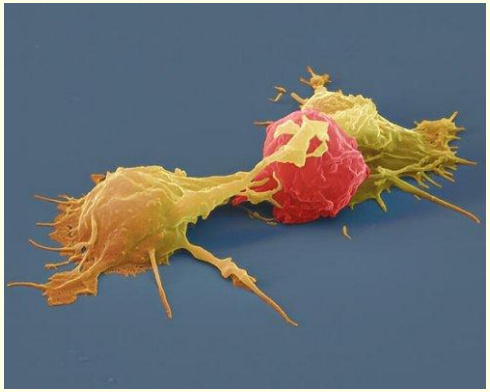
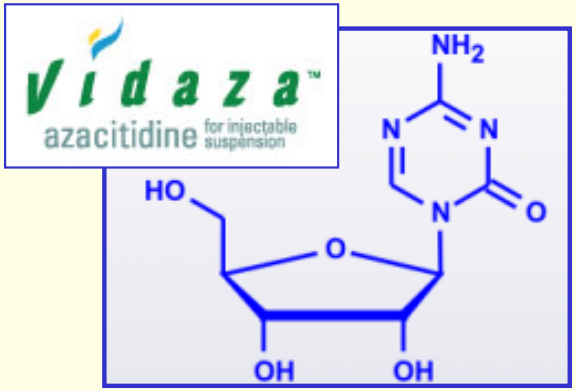
Epigenetic Cancer Diagnostics and Epigenetic Cancer Therapy

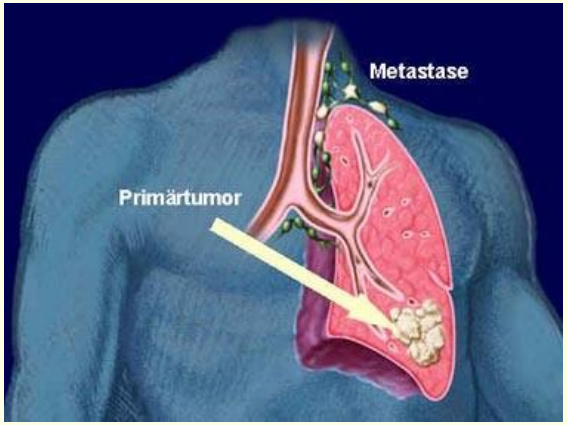
30.09.2021

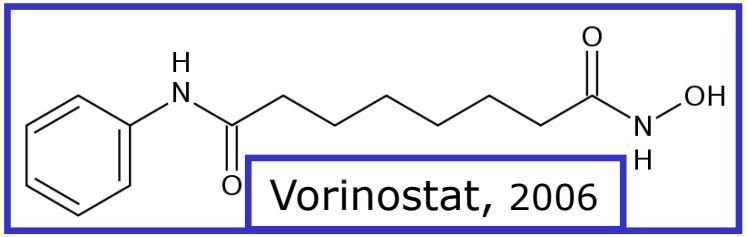




epi pro colon

epi pro lung





Prof. (emer.) Dr. Diethard Baron, Penzberg, diethard.baron@web.de

Program

Cancer

- development of cancer / causes of cancer / tumor types
- cancer genes (oncogenes, **tumor suppressor genes**)

Epigenetic Cancer Therapy

- inhibitors of epigenetic enzymes

Epigenetic Cancer Diagnostics

- diagnosis of colon cancer, lung cancer, breast cancer
- epigenetic clock helps in tumor therapy and tumor prognosis
- microRNA profiling detects breast cancer

Development of Cancer / Causes of Cancer

Cancer Genes

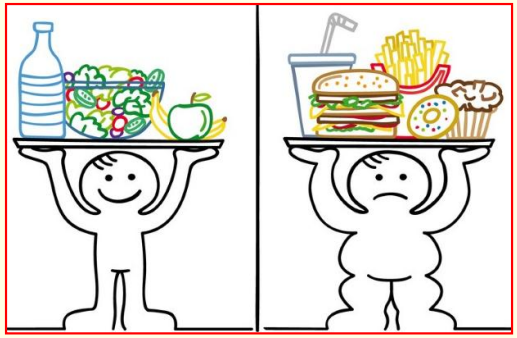
Cancer Types

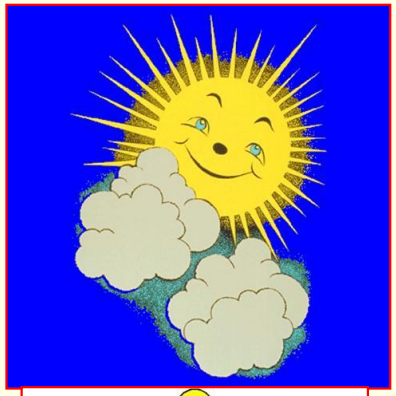
Causes of Cancer

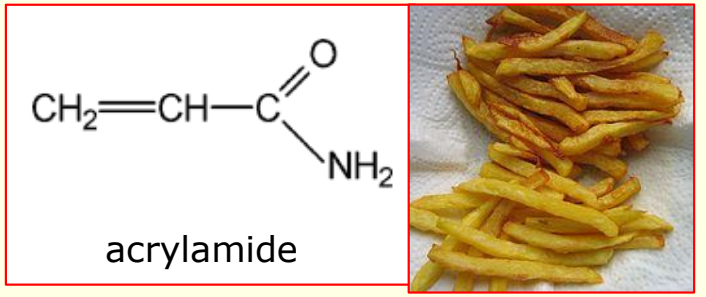
> 200 cancer types, multiple causes of cancer:

- smoking and nutrition (lung cancer, colon cancer...)
- genetic predisposition (breast cancer, ovarian cancer, colon cancer...)
- DNA damages / DNA mutations (majority of all cancer types)
- bacterial infections (stomach cancer, colon cancer)
- viral infections (cervix carcinoma [HPV], liver cancer [HBV], Hodgkin lymphoma [EBV]...)

HPV = human papilloma virus, HBV = hepatitis virus, EBV = Epstein-Barr virus)







Natural Immune Reactions against Cancer

- several hundreds or thousands of new cancer cells develop in our body every day
- seven different immune mechanisms are able to destroy the cancer cells to 100%
- **our immune system protects us against cancer**
- but: only as long as the immune system functions properly
- in case of immunosuppression / immunodeficiency, the **cancer risk** is increased

how can the immunsystem be suppressed?
multiple causes for immunosuppression

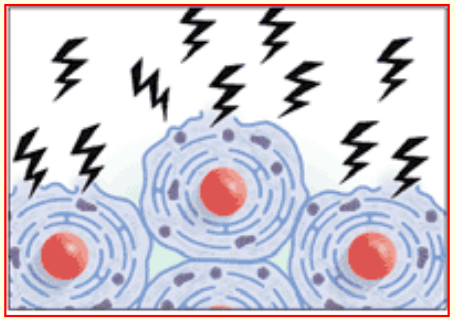
1. lifestyle / nutrition
2. environmental toxins (dioxin…)
3. age (> 60 years)
4. psychological problems
5. medical treatments (drugs, irradiation)
6. diseases…

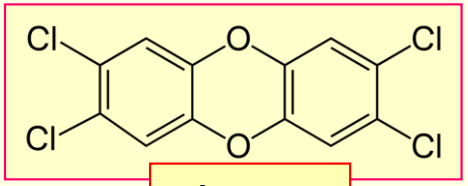
macrophages, natural killer cell, killer-T-cells, antibodies + complement, killer cells, macrophages, granulocytes

Mutations / DNA Damages promote Cancer

30.000 mutations per day and per cell

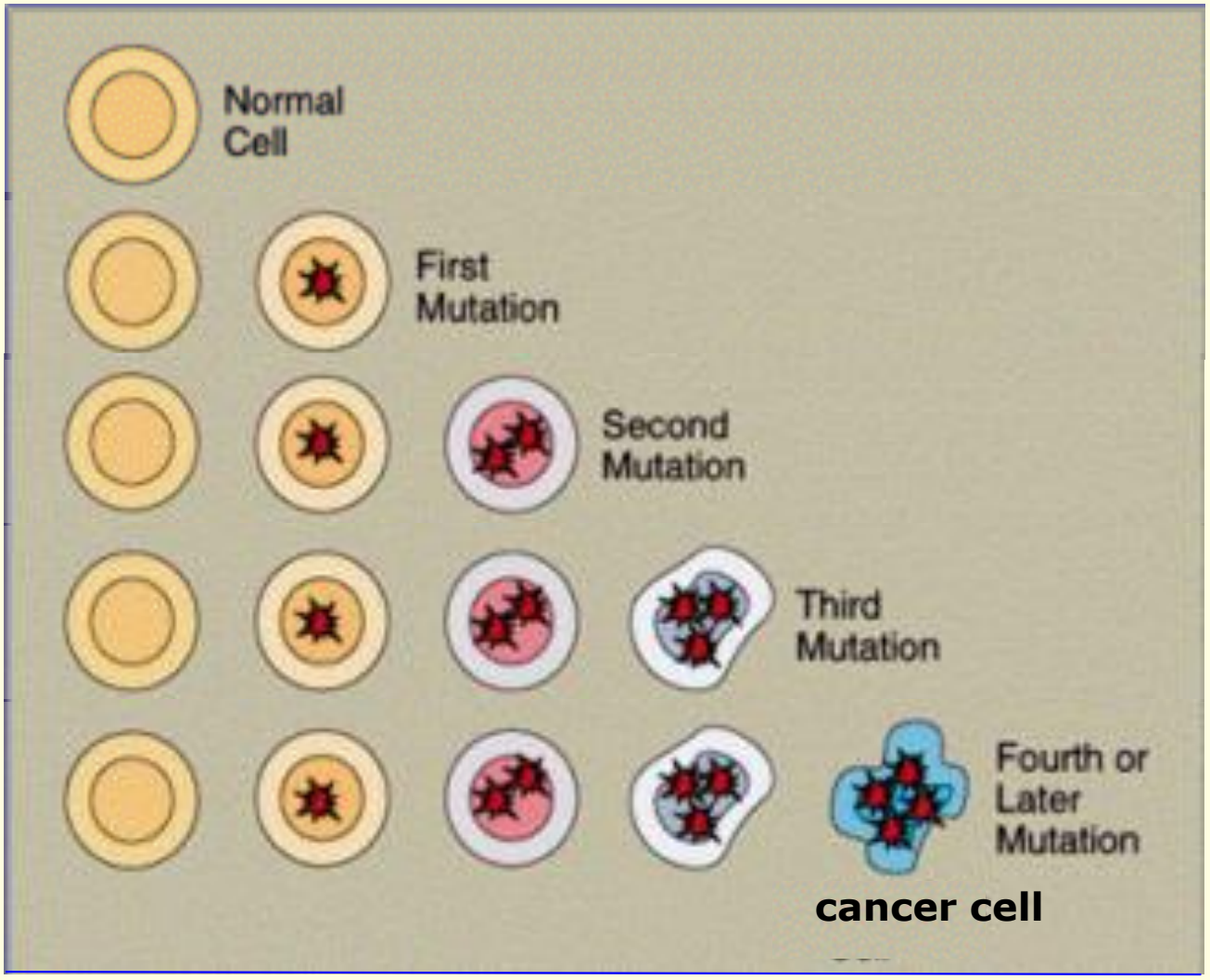
1. multiple causes for mutations
   - UV irradiation
   - radioactive irradiation
   - free radicals
   - chemicals and environmental toxins
   - virus infections
   - enzymatic failures
2. DNA damages/mutations must be continuously repaired
3. 173 DNA repairing enzymes in human body
4. when DNA damages are not repaired, the **cancer risk** is increased





dioxin

Multiple Mutations promote Cancer Cell Development



Cancer is a genetic Disease - 200 genes are involved

protooncogenes

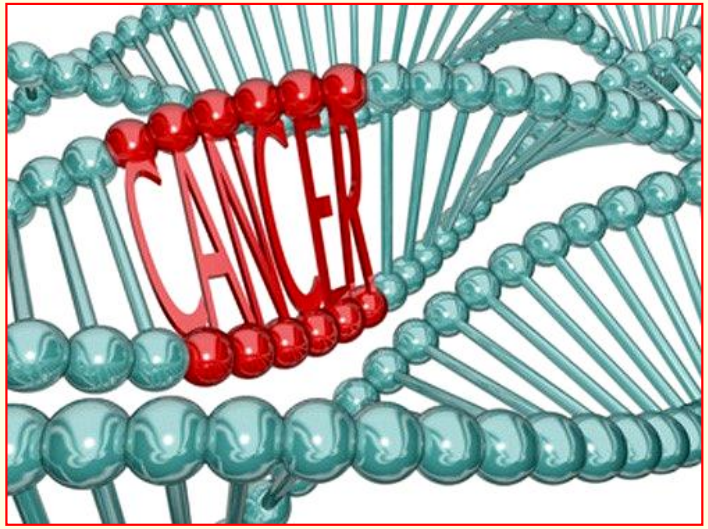
- protooncogenes do not cause cancer
- protooncogenes are in every normal cell and control **cell division** and **cell death**

oncogenes (>100)

- oncogenes are mutated protooncogenes and they cause cancer

tumor suppressor genes (30)

- they are in every normal cell and control **DNA repair** and **cell death**
- when tumor suppressor genes are mutated, DNA damages are no longer repaired and accumulate, resulting in cancer cells, and cancer cells no longer die



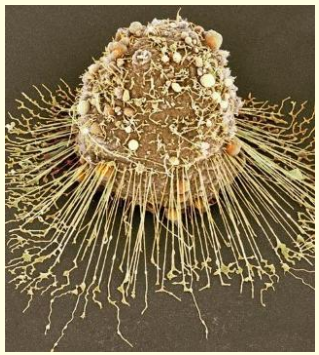
Benign and Malignant Tumors

malignant tumor	benign tumor
spreads in surrounding tissue	does not spread in surrounding tissue
destroys tissues and organs	does not destroy tissues or organs
forms metastases	does not form metastases
consists of **immortal** cells	consists of mortal cells

malignant tumor

benign tumor

HeLa cells // Henrietta Lacks // 1951



Examples for Benign Tumors

tumor	involved tissue / cells
hemangioma	blood vessels
osteoma	bones
myoma	muscle cells
lipoma	fat tissue

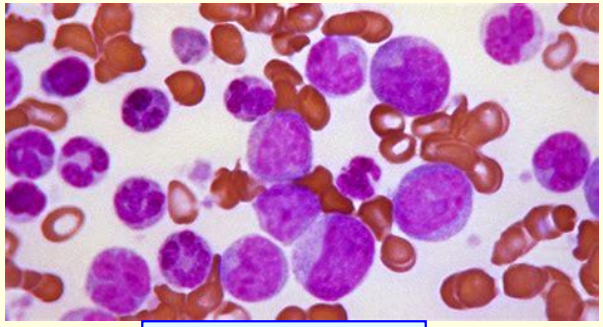
Examples for Malignant Tumors

tumor	involved organ / tissue
carcinoma	epithelial cells (skin cells) (**80%**)
lymphoma	lymphoid tissue / lymph nodes
sarcoma	connective tissue, bones, muscles
leukemia	blood cells

malignant melanoma skin cancer

lymphoma

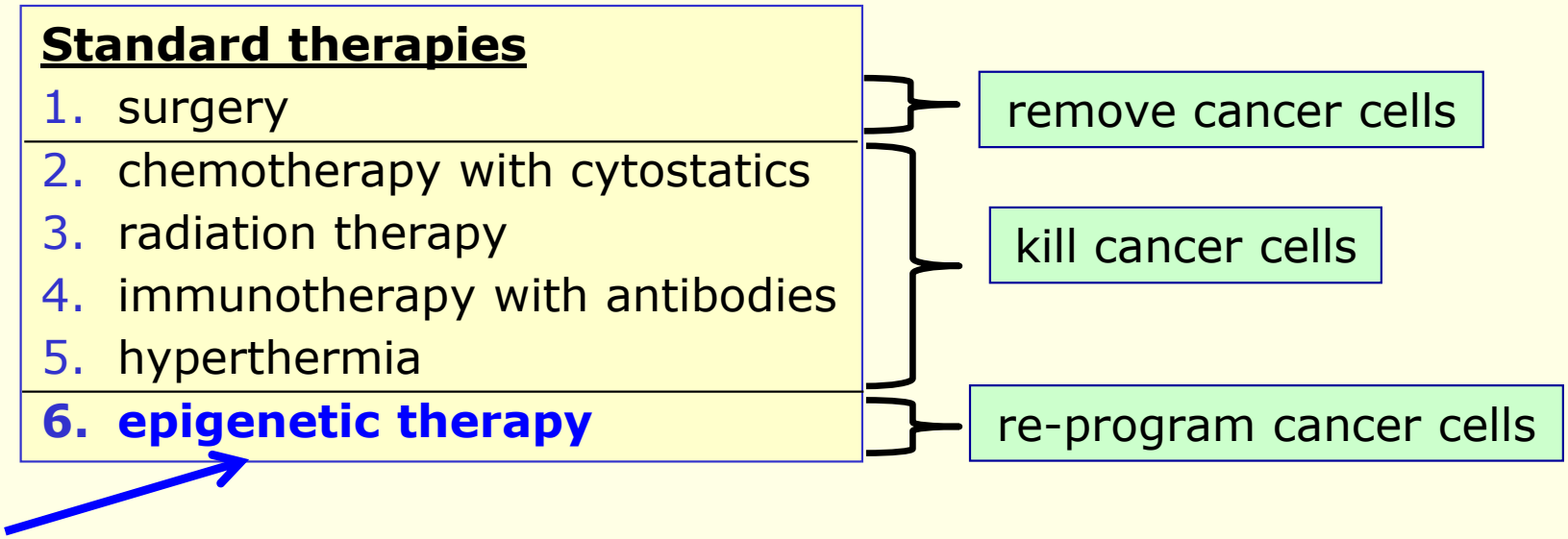
sarcoma



leukemia

Cancer Therapy
and
Epigenetic Cancer Therapy

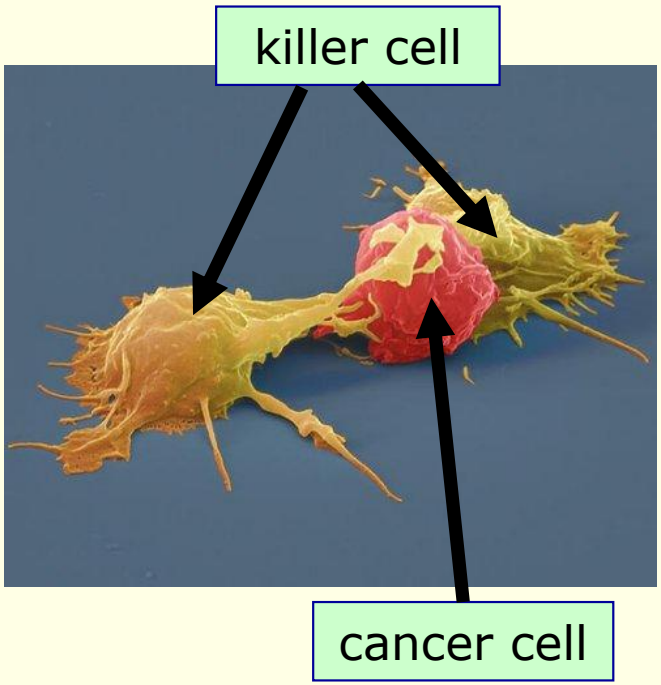
Cancer Therapy

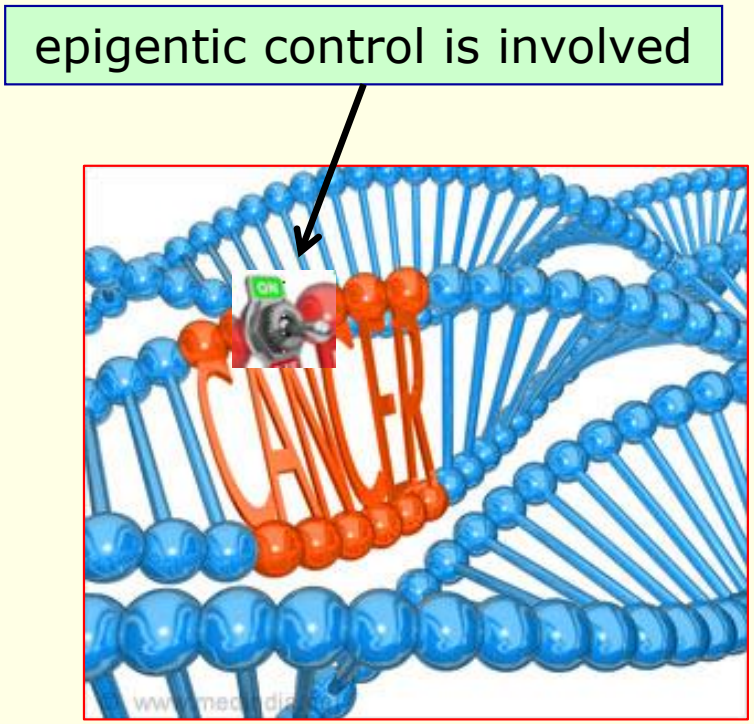


Epigenetics and **Cancer**

Two major causes of cancer:

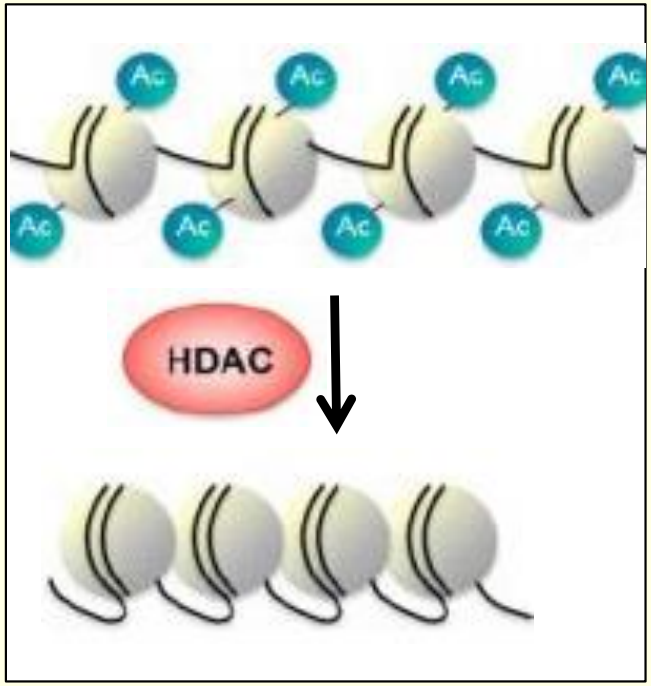
- normal cells become cancer cells by **mutations** (very old view, 70 years)
- normal cells become cancer cells without **mutations**
- normal cells become cancer cells by incorrect **epigenetic control** of genes **(epimutations)**
- **tumor suppressor genes** are **erroneously** switched off by epigenetic mechanisms



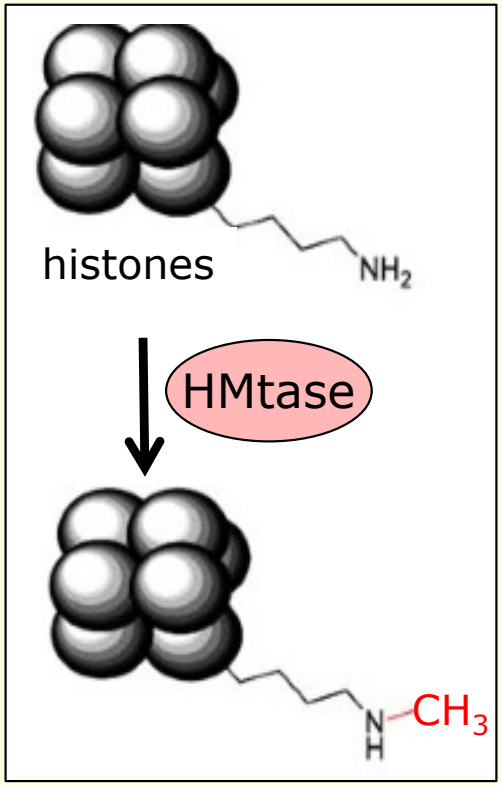


Epigenetics and **Cancer**

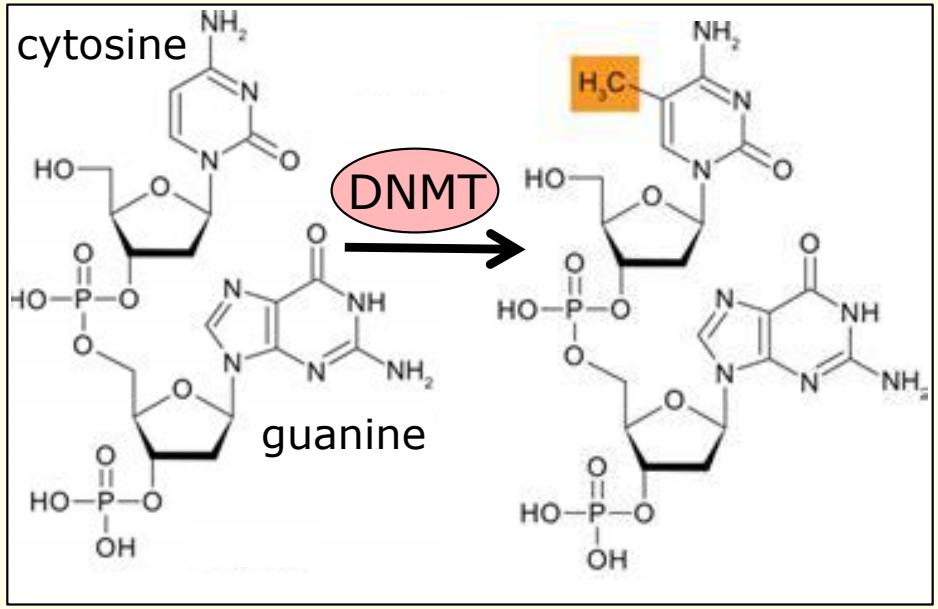
- **tumor suppressor genes** are **erroneously** switched off by epigenetic mechanisms
- these mechanisms are: histone deacetylation, histone methylation, DNA methylation
- such mechanisms are catalyzed by enzymes:
  1. histone deacetylation is catalyzed by histone deacetylase (HDAC)
  2. histone methylation is catalyzed by histone methyltransferase (HMtase)
  3. DNA methylation is catalyzed by DNA methyltransferase (DNMT)
- epigenetic cancer therapy is done by blocking these enzymes by **enzyme inhibitors**
- **9 inhibitors/drugs have been approved for epigenetic cancer therapy**



gene inactivation



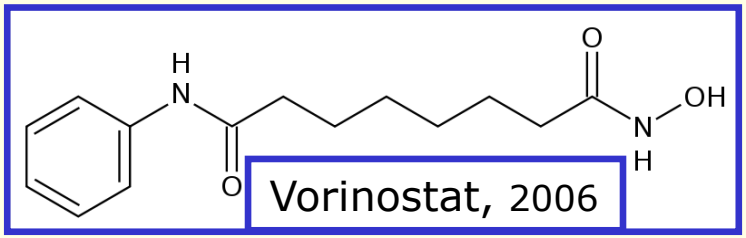
gene inactivation



gene inactivation

Therapy of Leukemia and Lymphoma by Inhibitors of Histone Deacetylase (HDAC)

6 drugs have been approved**:**
Vorinostat, Romidepsin, **Panobinostat, Belinostat, Chidamid, Tucidinostat**

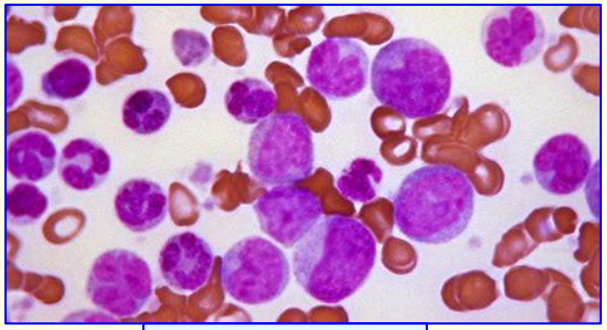


Vorinostat, 2006

Re-programming vs. **killing**

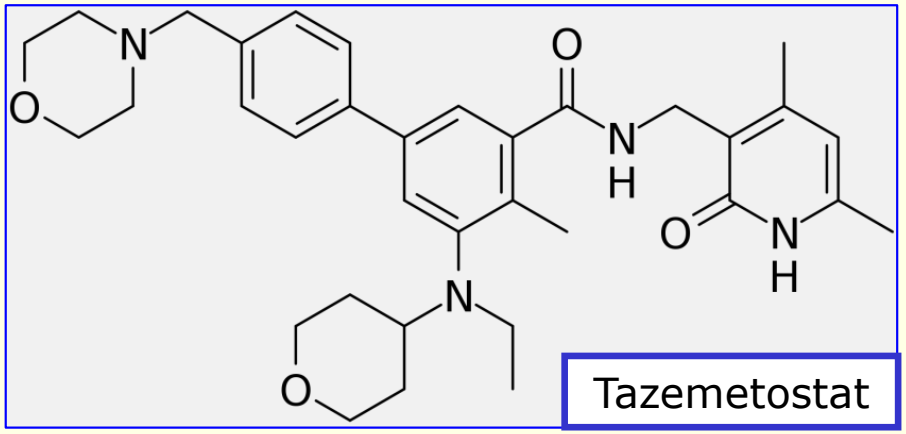
only for leukemias and lymphomas caused by epimutations


lymphoma


leukemia

Therapy of Sarcomas by Inhibitors of Histone Methyltransferase (EZH2)

1 drug has been approved: **Tazemetostat (Tazverik®)** (2020)



Tazemetostat

Re-programming vs. **killing**

only for sarcomas caused by epimutations

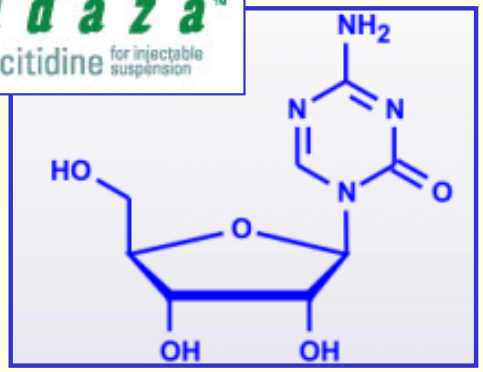
sarcoma

Merck & Co / MSD, Celgene, Novartis

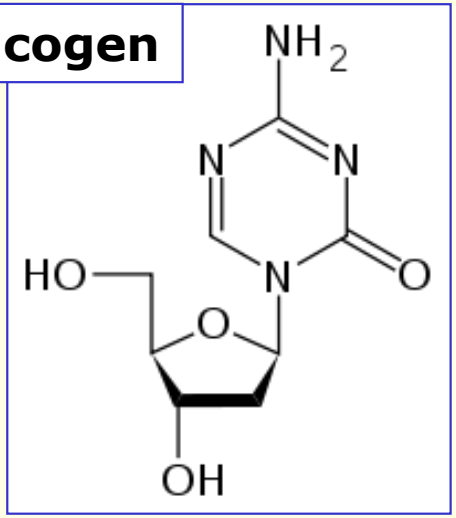
Therapy of Leukemias by Inhibitors of DNA Methyltransferase

- 2 drugs have been approved
- **Vidaza®** (2004) and **Dacogen®** (2006)

Vidaza™ azacitidine for injectable suspension

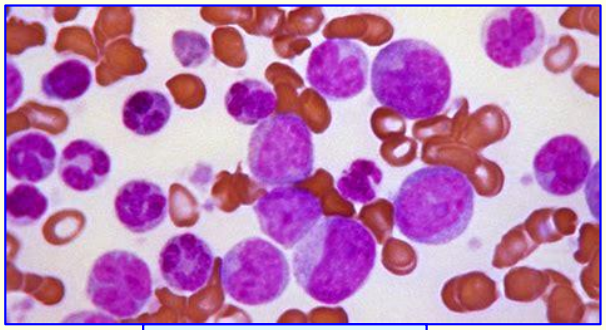


Dacogen



Re-programming vs. **killing**

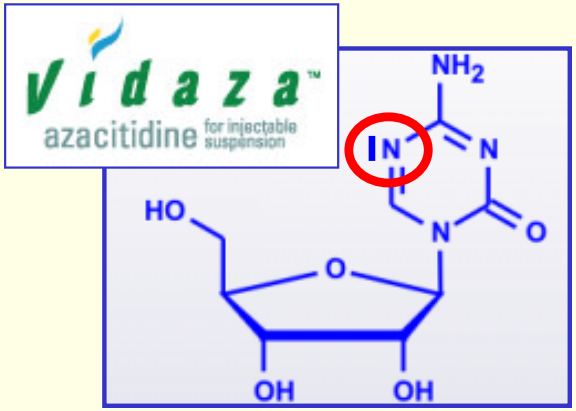
only for leukemias caused by epimutations

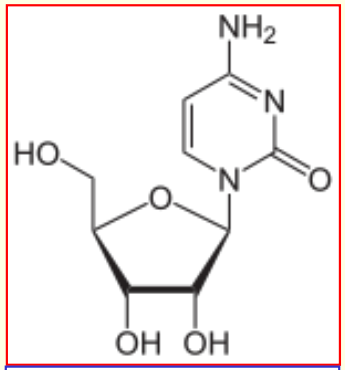


leukemia

Vidaza® - Inhibitor of DNA Methyltransferase (DNMT) Mode of Action

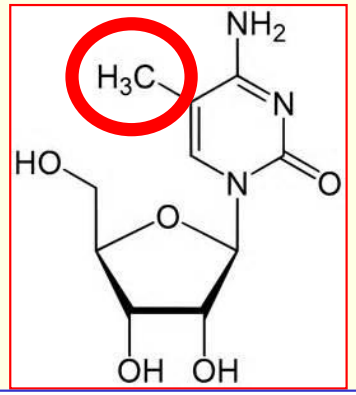
- **Vidaza™** cytidine analog (5-azacytidine)
- **Vidaza™** gets incorporated into DNA during DNA-replication
- **Vidaza™** reacts with amino acids in the active center of DNA methyltransferase **->**
- **Vidaza™** inhibits DNA methyltransferases (DNMT) by covalent linkage to DNMT **->**
- irreversible inhibition of DNMT **->** tumor suppressor gene can no longer be inhibited
- side effects: shortage of blood cells, confusion, anxiety, sleeplessness (insomnia)





cytidine

DNMT



5-methyl-cytidine

Cancer Diagnostics

Epigenetic Cancer Diagnostics

Diagnosis of Cancer

in vitro tests / laboratory tests

1. **analysis of tumor markers** in serum or urine
2. **genetic analysis of cancer cells**
3. **epigenetic analysis of cancer genes (epimutations)**

in vivo tests with patients / tumor imaging

Visualization of primary tumors and metastases by computer tomography (CT), magnetic resonance tomography (MRT), thermography, sonography

Epi proColon – Epigenetic Detection of Colorectal Carcinoma

- determination of methylation of **tumor suppressor gene** *Septin-9*
- *Septin-9* is **methylated** in cancer cells, but unmethylated in normal cells
- dead cancer cells release DNA in the blood ➔ blood plasma as sample material
- first approved **epigenetic** cancer test (2009)

epigenomics

epi pro colon

Epi proLung – Epigenetic Detection of Lung Cancer

- determination in blood plasma of methylation of *mSHOX2* gene and *PTGER4* gene
- *mSHOX2* and *PTGER4* are methylated in cancer cells, but unmethylated in normal cells
- dead cancer cells release DNA in the blood ➔ blood plasma as sample material
- since 2010 in EU approved epigenetic cancer test

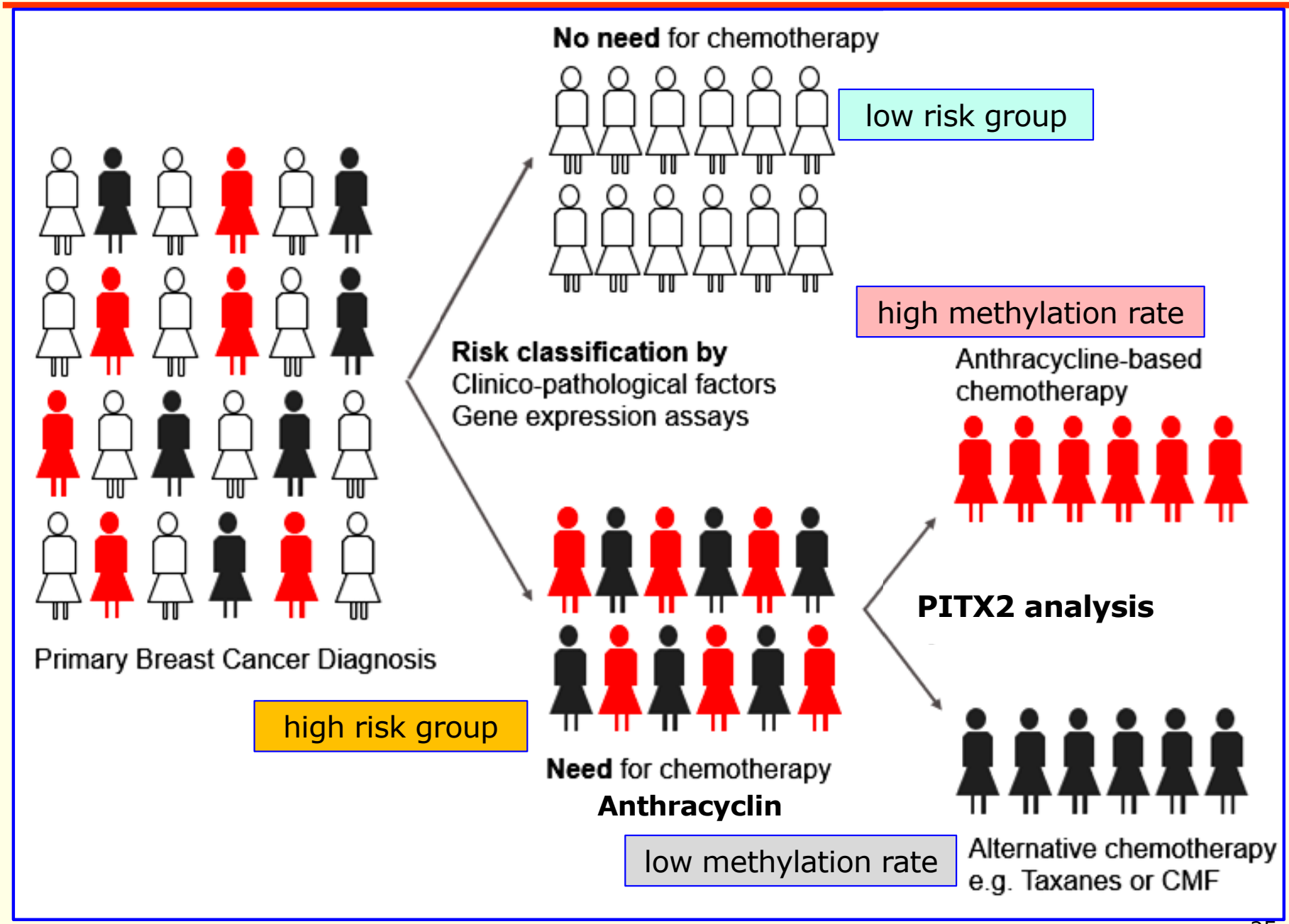
epigenomics

epi pro lung

Therascreen PITX2 - Epigenetic Breast Cancer Test

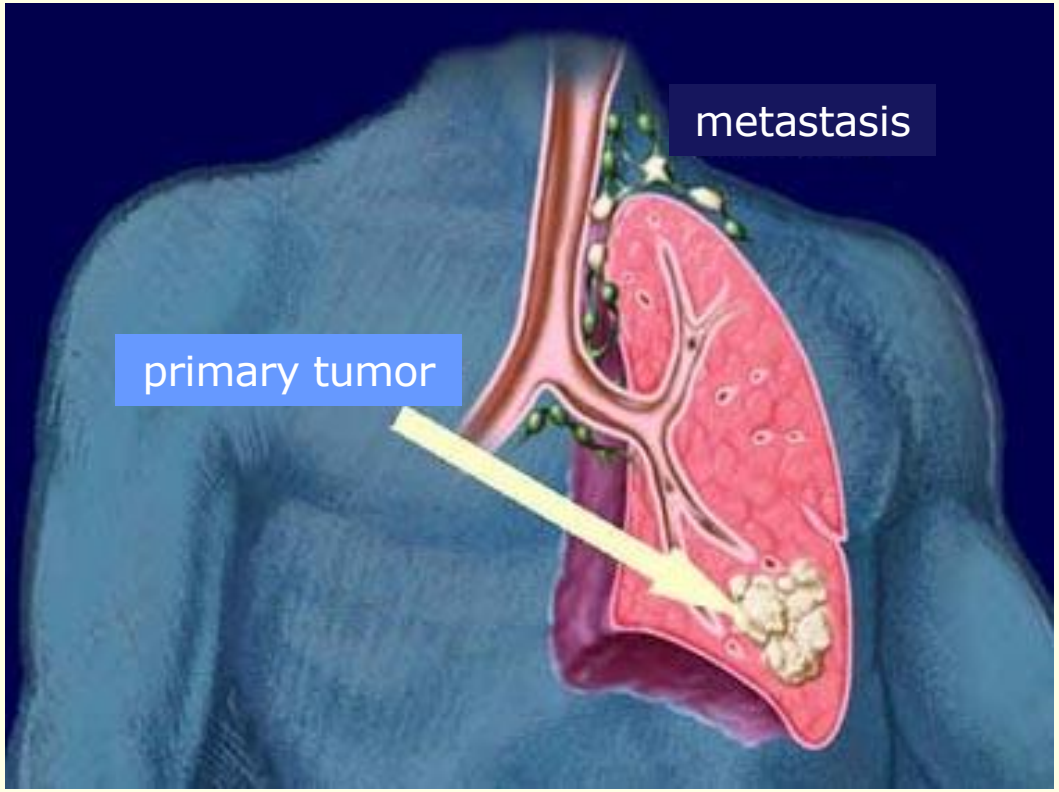
- breast cancer patients are classified into low risk, medium risk and high risk groups
- high risk breast cancer patients are treated with anthracycline chemotherapy
- severe side effects of anthracycline: heart and kidney failure, leukemia, anemia...
- is the therapy with anthracycline really necessary for all high risk patients?
- or is it possible to further subclassify the high risk group in a lower risk and higher risk group?
- lower risk group would not need anthracycline, only the higher risk group
- yes, a subclassification is possible by analysis of **methylation** of *PITX2* gene
- low **methylation rate** indicates a lower cancer risk **->** no anthracycline therapy is needed, milder chemotherapy is sufficient
- high **methylation rate** indicates a high cancer risk **->** anthracycline chemotherapy must be applied

Therascreen PITX2



Epigenetic DNA Blood Test detects Hidden Tumor

- 15% of cancer patients have hidden primary tumors
- metastases are detected first, where is the primary tumor?
- detection of primary tumor by analysis of **methylation** of free DNA in blood plasma
- free DNA from **normal cells** which were destroyed by matastasizing tumor cells
- **methylation pattern is tissue/cell specific**



How old am I? The Epigenome tells your true Age / Horvath Clock

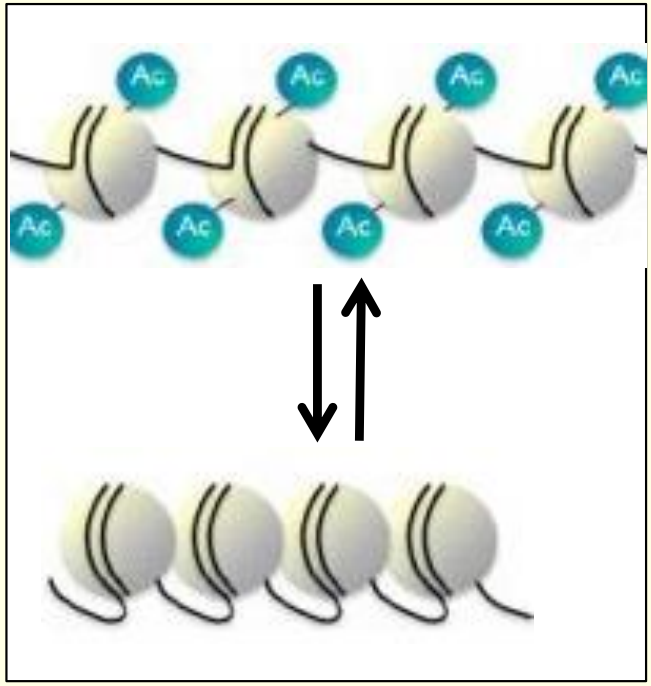
- with increasing age „aging genes" are switched off by **DNA methylation**
- „aging genes" control DNA repair, stem cell proliferation, free radical degradation…
- age determination by analysis of methylation in 353 positions in the genome
- precise age determination of cells and people +/- 1.5 years

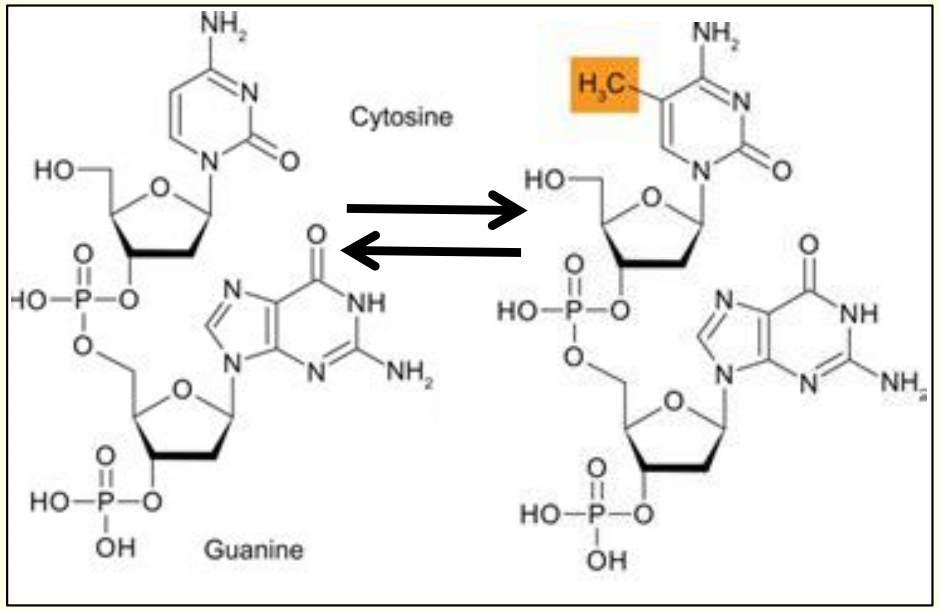
Application

- Forensics: age determination of criminals or victims
- calculation of life span (GrimAge)
- **cancer therapy**: cancer cells are up to 40% epigenetically older than normal cells of the patient
- epigenetically older cancer cells grow more aggressively, acquire faster drug resistance, allow a more precise prognosis for the patient

Epigenetic Gene Control Mechanisms

1. **Histon-Modifizierung**
2. **DNA-Methylierung**
3. **microRNA (miRNA)** ←
4. **Chromosome Distribution**
5. **RNA Methylation**





Epigenetic Gene Control by microRNA = miRNA

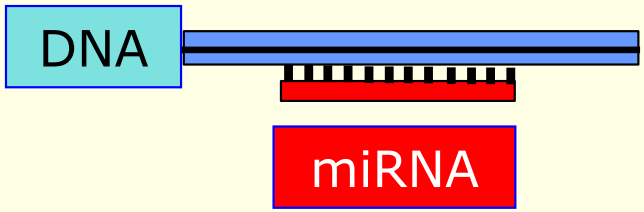
- **microRNA** are found in plants, worms, flies, mice, and humans
- > 40% of the human genes are regulated by **miRNAs**
- > 5.000 different **miRNAs** in humans
- **miRNA** genes are coding for miRNA
- miRNAs control transcription und translation

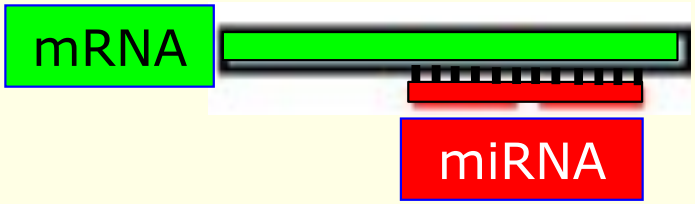
5′ cuccuacauauuagcauuaaca 3′

miRNA (22 nucleotides)

Control of Transcription and Translation by miRNA

- when a miRNA is complementary to a specific **DNA** sequence, the miRNA binds to the DNA and inactivates the gene
- when a miRNA is complementary to a specific **mRNA**, the miRNA binds to the mRNA and inhibits translation





Breast Cancer Detection by miRNA - miRNA Urine Test

- breast cancer has a typical miRNA profile:
- concentration of microRNA-155 (miR-155) is strongly **increased** in urine
- concentration of miR-21, miR-125b, miR-451 is strongly **decreased** in urine
- urine as sample material, fast, easy, not painfull
- application: early diagnosis of breast cancer, survey of cancer therapy

5′ cuccuacauauuagcauuaaca 3′

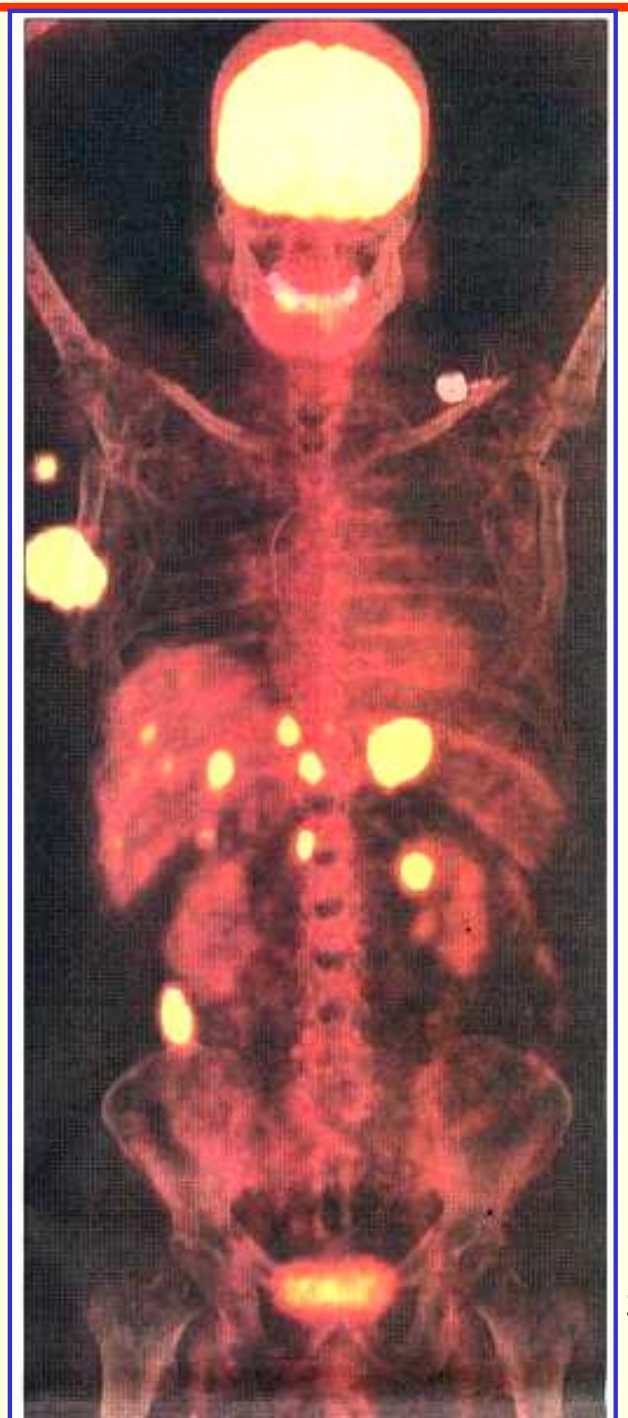
miR-155 (22 NT)

Supplement

Visualization of Metastases of Malignant Melanoma by PET-CT

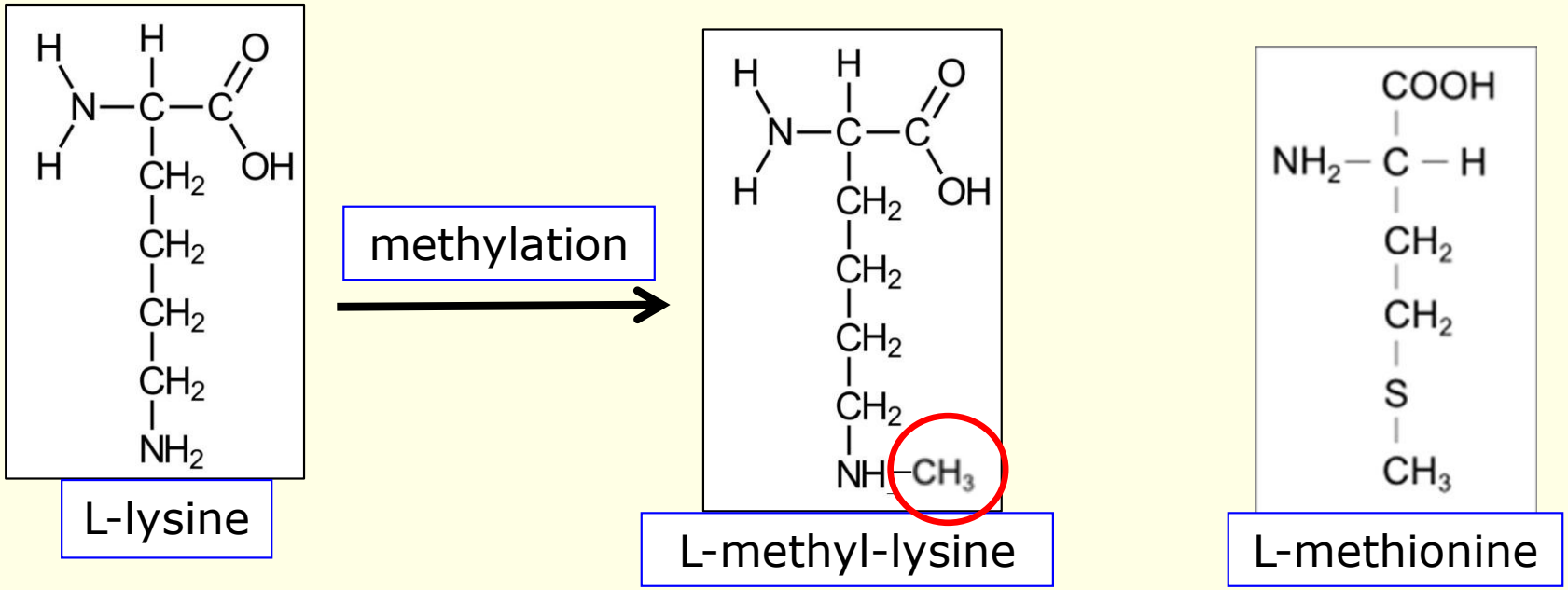
PET: Positron Emission Tomography
CT: Computer Tomography

1. i.v. injection of radioactive desoxyglucose
2. glucose accumulates in cancer cells
3. whole body scan after 10 to 20 min.



Brain Cancer (Glioma) caused by false epigenetic Regulation

- Glioma: very aggressive infantile brain cancer, no treatment available
- medium survival time is 9 months after diagnosis
- mutation in **histon H3**, lysine replaced by methionine, methylation no longer possible
- no methylation = genes for cell proliferation can no longer be swiched off
  -> uncontrolled cell growth **->** brain cancer

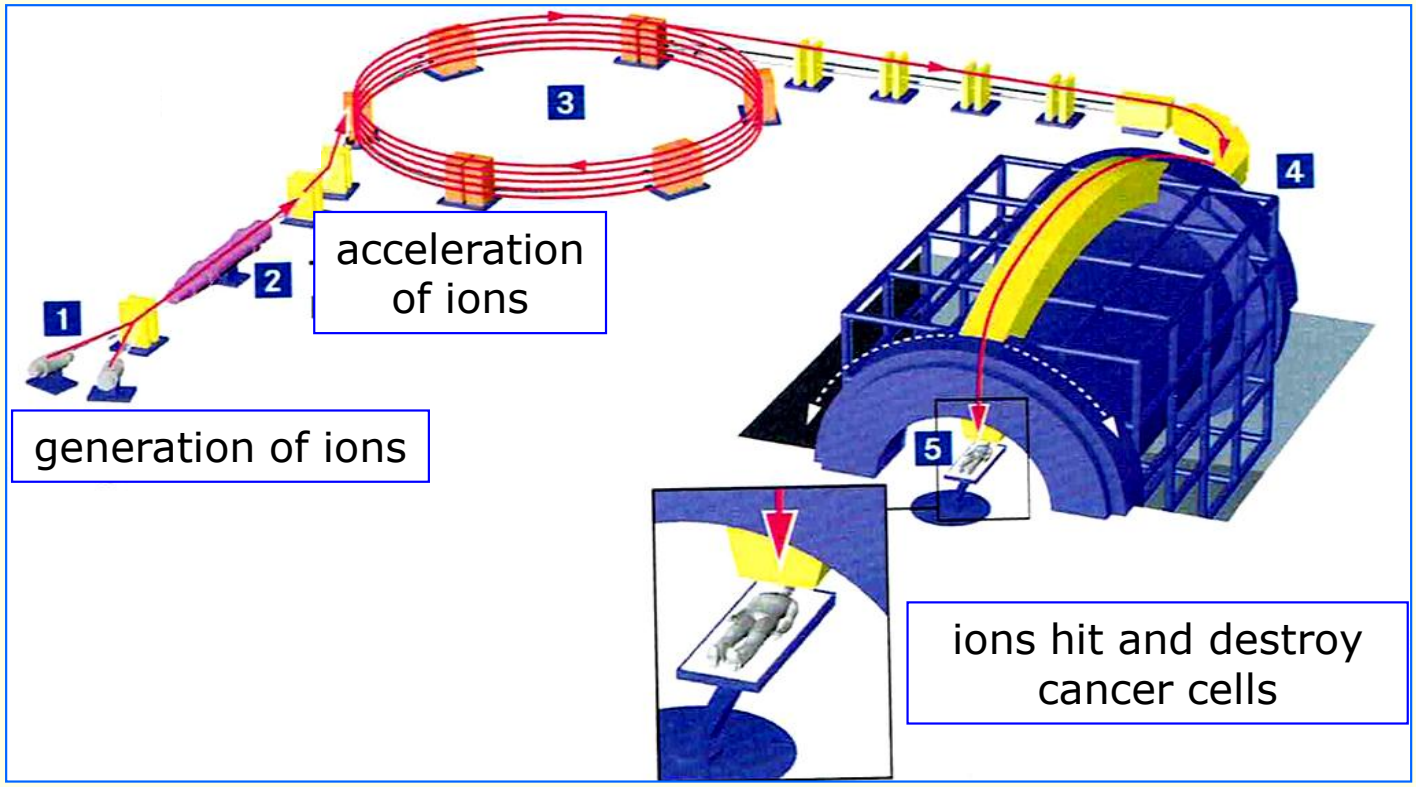


Cancer and Tumor - it‘s not the same

- **cancer** and **tumor** are not identical
- **tumor** is the increase of tissue volume
- **cancer** is the malignant formation of new tissue
- what is the origin of the word „cancer“?
- the greek doctor **GALENOS** (129-216 A.C.) introduced the word „cancer“
- the branched cell structures of breast cancer look like the animal cancer/shellfis)

Heavy Ion Therapy

- 600 tons, 100 million Euros, 7 hospitals in Germany, 58 hospitals world wide
- precise irradiation of the cancer
- carbon atoms or protons, speed of 200.000 km/sec.
- 20.000 patients are treated per year in 7 hospitals
- for treatment of bone-, lung-, and liver cancer

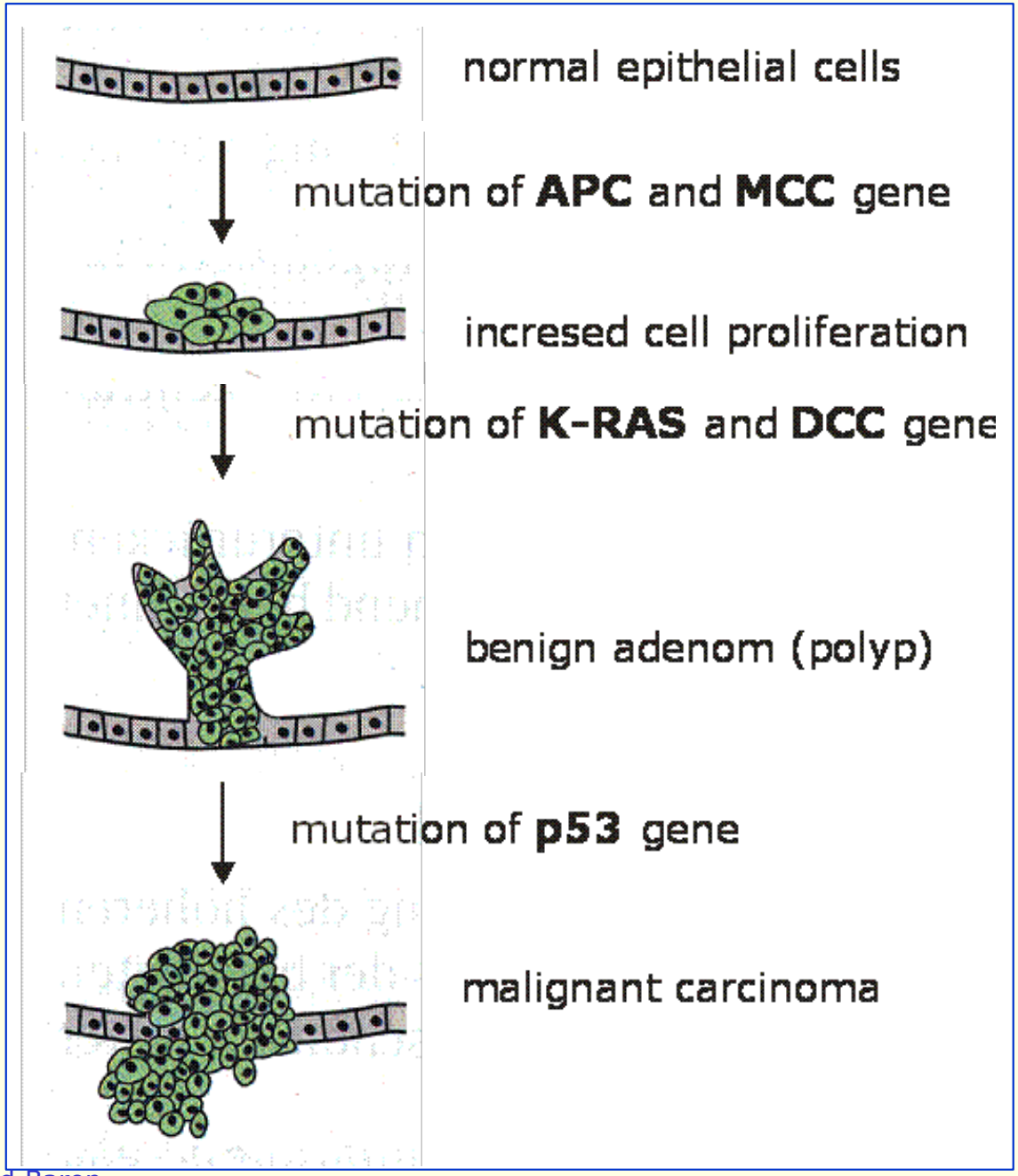


Why is Cancer Therapy so difficult?

- 50% chance to be cured for cancer (von 3% bis 90%)
- what does it mean „cure of cancer"?

1. each cancer differs from person to person
2. permanent mutations **->** heterogeneity of cancer cells **->** resistance to drugs
3. escape mechanisms (15)
4. cancer stem cells
5. cancer vascularization

Five Mutations lead to Colon Cancer



The End