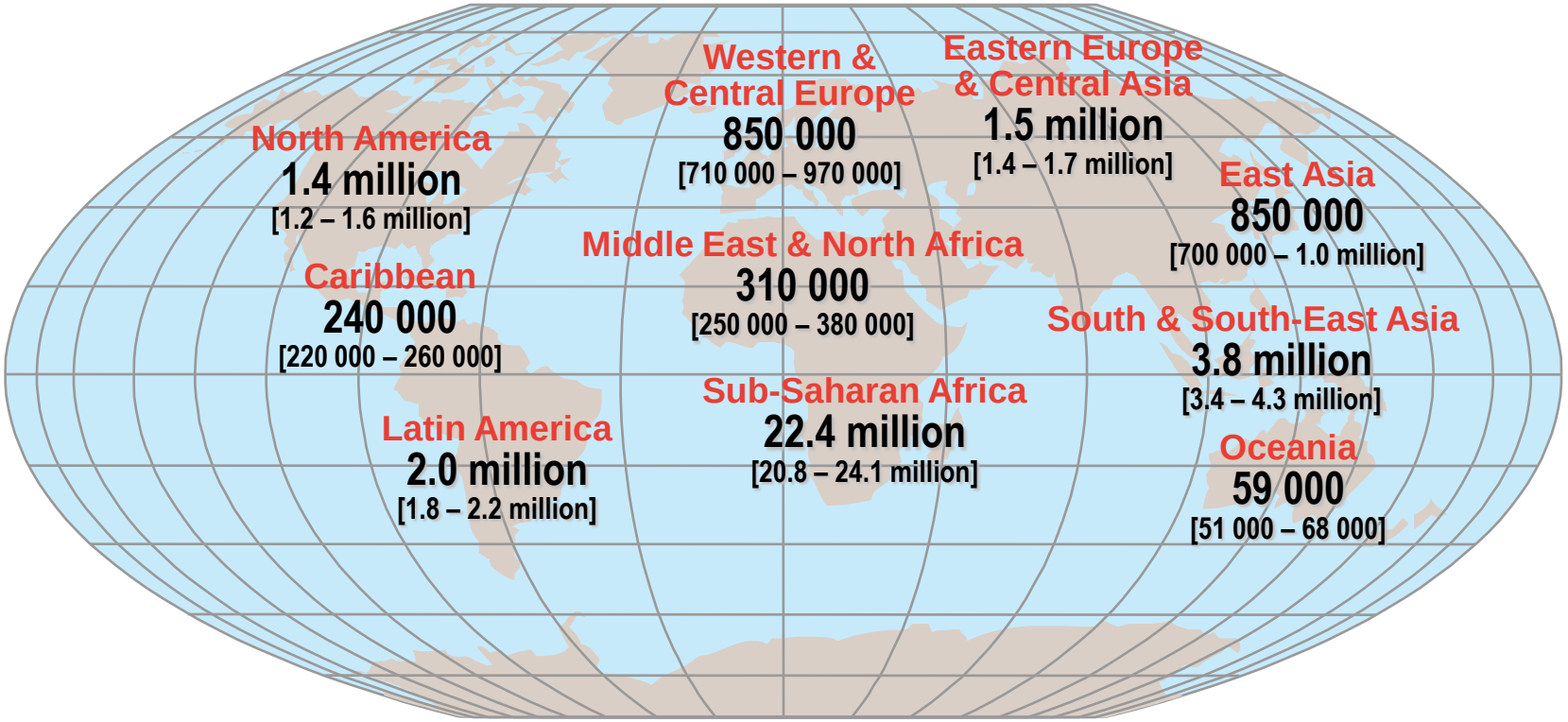
Mechanism of action of ACV

- Acyclovir enters virus-infected cell
  - Virus-specific thymidine kinase converts ACV to ACV-monophospate (ACV-MP)
  - ACV-MP further phosphorylated by cellular enzymes to ACV-triphosphate (ACV-TP)
  - ACV-TP binds viral DNA polymerase acting as a DNA chain terminator
- Viral DNA polymerase has a 10-30-fold greater affinity for ACV-TP than cellular DNA polymerase

Drug resistance

- Drug resistance may occur but is mostly seen in immunocompromised hosts
- Mutations in
  - Viral thymidine kinase (most common)
  - Viral DNA polymerase
- Drug resistant strains tend to be less virulent

Adults and children estimated to be living with HIV, 2008

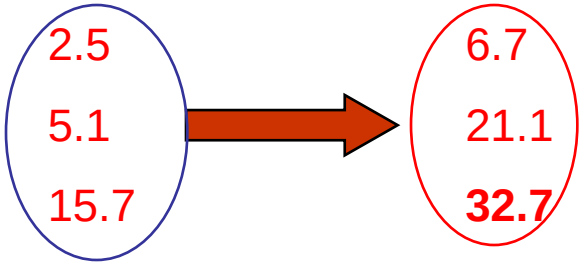


Total: 33.4 million (31.1 – 35.8 million)

December 2009

Estimated HIV prevalence among South Africans, by age and sex: 2008

Age	Male prevalence %	Female prevalence %
2-14	3.0	2.0
15-19	2.5	6.7
20-24	5.1	21.1
25-29	15.7	**32.7**
30-34	**25.8**	29.1
35-39	18.5	24.8
40-44	19.2	16.3
45-49	6.4	14.1
50-54	10.4	10.2
55-59	6.2	7.7
60+	3.5	1.8
Total	7.9	13.6



Women are affected to a greater extent by the AIDS epidemic in SA

- HIV prevention strategies that may be effective
  - Abstinence – 100% reduction
  - Condom usage – 100% reduction

  - Male circumcision – 57% reduction
  - STD treatment (Tanzania) – 42% reduction
  - HIV vaccine (Thailand) – 31% reduction

  - ARV treatment of infected individual reduces risk of uninfected partner

Sciencexpress Research Article

Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women

Quarraisha Abdool Karim,[1,2]*† Salim S. Abdool Karim,[1,2,3]* Janet A. Frohlich,[1] Anneke C. Grobler,[1] Cheryl Baxter,[1] Leila E. Mansoor,[1] Ayesha B.M. Kharsany,[1] Sengeziwe Sibeko,[1] Koleka P. Mlisana,[1] Zaheen Omar,[1] Tanuja N Gengiah,[1] Silvia Maarschalk,[1] Natasha Arulappan,[1] Mukelisiwe Mlotshwa,[1] Lynn Morris,[4] Douglas Taylor,[5] on behalf of the CAPRISA 004 Trial Group‡

[1]Centre for the AIDS Program of Research in South Africa (CAPRISA), Durban, South Africa. [2]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA. [3]University of KwaZulu-Natal, Durban, South Africa. [4]National Institute for Communicable Diseases, Johannesburg, South Africa. [5]FHI, North Carolina, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: caprisa@ukzn.ac.za

‡The members of the CAPRISA 004 Trial Group appear at the end of this paper.

Tenofovir: **Adenosine nucleotide analogue**
Oral ARV
1st ARV to be formulated as a microbicide gel

The CAPRISA 004 trial assessed effectiveness and safety of a 1% vaginal gel formulation of tenofovir, a nucleotide reverse transcriptase inhibitor, for the prevention of HIV acquisition in women. A double-blind, randomized controlled trial was conducted comparing tenofovir gel (n = 445) with placebo gel (n = 444) in sexually active, HIV-uninfected 18 to 40 year-old women in urban and rural KwaZulu-Natal, South Africa HIV serostatus, safety, sexual behavior and gel and condom use were assessed at monthly follow-up visits for 30 months. HIV incidence in the tenofovir gel arm was 5.6 per 100 women-years, i.e. person time of study observation, (38/680.6 women-years) compared to 9.1 per 100 women-years (60/660.7 women-years) in the placebo gel arm (incidence rate ratio = 0.61; P = 0.017). In high adherers (gel adherence > 80%), HIV incidence was 54% lower (P = 0.025) in the tenofovir gel arm. In intermediate adherers (gel adherence 50 to 80%) and low adherers (gel adherence < 50%) the HIV incidence reduction was 38% and 28% respectively. Tenofovir gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence. No increase in the overall adverse event rates

Safety and efficacy

Approx 900 participants
445: tenofovir
444: placebo

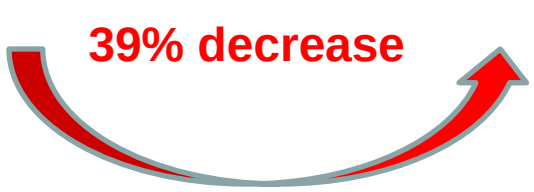
Uninfected women
Urban and Rural

30 month period
Monthly follow-up

Tenofovir gel decreased HIV incidence by between 28% and 54% depending on adherence (i.e. whether the individual used the gel each time)

Average decrease: 39%

	Tenofovir gel N = 445	**Placebo gel** N = 444
No. of HIV infections	**38**	**60**
HIV incidence (%) (per 100 women-years)	**5.6**	**9.1**



Adherence increases the protective effect

-If the gel is used in more than 80% of sex acts, the HIV incidence decreases by 54%

-- If the gel is used in less than half the sex acts, the reduction in HIV is only 28%

	N	HIV incidence		Effect
		Tenofovir	Placebo	
High adherers (>80% gel adherence)	336	4.2	9.3	**54%**
Intermediate adherers (50-80% adherence)	181	6.3	10.0	**38%**
Low adherers (<50% gel adherence)	367	6.2	8.6	**28%**

Summary

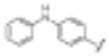
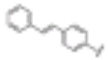
- Safety
  - No major safety concerns
  - No resistance to tenofovir
  - Use of gel was acceptable to participants and partners
- Proof of concept:
  - Tenofovir gel can prevent HIV infection in women when used within a microbicide gel

Nucleotide analogues and stem cells

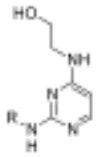
- Cardiogenol:
  - Pyrimidine analogue
  - induces embryonic stem cells to differentiate into heart cells
- Reversine:
  - Purine analogue
  - dedifferentiates (convert into a less specialized form) muscle cells into stem cells

Pyrimidine analogue: Cardiogenol

Table 1. Chemical Structures and Biological Activities of Cardiogenols

	R	EC_{50}	Optimal Activity
Cardiogenol A		1 μM	++
Cardiogenol B		0.5 μM	+++
Cardiogenol C		0.1 μM	++++
Cardiogenol D		0.1 μM	++++

++: 10-25% cells are positive for MHC after 7 days.
+++: 25-40% cells are positive for MHC after 7 days.
++++: 40-55% cells are positive for MHC after 7 days.



CARDIOGENOL

Embryonic stem cell → →→ → → Heart cells

Purine analogue: Reversine