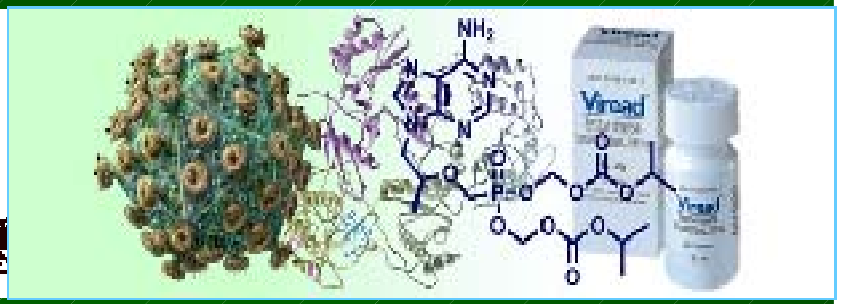
VÝZKUMNÉ CENTRUM ÚOCHB & GILEAD SCIENCES

GSRC

GILEAD SCIENCES & IOCB RESEARCH CENTRE




# Therapeutics from the family of nucleoside and nucleotide analogs:

# A contribution of the Czech science to world medicine

**Marcela Krečmerová**

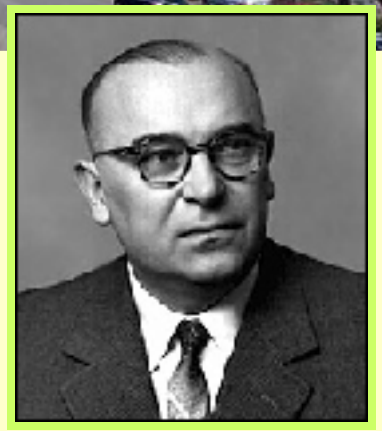
Institute of Organic Chemistry and Biochemistry ASCR, v.v.i.

Gilead Sciences & IOCB Research Centre

**25th SVU Congress, June 27- July 3 2010, Tábor**

**Institute of Organic Chemistry and Biochemistry ASCR, v.v.i.**

- established 1953



The greatest credit for estabilishing and developing the Institute belongs to Professor **František Šorm** (1913-1980).

Scientific interests: Natural compounds – terpenes, biologically active components of plants, antimetabolites of nucleic acid constituents as potential cancerostatics or antivirals.

## Scientific history

**The sixties, 20th century**

- IOCB is one of 4 world institutions successful in **oligonucleotide synthesis** (J. Smrt, S. Chládek)

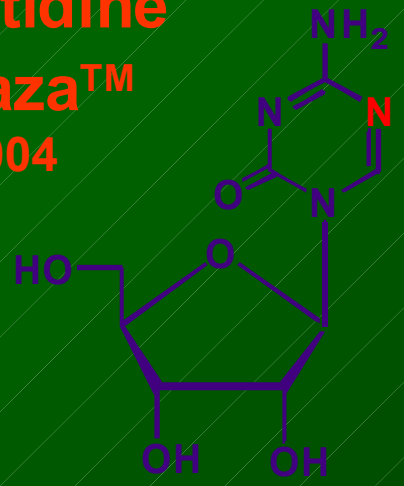
- Synthesis and studies of chemically modified nucleosides and their metabolites - F. Šorm, A. Píškala, J. Žemlička, J.Pitha, J. Veselý, A. Čihák, K.Raška:
  **antileukemic agents 5-azacytidine (1964)**
  **2´-deoxy-5-azacytidine (1964)**

Approved by FDA for the treatment of acute myelodysplastic syndrom - 40 years later (!!!)
2004: 5-azacytidine, Vidaza™
2006: 2´-deoxy-5-azacytidine, Dacogen™

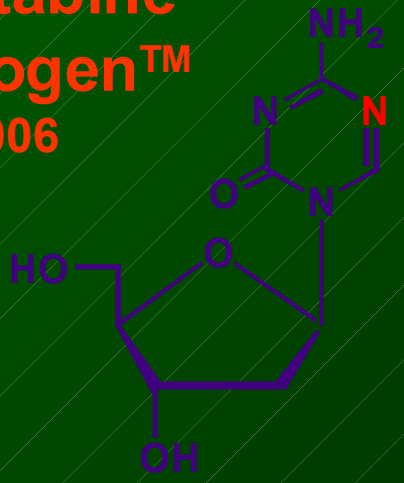
At present – Clinical investigation of 5-azacytidine in the treatment of solid tumors

**RNDr. Alois Píškala, CSc.**

**azacitidine**
**Vidaza™**
**2004**




**decitabine**
**Dacogen™**
**2006**




Produced by SuperGen, U.S.A.

# BIOCHEMICAL PROPERTIES OF 5-AZACYTIDINE – DISCOVERIES MADE IN IOCB:

- The compound has an antiproliferative activity

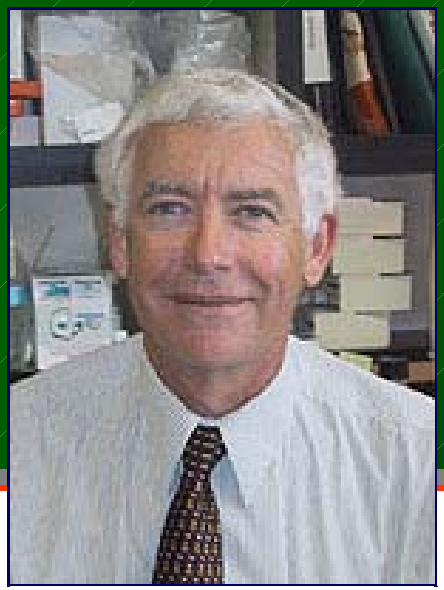
- Phosphorylation in the cell to mono- di- and triphosphate
- 5-Azacytidine triphosphate is an substrate for RNA polymerases.

- Incorporation of 5-azacytidine triphosphate to t-RNA, mRNA a preribosomal RNA

inhibition of synthesis of ribosomes and proteins

*Píškala, Šorm, Veselý, Čihák, Zadražil, Fučík, Pačes (1964-1978)*

# 1980, Peter A. Jones
## USC School of Medicine, L.A., USA:



Synthesis of 5-azacytidine according to an original paper of Alois Píškala (*Collection Czech Chem. Commun.*1964, *29*, 2060-2076) and investigation of its mechanism of action.

## 5-AZACYTIDINE WORKS ON EPIGENETIC PRINCIPLE AS AN INHIBITOR OF DNA METHYLATIONS.

Undesired methylations in DNA = the addition of Me group to a stretch of DNA which can lock or silence genes which are normally responsible for the cell growth control.

This process can be reversible.

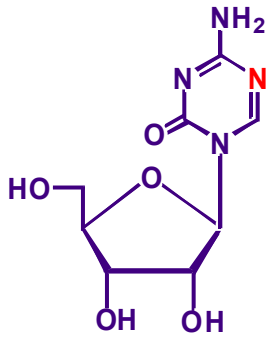
**Possibility of transformation of cancer cells to normal healthy cell by the action of inhibitors of DNA methylations.**

**It is not necessary to kill the cancer cell, it can be repared.**

**Vidaza May Be Safe And Effective In MDS Patients Of All Ages (ASH 2009)**

By Gillian Losh

Published: Jan 22, 2010 5:54 pm

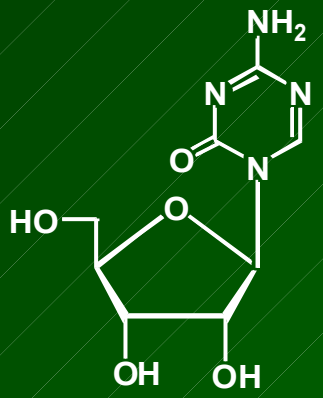



Vidaza (azacytidine) may be as effective and well tolerated in myelodysplastic syndromes (MDS) patients aged 80 years and above as compared to patients less than 80 years old, according to a retrospective analysis by French researchers.

The findings were presented at the 51st Annual Meeting of American Society of Hematology (ASH) meeting in December 2009.

MDS patients aged 80 years and above make up 30 to 35 percent of all MDS patients. These patients are usually not candidates for chemotherapy, even at low doses, because older patients typically do not respond well to the side effects of chemotherapy. Instead, they generally only receive supportive care, which may help improve quality of life but does not treat MDS.

Vidaza®
azacitidine for injection

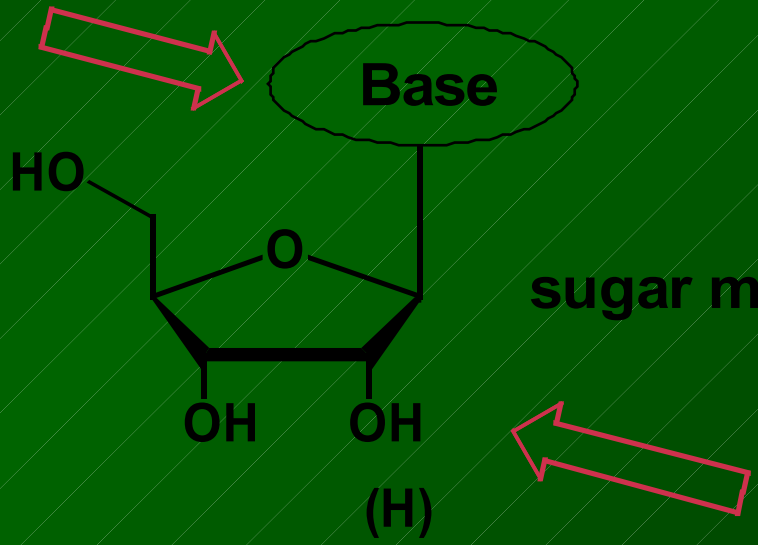



VIDAZA is the first and only agent proven to extend overall survival vs CCR in patients with higher-risk MDS

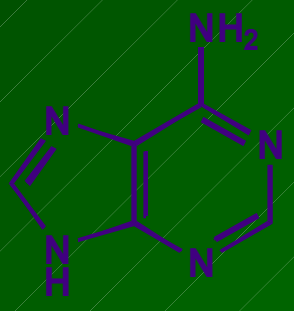
In the largest randomized study ever conducted in patients with higher-risk MDS, VIDAZA significantly extended overall survival vs conventional care regimens.

**MOST OF RATIONALLY DEVELOPED ANTIVIRALS AND MANY CYTOSTATICS ARE MODIFIED NUCLEOSIDES**

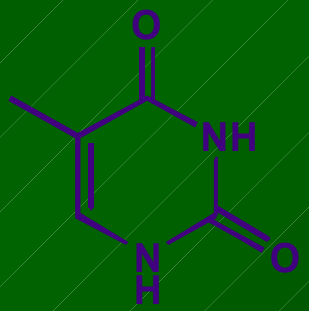
**Possibilities of chemical modifications of nucleosides:**



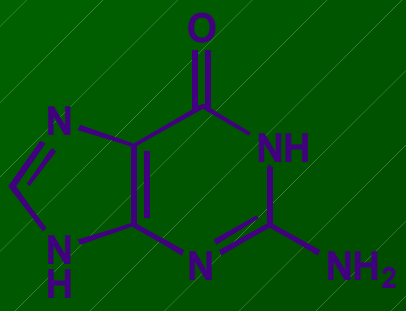

**Base:**



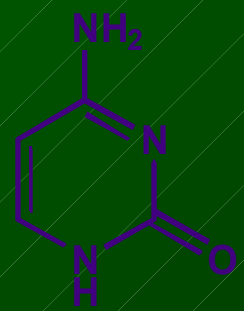

Adenine



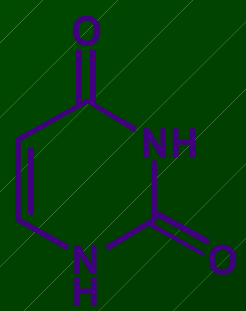

Thymine




Guanine




Cytosine




Uracil

Chemically modified nucleosides, nucleotides and nucleobases can work as **antimetabolites** in the process of nucleic acid metabolism.

**ANTIMETABOLITE** is a CHEMICALLY MODIFIED MOLECULE OF A NATURAL METABOLITE ABLE TO INFLUENCE SOME ENZYME REACTIONS.

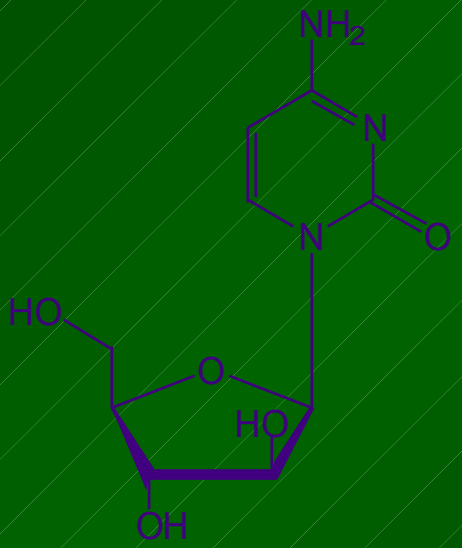
ANTIMETABOLITES CAN INFLUENCE PROCESSES IN CELLS (NEOPLASIA) AS WELL AS IN CELL PARASITES (VIRUSES, PARASITES, FUNGI).

## First generation antimetabolites

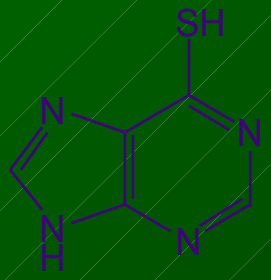
Maximum structural resemblance to natural metabolites

**TREATMENT OF LEUKEMIA**

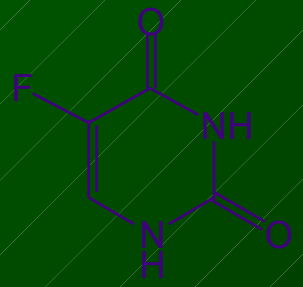
**ANTITUMOR THERAPEUTICS**




Cytosine arabinoside




6-Mercaptopurine




Fluorouracil

## 2nd Generation of antimetabolites

Structural resemblance to natural metabolite is only in some basic aspects

# ACYCLIC NUCLEOSIDE ANALOGS

The seventies of the 20th century

Carbohydrate moiety of the molecule is substituted with an aliphatic chain containing OH groups

# **ANTIVIRALS** made in IOCB

## HISTORY

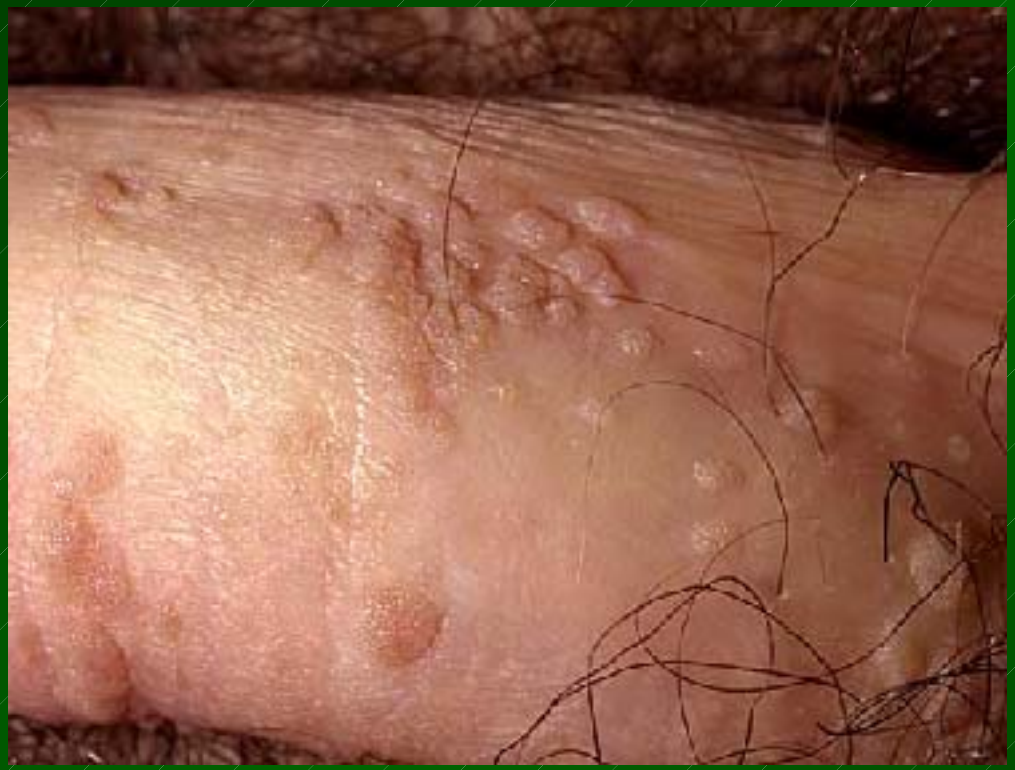
The sixties of the 20$^{th}$ century – nucleoside analogs mostly developed as potencial cytostatics

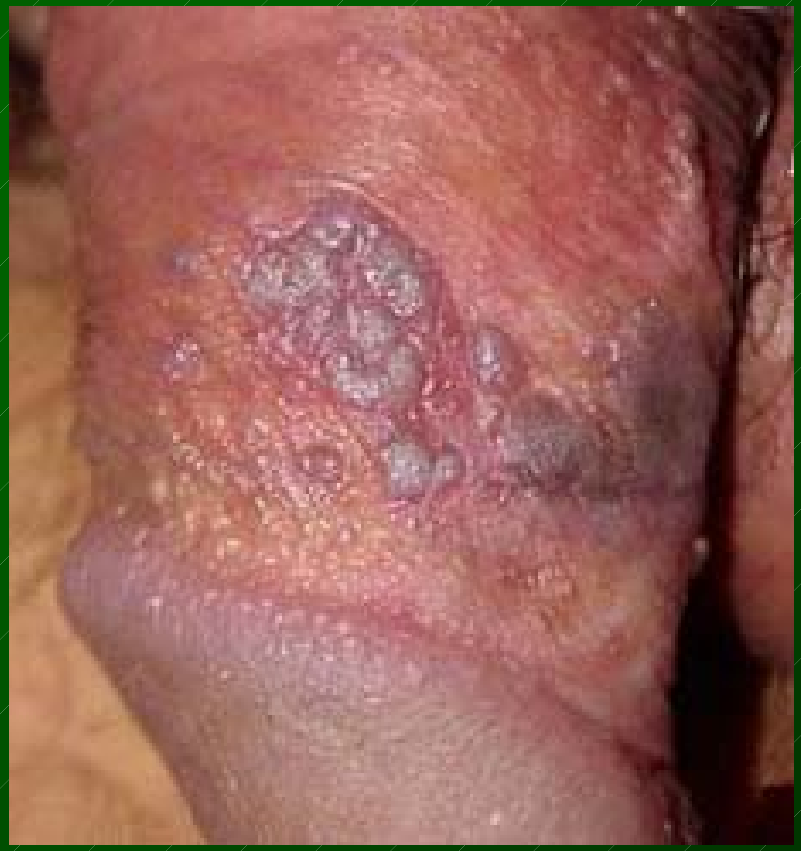
Tepid interest of pharmaceutical companies to develop antivirals

Turning point – the beginning of the seventies:

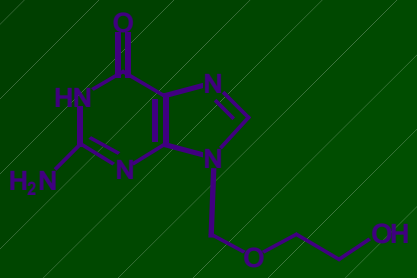
A large „epidemy“ of genital herpes (HSV-2)
in USA due to a promiscuous life style of then society

# Genital herpes caused by HSV-2

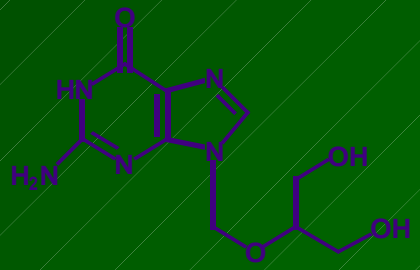




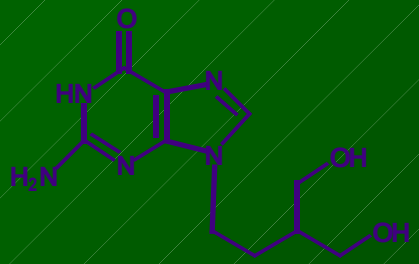
# ACYCLIC NUCLEOSIDE ANALOGS DERIVED FROM GUANINE



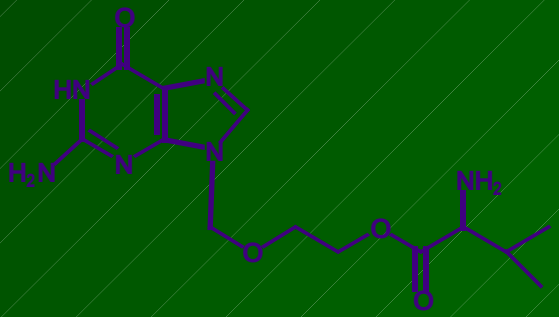
ACYCLOVIR



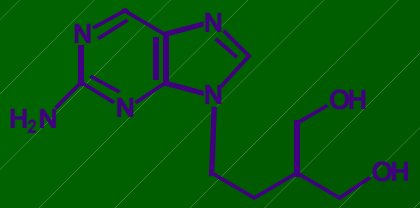
GANCICLOVIR



PENCICLOVIR



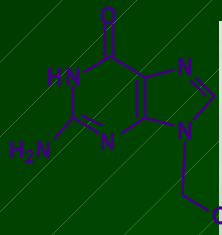
VALACYCLOVIR



FAMCICLOVIR

**ANTIHERPETIC AGENTS**

**Gertrude B. Elion** (1918-1999) – development of acyclovir (ZOVIRAX)

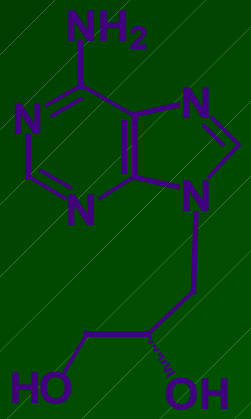



The fifties

1988

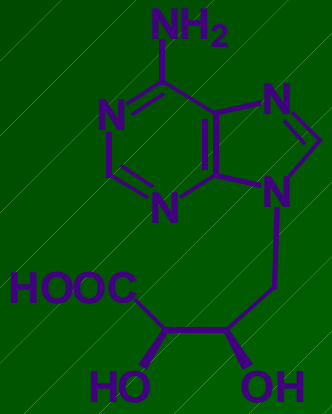
1988 – Nobel Prize for medicine (together with Georg Hitchings)
The first Nobel Prize targeted to pharmaceutical industry

# 1970-1985, IOCB, **Antonín Holý**: Acyclic analogs of adenosine

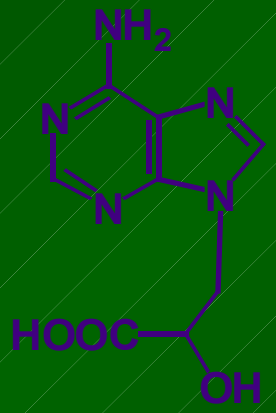


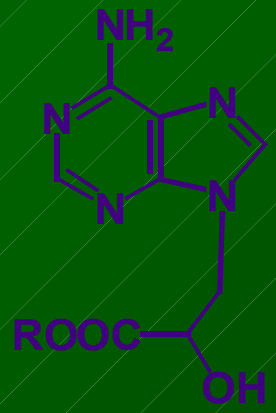

(S)-DHPA

9-(2,3-Dihydroxypropyl)adenine




D-eritadenine




AHPA a its esters

3-(Adenin-9-yl)-2-hydroxy-propanoic acid


**DHPA - antiherpetic drug Duviragel**

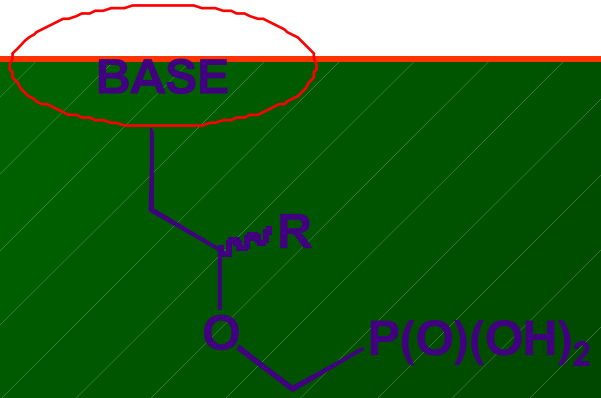
**(Only S-enantiomer is antivirally active).**

# ACYCLIC NUCLEOSIDE PHOSPHONATES (ANPs)

1986 Antonin Holy reports ANPs as a new type of nucleotide antimetabolites

## Biological activities:

ANTIVIRALS
CYTOSTATICS
ANTIPARAZITIC ACTIVITY
(MALARIA, *Trypanosoma brucei*)
IMMUNOMODULATORY ACTIVITY

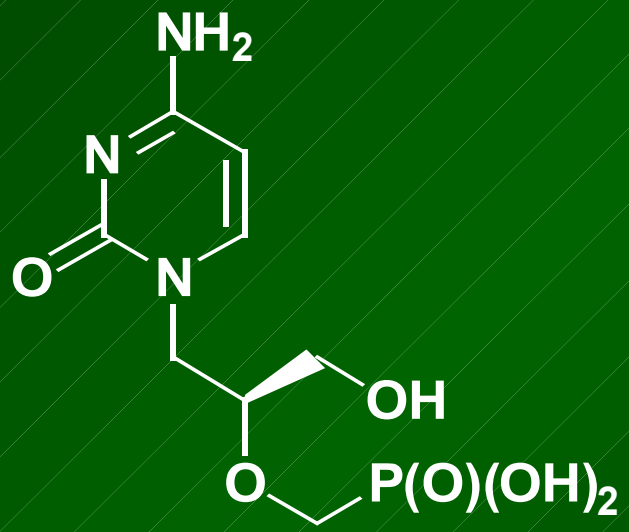



R = H, OH, $CH_3$, $CH_2F$

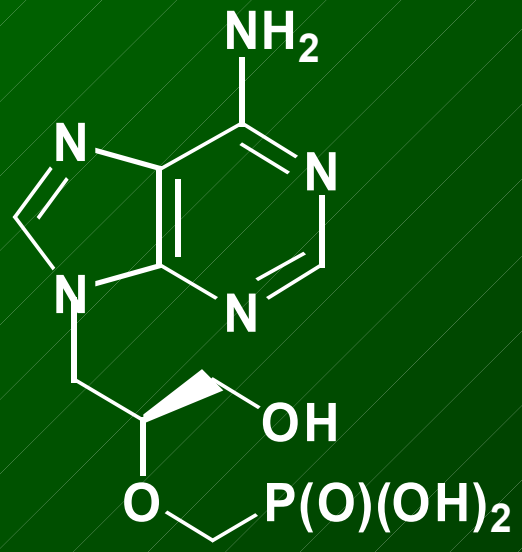
# STRUCTURAL TYPES OF ACYCLIC NUCLEOSIDE PHOSPHONATES

## 1. HPMP DERIVATIVES

### (S)-3-Hydroxy-2-(phosphonomethoxy)propyl derivatives



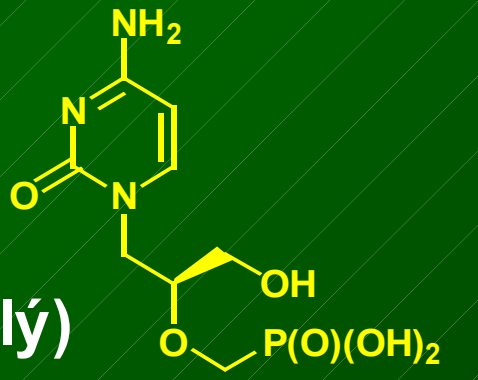

(S)-HPMPC




(S)-HPMPA

# (S)-HPMPC: CIDOFOVIR, VISTIDE™

(Gilead Sciences)

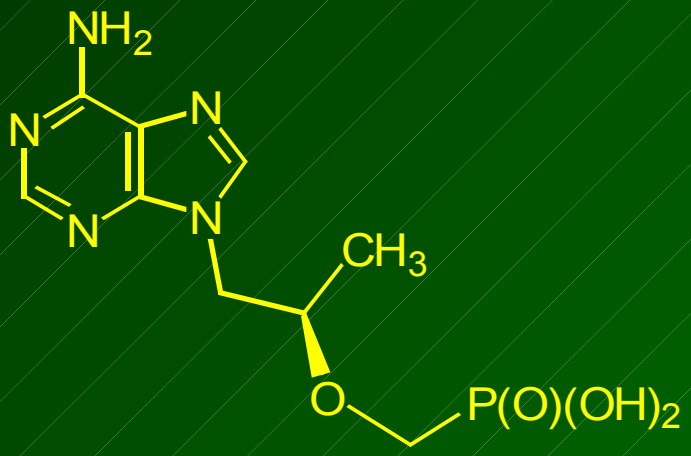

- Synthesis: 1986 (A. Holý)
- Approved by FDA: 1996
  for the treatment of cytomegalovirus retinitis in AIDS patients
- Intravenous application

**Activity: all DNA viruses**

**„Out of label“ used also for treatment of HSV infections (herpes genitalis), papilomavirus infections, progressive multifocal leukoencephalopathy, molluscum contagiosum, orf (and other poxvirus infections), adenovirus infections, Kaposi sarcoma**

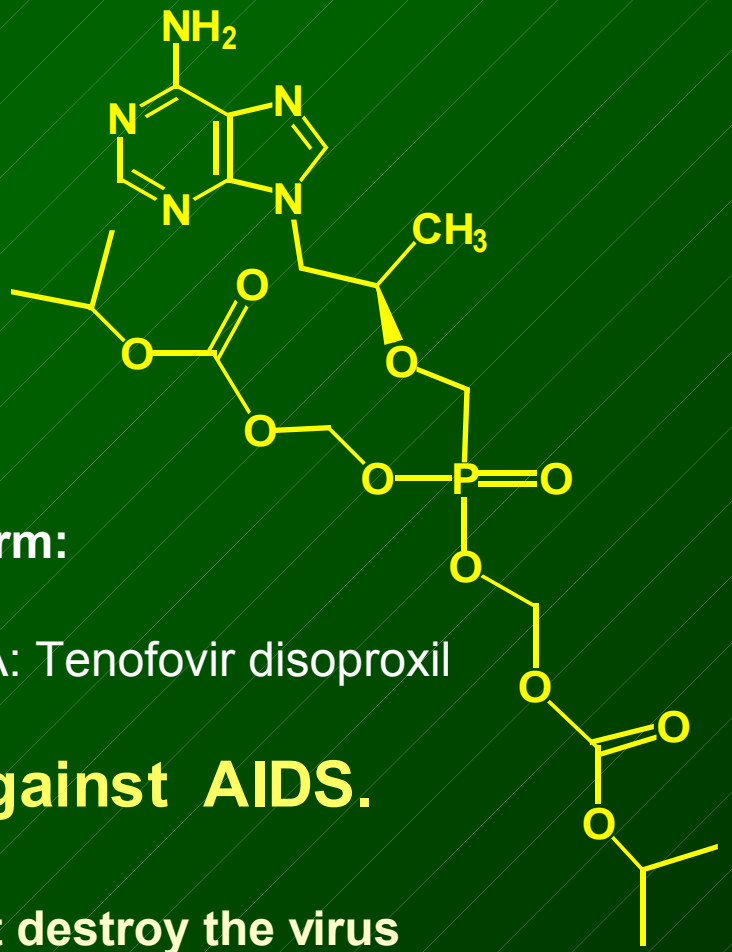
# 2. PMP- DERIVATIVES

## (R)-2-(Phosphonomethoxy)propyl derivatives




**PMPA – tenofovir**

**Prodrug form:**




Bis-(POC)-PMPA: Tenofovir disoproxil

**At present the best selling drug against AIDS.**

**The drug inhibits HIV multiplication. It cannot destroy the virus altogether, but delays AIDS development in HIV-infected patients.**

**Newly approved for the treatment for Hepatitis B infections.**


**Viread™ (tenofovir disoproxil)**

approved 2001


**Truvada™ (tenofovir disoproxil + emtricitabine)** approved 2004


**Atripla™ (tenofovir disoproxil + emtricitabine + efavirenz)**

approved 2006

# Antiretroviral drugs – the present state:
# 28 Compounds and combinations available

- Affecting diverse stages of virus development
- Devided to several groups according to mechanism of action:

**Reverse transcriptase inhibitors**

Nucleoside: AZT, didanosine, zalcitabine,...

Nucleotide: tenofovir (Viread),

Non-nucleoside: efavirenz (Sustiva®)

**HIV protease inhibitors** (ritonavir, nelfinavir)

**Fusion inhibitors** block virus entry to the cell through the cell membrane

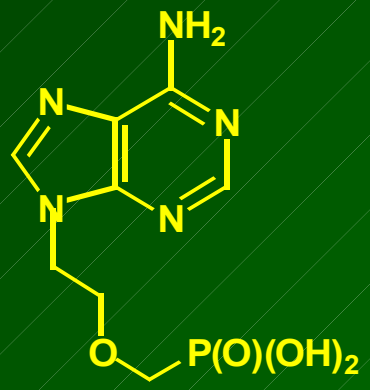
---

Combined therapy – several compounds in 1 product
(Atripla = efavirenz + tenofovir DF + emtricitabine)
1 tablet daily

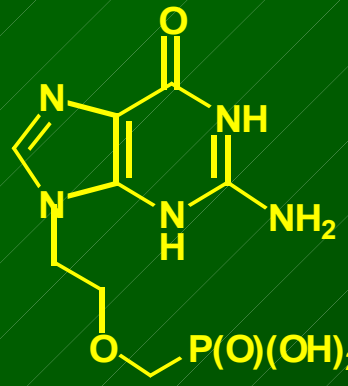
# 3. PME- DERIVATIVES

## 2-(Phosphonomethoxy)ethyl derivatives

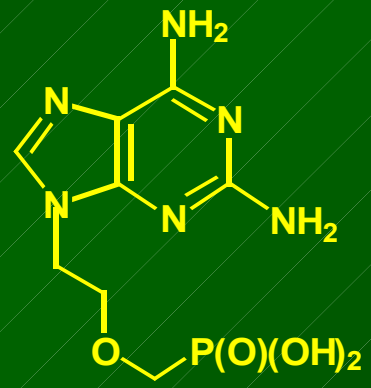
PMEA



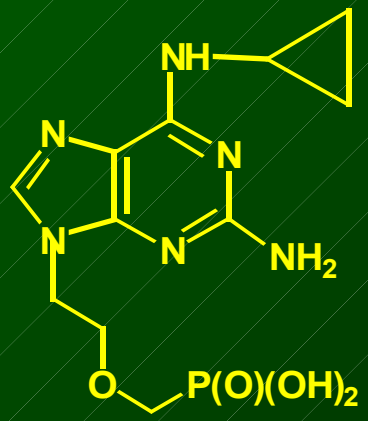

PMEG



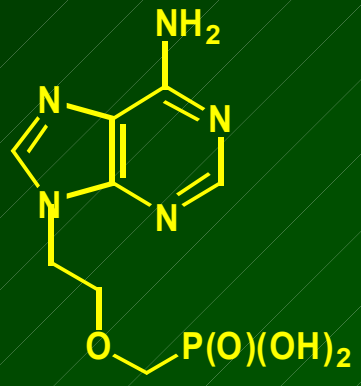

PMEDAP




cPrPMEDAP



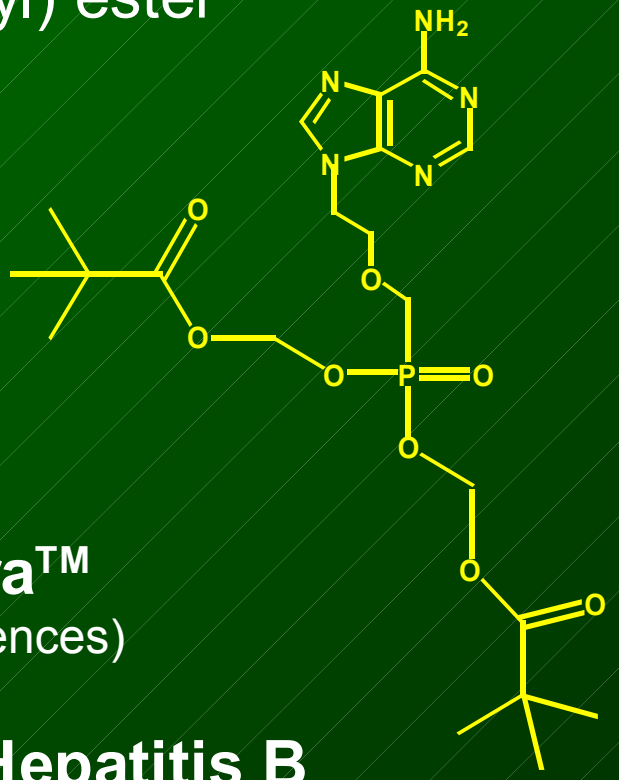
# PMEA, 9-(2-Phosphonomethoxyethyl)adenine (adefovir)




**Clinically used in a form of the prodrug**

**Adefovir Dipivoxil** (Bis-POM-PMEA)

= bis(pivaloyloxymethyl) ester

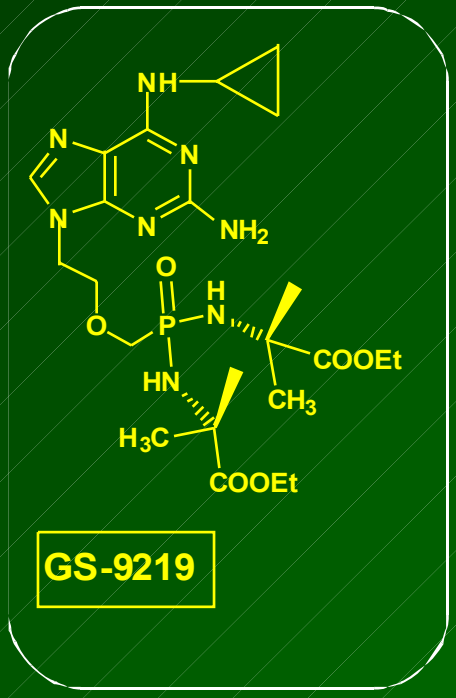

**Hepsera™**
(Gilead Sciences)

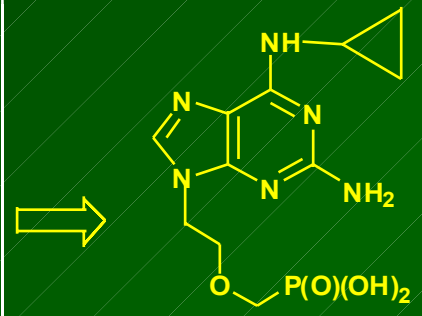
**Treatment of Hepatitis B**

# GS-9219

**Compound developed in collaboration of IOCB (A.Holý) and Gilead Sciences**

## Clinical Phase I





PMEG

GS-9219 ⇒

**PMEG** in cancer cell:

- Phosphorylation
- Incorporation to the nucleic acid
- Completion of DNA replication
- Cancer cell death

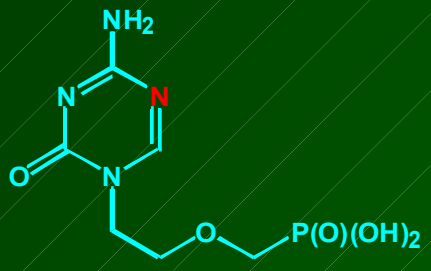
**Utilization of GS-9219:**

- non-Hodgkin lymphoma and chronic lymphatic leukemia
- High effectivity *in vivo*: 1 injection of GS-9219 caused a complete disappearance of tumors after 6 days (Beagle dogs)

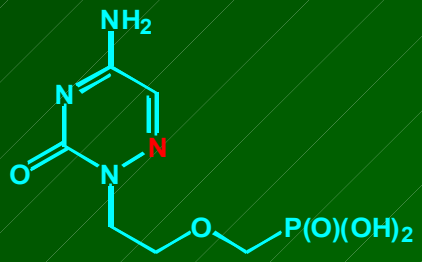
**Present state: Clinical Phase I**

Drug in the market: ??? 6-8 years

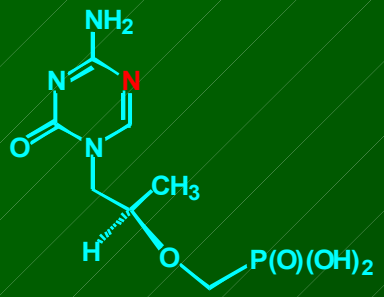
# ACYCLIC NUCLEOSIDE PHOSPHONATES WITH A TRIAZINE BASE - antiviral activity



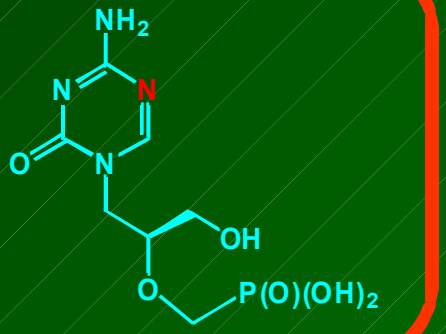

low activity



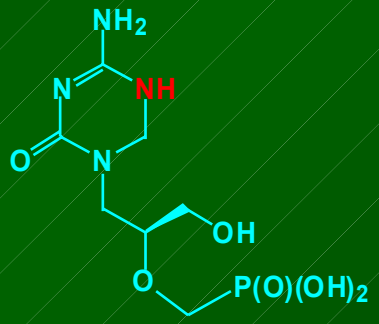

no activity




inactive




**strong activity against all DNA viruse**

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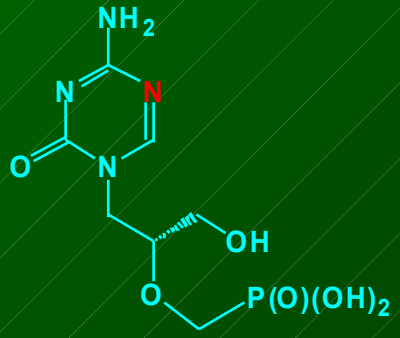


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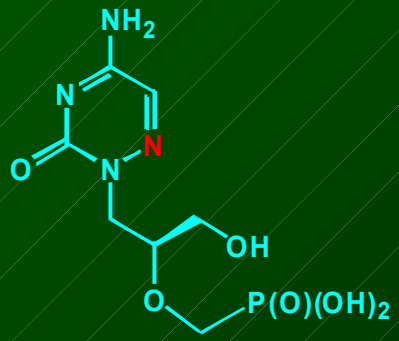
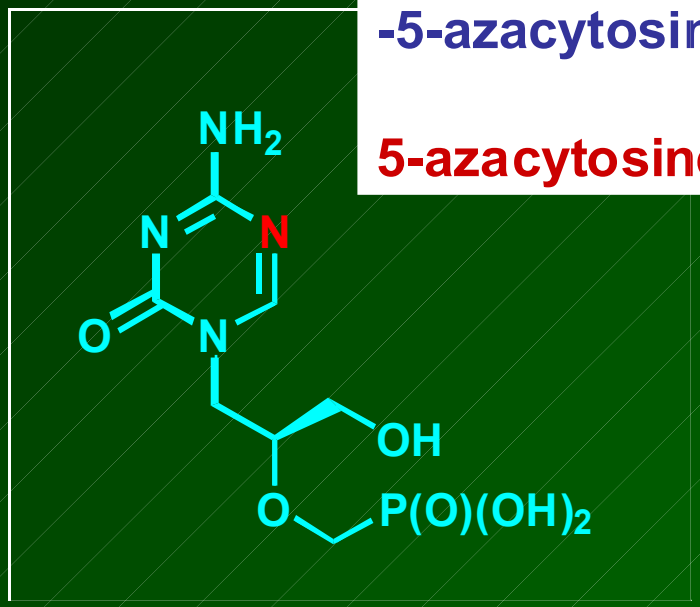


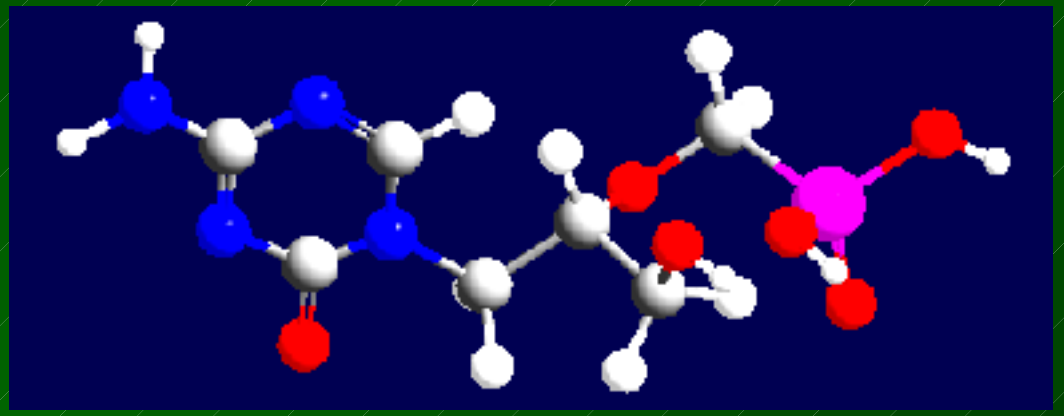

low activity

Krečmerová M., Holý A. et al. : *J. Med. Chem.* **2007**, *50*, 1069-1077.

# 1-(S)-[3-Hydroxy-2-(phosphonomethoxypropyl]--5-azacytosine (HPMP-5azaC)

**5-azacytosine analog of cidofovir**



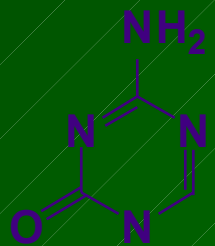



**A selective activity against DNA viruses:** adenovirus, poxviruses (vaccinia virus, cowpox virus, orf virus), herpesviruses (HSV-1, HSV-2, VZV, cytomegalovirus, HHV-6)

**Comparison with cidofovir:**

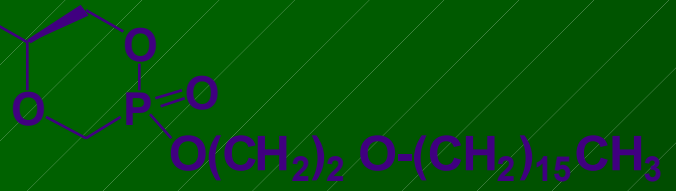
- Similar activity ($EC_{50}$): HSV-1, HSV-2, vaccinia
- Higher activity (2-7 fold ): VZV, HCMV, Ad2, HHV-6
- Lower toxicity ($CC_{50}$)

→ 2-12 fold higher selectivity index (i.e. ratio of $CC_{50}$ to $EC_{50}$ )


Lipophilic prodrug of HPMP-5-azaC

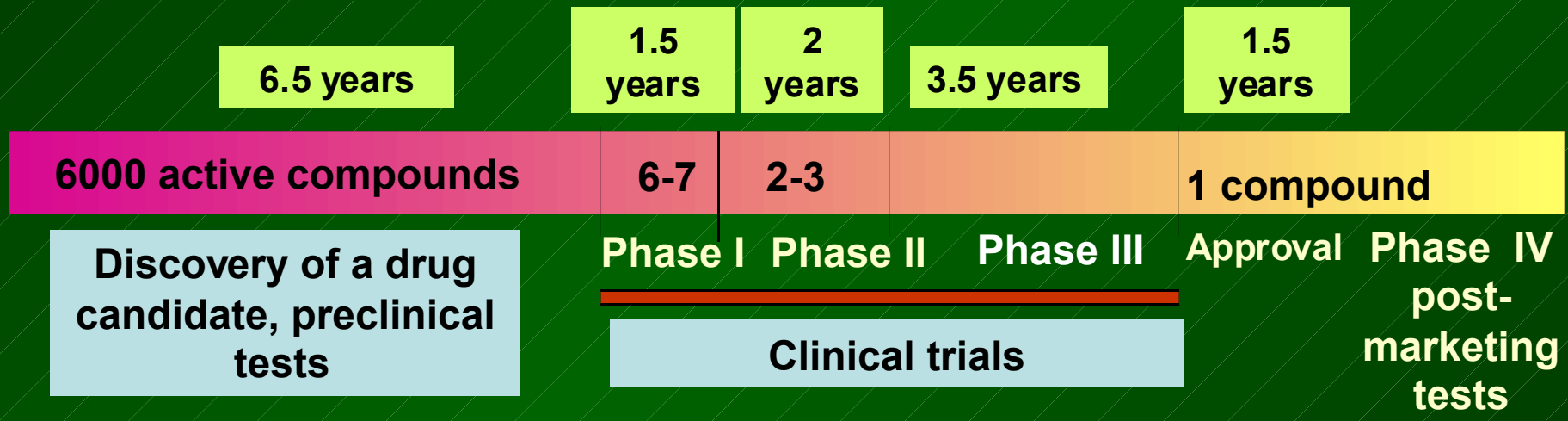
MK-612




**The most active compound against cytomegalovirus: $EC_{50}$ 0.00026 nmol/mL (HCMV - Davis strain)**

- preclinical investigations finished
- complicated metabolic profile

# Development of a new drug is a 15 years lasting process ...




## ... and costs 800 million to 1 billion $

# Thank you for your attention !