Therapeutics from the family of nucleoside and nucleotide analogs:

A contribution of the Czech science to world medicine

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The greatest credit for establishing 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. Pískala, 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
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 a substrate for RNA polymerases.

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

  inhibition of synthesis of ribosomes and proteins

Pískala, Šorm, Veselý, Čihák, Zadražil, Fučík, Pačes (1964-1978)
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 repaired.
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 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.
Possibilities of chemical modifications of nucleosides:

**Base:**
- Adenine
- Thymine
- Guanine
- Cytosine
- Uracil

**Base:**
- Adenine
- Thymine
- Guanine
- Cytosine
- Uracil

**Sugar moiety:**
- HO
- O
- OH

**Most of rationally developed antivirals and many cytostatics are modified nucleosides.**
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
2\textsuperscript{nd} Generation of antimetabolites

Structural resemblance to natural metabolite is only in some basic aspects

\begin{center}
\textbf{ACYCLIC NUCLEOSIDE ANALOGS}
\end{center}

The seventies of the 20\textsuperscript{th} century

Carbohydrate moiety of the molecule is substituted with an aliphatic chain containing OH groups
ANTIVIRALS made in IOCB
HISTORY

The sixties of the 20th century – nucleoside analogs mostly developed as potential 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 – Nobel Prize for medicine (together with Georg Hitchings)
The first Nobel Prize targeted to pharmaceutical industry

(S)-DHPA                              D-eritadenine                             AHPA and its esters

9-(2,3-Dihydroxypropyl)adenine                        3-(Adenin-9-yl)-2-hydroxypropanoic acid

DHPA - antiherpetic drug Duviragel
(Only S-enantiomer is antivirally active).
ACYCLIC NUCLEOSIDE PHOSPHONONATES (ANPs)

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

Biological activities:

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

\[ \text{BASE} \]

\[ \text{O} \]

\[ \text{P(O)(OH)}_2 \]

\[ \text{R} = \text{H, OH, CH}_3, \text{CH}_2\text{F} \]
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
- Divided 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
**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 viruses

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$_{50}$)
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 ...

<table>
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<tr>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Approval</th>
<th>Phase IV post-marketing tests</th>
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<td>1.5 years</td>
<td>2 years</td>
<td>3.5 years</td>
<td>1.5 years</td>
<td>1 compound</td>
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6000 active compounds

Discovery of a drug candidate, preclinical tests

Clinical trials

... and costs 800 million to 1 billion $
Thank you for your attention!