“History of cancer”

3000-1500 BC, Egyptian papyrus (via Latin, from Greek πάπυρος, papyros) (George Ebers, Edwin Smith), Pre Columbian mummies and artefacts

500 BC, Hippocrates (melanoma, melas, "dark" and oma “tumor”)  

460 – 370 BC, Hippocrates:  
- Wording: “carcinos” (crab or crayfish, Greek)  
- Skin, nose, breast

25 BC – 50 AD, Celsus: “cancer” (crab, Latin)

129-199, Galenus: book on oncology  
Distinction between benign (oncos) vs malignant (carcinos) tumour

XVII., Adrian Helveticus, surgery  
1761, Giovanni Morgagni, Padua: postmortem biopsy  
1896, X-ray
Diagnosis of Tumour

- "if it hurts"
- **Perception** (detected by the five senses)
  - Biopsy, histology
  - X-ray (chest, mammography, etc.)
  - Scintigraphy ("scint," Latin scintilla, spark)
  - CT - SPECT (Single photon emission computed tomography)
    - Contrast material remains in the bloodstream
  - PET (Positron emission tomography)
    - Tissue absorption of the labelled (contrast) material
- Immundiagnosis - tumormarkers
- DNS chip/DNS array
Melanoma, lymphoma, breast cancer

http://lymphomacancer.co.uk/lymphoma-cancer-types/burkitts-lymphoma-cancer.html

http://daganatok.hu/melanoma/

http://www.naturalnews.com/051601_breast_cancer_Komen_Foundation_industry.html
### 5-year prevalence (%) vs stages in case of breast cancer

<table>
<thead>
<tr>
<th>Stadium (phase)</th>
<th>5-year relative prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>I</td>
<td>98%</td>
</tr>
<tr>
<td>IIA</td>
<td>88%</td>
</tr>
<tr>
<td>IIIA</td>
<td>76%</td>
</tr>
<tr>
<td>IIIIB</td>
<td>56%</td>
</tr>
<tr>
<td>IIIIB</td>
<td>49%</td>
</tr>
<tr>
<td>IV</td>
<td>16%</td>
</tr>
</tbody>
</table>

Source: American Cancer Society  
[https://www.cancer.org/research.html](https://www.cancer.org/research.html)
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Biopsy, histology, histopathology
(from Greek histos, tissue, pathos, suffering and logos, study)

a) Atypical structure of the tissue

b) Pleomorphism (pleomorphic): Occurring in various distinct forms.
   -- In terms of cells, having variation in the size and shape
     of cells or their nuclei.
   -- Different tissue structures within a tumour.

c) High level of cell division.

d) Infiltration: Cancer that has spread beyond the layer of tissue
   in which it developed and is growing into surrounding, healthy tissues.
   Also called invasive cancer.
Forms typical for inflammation

Forms typical for normal tissue

Forms typical for cancer suspect

Forms typical for cancer
Prostate cancer: is it time to expand the research focus to early-life exposures?
S. Sutcliffe & G. A. Colditz
Nature Reviews Cancer 13, 208-518
(March 2013)
Diagnosis of Tumour

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1896: W. C. Röntgen: **Cathode rays** (emitted by the negative electrode, or cathode, in a vacuum tube) for biological and medical imaging (Nobel prize, 1901)

1903: A. H. Becquerel, M. Sklodowska-Curie, P. Curie: Radioactive decay, also known as nuclear decay or **radioactivity**, and isotopes in nature (Nobel prize, 1903)

1910-15: Gy. Hevesy György: **Isotopes as biological labels, „reporters“** (Nobel prize, 1943)

Using particle accelerator (machine that uses electromagnetic fields to propel charged particles to nearly light speed and to contain them in well-defined beams) for **creation of non-natural isotopes**. Based on the idea of L. Szilárd, E. O. Lawrence (Nobel prize, 1939)

1932: C. D. Anderson: **Discovery of positron** as novel particle (Nobel prize, 1936)

1934: F. Joliot and I. Joliot-Curie: **Discovery of the first non-natural isotope emitting positron** (Nobel prize, 1935)

1975: M. Ter-Pogossian et al.: **the first PET camera**, $^{18}$F-fluoro-desoxy-glucose

(www.teppet.org)
**X-ray**

Radiograph
Light/dark, depending on the absorption rates of the various tissues.
Dense materials (e.g. bone): white,
Soft materials (e.g. fat, muscle): in varying shades of gray.

Originally, X-rays for imaging bones,
Today, improvements:
- better photographic films,
- more accurate focusing systems
- more sensitive detection
At lower-exposure levels, fine detail and subtle differences in tissue density.


Diagnosis of lung cancer - comparison
Computed tomography (CT): combines multiple X-ray images into a 3D model

Small (A) and large (B) tumor in the upper right lung

Left Upper Lobe Cancer note how much more obvious the tumor is on the CT scan compared to chest Xray

Slices: 1-10 millimeters thick.
G. N. Hounsfield: The first CT built (1971)
A. M. Cormack: Theoretical background (1963-64)

- Moving X-ray source and detector
- Covering of the whole body
- 1 sec scanning time

The Nobel Prize in Physiology or Medicine 1979
http://www.nobelprize.org
Normal mammogram

Mammogram showing carcinoma

Oncology in Practice p.4, 3/1994
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Bone scintigraphy

Phosphorus metabolism disorders are the result of abnormal serum phosphate levels, caused by defects in the intake, excretion and cellular utilization of phosphate.

Increased phosphor metabolism in tumorous bone tissues

Increased incorporation of phosphor derivatives.

Application of $^{99m}$Tc-labelled phosphoric acid.
Bone scan (bone scintigraphy) with Tc⁹⁹-jelzett phosphoric acid derivatives

Methane-diphosphonic acid (MDP)

Michaelis-Arbusov reaction (R = ethyl)

\[
P(OR)_3 + \text{ClCH}_2P(OR)_2 \xrightarrow{180^\circ} (RO)_2P-\text{CH}_2 - P(OR)_2 \xrightarrow{\text{HCl}} (HO)_2P-\text{CH}_2 - P(OH)_2
\]

triethylphosphit  methane diphosphoric acid
diethyl ester               diethyl ester

chlormethane phosphoris acid
methane diphosphoric acid
Bone scan (bone scintigraphy) with Tc99-jelzett phosphoric acid derivatives

3,3-diposphono-1,2-propane dicarboxylic acid, DPD

Synthesis (R = ethyl)

\[
\begin{align*}
\text{MDP-tetraalkyl ester} & \quad (\text{RO})_2P-\text{CH}_2 - P(\text{OR})_2 \\
\text{Maleic acid-dialkyl ester} & \quad \text{CH}_2 - \text{COOR} \\
\text{RONa} & \\
\text{HCl} & \rightarrow \text{CH} - \text{COOH}
\end{align*}
\]

Femur-to-blood uptake ratio (Q) of Tc\textsuperscript{99m} carboxyphosphonates as function of time after i.v. in the rat.
Detection of bone metastasis (breast cancer)
Detection of bone metastasis (carcinoma)  
Bone inflammation (periostitis)  

Tc$^{99m}$ - 3,3-diphosphono-1,2-propane dicarboxylic acid (DPD)
Diagnosis of Tumour

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## Comparison

<table>
<thead>
<tr>
<th></th>
<th>MRI</th>
<th>CT</th>
<th>SPECT</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principle</strong></td>
<td>Nuclear magnetic resonance</td>
<td>X-ray transmission</td>
<td>photon emission</td>
<td>pozitron emission</td>
</tr>
<tr>
<td><strong>Frequent isotopes</strong></td>
<td>Tc</td>
<td>Ga</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>structure</td>
<td>structure</td>
<td>function</td>
<td>function</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>&lt; 1 mm</td>
<td>1 mm</td>
<td>4-5 mm</td>
<td>2,8 mm</td>
</tr>
<tr>
<td><strong>Data collection</strong></td>
<td>20 min</td>
<td>2 min</td>
<td>15 min</td>
<td>20 min</td>
</tr>
<tr>
<td><strong>Exposure (mSv)</strong></td>
<td>2-8</td>
<td>6-10</td>
<td></td>
<td>2-10</td>
</tr>
</tbody>
</table>

Gulyás B. Magyar Tudomány, 1999
PET - Positron emission tomography

Photon-pair detection
**P(béta⁺)ET radionuclides commonly used in oncology**

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>Used to measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{15}$O$_2$</td>
<td>$^2$ min</td>
<td>Blood flow, Oxygen metabolism</td>
</tr>
<tr>
<td>$^13$N</td>
<td>$^{10}$ min</td>
<td>Blood flow</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>$^{20}$ min</td>
<td>Amino acid uptake, Glucose utilisation, Proliferation, Somatostatin receptor</td>
</tr>
<tr>
<td>$^{68}$Ga</td>
<td>$^{68}$ min</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>$^{110}$ min</td>
<td>Glucose utilisation, Pyridine uptake, Drug uptake, Estrogen receptors, Monoclonal antibodies</td>
</tr>
<tr>
<td>$^{124}$I</td>
<td>4 days</td>
<td>Monoclonal antibodies</td>
</tr>
</tbody>
</table>

Production and characterization of P(beta⁺)ET isotopes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Distance</th>
<th>Half-life</th>
<th>Max energy</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>^15O</td>
<td>8 mm</td>
<td>2 min</td>
<td>1.74 MeV</td>
<td>14N(d,n) → ^15O</td>
</tr>
<tr>
<td>^13N</td>
<td>5 mm</td>
<td>10 min</td>
<td>1.20 MeV</td>
<td>12C(d,n) → ^13N</td>
</tr>
<tr>
<td>^11C</td>
<td>4 mm</td>
<td>20 min</td>
<td>0.97 MeV</td>
<td>14N(p,a) → ^11C</td>
</tr>
<tr>
<td>^18F</td>
<td>2 mm</td>
<td>110 min</td>
<td>0.64 MeV</td>
<td>18O(p,n) → ^18F</td>
</tr>
</tbody>
</table>
### Radiolabelled drugs for use in PET

<table>
<thead>
<tr>
<th>Radiolabelled drugs</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$N-cisplatin</td>
<td>kinetic studies</td>
</tr>
<tr>
<td>$^{13}$N or $^{11}$C-carmustine</td>
<td>kinetics in normal brain and glioma</td>
</tr>
<tr>
<td>($^{11}$C-$^N$-methyl)temozolomide</td>
<td><em>in vivo</em> mechanism of action</td>
</tr>
<tr>
<td>$^{11}$C-adriamycin</td>
<td><em>in vivo</em> quantify MDR</td>
</tr>
<tr>
<td>$^{57}$Co-bleomycin</td>
<td>tumour and normal tissue kinetics</td>
</tr>
<tr>
<td>$^{16}$-$^{(18}$F)-fluoroestradiol-17-β</td>
<td>measurements of receptor concentration</td>
</tr>
<tr>
<td>$^{18}$F-5-fluorouracil</td>
<td>kinetics, predicting response</td>
</tr>
</tbody>
</table>
Radiolabelled FDG for use in PET

2-Deoxy-2-[\(^{18}\)F]fluoroglucose (FDG) → 2-Deoxy-2-[\(^{18}\)F]fluoroglucose-6-phosphate

- a glucose analog,
- taken up by high-glucose-using cells such as brain, kidney, and cancer cells
- phosphorylation prevents the glucose from being released from the cell

J.F. Vansteenkiste, S.G. Stroobants Eur. Respir J. 2001;17:802-820
Detection of lung cancer: $^{18}$F-deoxyglucose - PET

Lymph node metastasis - brain/bladder (normal)

Radioactive isotope conjugates

- Biosynthetic approach
  - $^{15}C, ^3H, ^{14}C, ^{32}P, ^{35}S$

- Chemical synthesis
  - Direct
    - e.g. Tyr, His modification
  - Indirect
    - a) Covalent linkage
    - b) Complex formation
Considerations

Example: $^{125}\text{I}$, $^{131}\text{I}$
(17 iodine, 13 bromine-, 6 chlorine-, 2 fluor isotopes $t_\frac{1}{2} > 3$ min)

- In vitro
  - Long half-lifetime
  - Low energy emission (photon)

- In vivo „IMAGING“
  - X-ray or $\gamma$-emission
  - SPECT, PET
  - $\gamma$-camera
  - Relatively high energy, short half life

- In vivo THERAPY
  - No optimal combination (high energy: threat)
  - Low energy, long half-life
## Selection of nuclei

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Availability</th>
<th>Cost</th>
<th>Half life</th>
<th>Gamma energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{123}$I</td>
<td>low</td>
<td>high</td>
<td>13 hr</td>
<td>159</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>good</td>
<td>low</td>
<td>8 day</td>
<td>364</td>
</tr>
<tr>
<td>$^{111}$In</td>
<td>good</td>
<td>medium</td>
<td>67 hr</td>
<td>173, 247</td>
</tr>
<tr>
<td>$^{67}$Ga</td>
<td>good</td>
<td>medium</td>
<td>78 hr</td>
<td>185, 300</td>
</tr>
<tr>
<td>$^{99m}$Tc</td>
<td>good</td>
<td>low</td>
<td>6 hr</td>
<td>141</td>
</tr>
</tbody>
</table>
Direct incorporation

Incorporation of iodine isotope into protein
1. Chloramine-T method

\[
\text{His} \quad \text{Tyr}
\]

- 30 s - 30 min
- water-soluble
- pH 7 phosphate buffer 0.05 M

Greewood, FC et al. Biochem J 89 114 (1963)
Wilbur, DS Bioconjugate Chem 3 433 (1992)
2. Immobilised Chloramine-T (IODO-BEADS)

- Polystyrene matrix
- 3 mm
- U.S. Patent 4448764 és 4436718
- 2 - 5 min
- Good protein recovery
- Mild conditions
- pH 7.2 - 8.4

3. IODO-GEN

Fraker, PJ és Speck, JC BBRC 80 849 (1978)

- Good water solubility
- Surface adsorption
- Quick
- Termination by solvent removal

1,3,4,6-Tetrachloro-3a,6a-diphenylglycouril

\[
\text{IODO-GEN} + \text{Na}^{125}\text{I} + \text{H}^+ \rightarrow \text{IODO-GEN} + \text{125Cl}^- + \text{125I}^- + \text{125H}^+
\]
Indirect incorporation
Preparation of conjugates with radiolabel - The Bolton-Hunter reagent

\[
\text{N-Succinimidyld-3-(4-hydroxyphenyl) propionate} \quad \xrightarrow{\text{Chloramine-T iodination reaction}} \quad \text{Iodinated propionate (Bolton and Hunter reagent)}
\]

\[
\text{I}^{125}_\text{i}\text{-labelled protein} \quad \xrightarrow{\text{Conjugation reaction}} \quad \varepsilon\text{-Amino group of lysine in protein to be labelled}
\]
Indirect incorporation by chelators

Linear chelators

Diethylenetriamine pentaacetic acid (DTPA)

DTTA amint tartalmazó molekula tiourea kötés képz dés

Inαirect incorporation by chelators
The principle

\[
\begin{align*}
\text{NH}_2 & + \quad \text{COOH} \\
\text{COO}^- & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{COO}^- \\
\text{CH}_2 & \quad \text{COO}^- \\
\text{In}^{3+} & \quad \text{COO}^- \\
\text{CH}_2 & \quad \text{COO}^- \\
\text{CH}_2 & \quad \text{COO}^- \\
\text{In}^{3+} & \quad \text{COO}^{-}
\end{align*}
\]

D.J. Hnatowich et al., Science 220, 613, 1983

\[
\text{pH 8.2, 0.05 M NaHCO}_3
\]

\[
\text{pK}_s = 28.4
\]
Linear chelators

DFA
deferoxamin
Cyclic chelators

1,4,7-triazacyclononane-1,4,7-triacetic acid

1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

1,4,5,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
**FDA* approved monoclonal antibody conjugates**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Specificity</th>
<th>Type</th>
<th>Indication</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mylotarg</td>
<td>CD33</td>
<td>humanized</td>
<td>acute myeloid leukemia</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>toxin-linked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zevalin</td>
<td>CD20</td>
<td>mouse</td>
<td>non-Hodgkin lymphoma</td>
<td>2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>radioligand-linked</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Food and drug administration

*Nature Med. 9:129 (2003)*
Tc99-radiolabeled mAb against the cellular membrane of the Raji cell component of B cell lymphoma of patients with non-Hodgkin's B-cell lymphoma.

41 year old showing relatively normal distribution of tracer A) Nasopharynx B) Heart C) Liver, Spleen, and Kidney D) Testicles, Bladder and Bone Marrow
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Tumour markers

**Tumour associated antigens**
- In healthy tissue as well
- Less on normal cells
  
  *e.g.* Carcinoembryonic antigens (CEA)

**Tumour specific antigens**
- Only in tumour tissue (cells)
  
  - Induced by carcinogens (chemical, physical or virus)

**In serum**
Antigens: CEA (1965), alpha-fetoprotein, CA19-9, CA125, CA15-3 glycoproteins, psotate/-specific antigen (PSA)
Hormones: human chorionic gonadotropin (HCG), insulin, calcitonin, ACTH
Enzymes: Acid phosphatase (ACP), alkaline phosphatase (ALP), catepsin D
Adhesion molecules: ICAM-1, integrin, cadherin

**In urine**
(bladder cancer) - Mcm5 protein, nuclear matrix protein 22 (NMP22) etc.
**Tumormarkers in clinical practice**

<table>
<thead>
<tr>
<th>Location</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>CEA</td>
</tr>
<tr>
<td>Breast</td>
<td>CEA, CA 15-3, ferritin</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>CEA</td>
</tr>
<tr>
<td>Colon</td>
<td>CEA, CA 19-9, lactate dehydrogenase</td>
</tr>
<tr>
<td>Stomach</td>
<td>CEA, CA 19-9, CA 72-4</td>
</tr>
<tr>
<td>Leukemia</td>
<td>ferritin, lactate dehydrogenase</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>β-2-microglobulin, ferritin, lactate dehydrogenase</td>
</tr>
<tr>
<td>Lung (kissejtes)</td>
<td>CEA, bombesin, calcitonin</td>
</tr>
<tr>
<td>Ovarium</td>
<td>CA 125, CEA</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>thyreoglobulin, calcitonin</td>
</tr>
<tr>
<td>Kidney</td>
<td>erythropoietin, renin</td>
</tr>
<tr>
<td>Testis</td>
<td>α-fetoprotein, lactate dehydrogenase</td>
</tr>
</tbody>
</table>

Not suitable for the screening healthy population.
Suitable for monitoring after surgery/treatment to detect relapse.

„Fingerprint“

*Mucin glycoproteins*
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    tissue absorption of the labelled (contrast) material
- Immundiagnosis - tumormarkers
- DNA chip/DNA array
DNA probe array

DNA chip produced using Bubble Jet printing technology

DNA chip
- Formed through covalent binding
- Homogeneous
- Temperature stability

Cancer cell

Fluorescence DNA

Identification
- Ultraprecise detection
- On/off determinant
DNA (oligonucleotid) chip
Expression levels of 50 genes most highly correlated with the acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

Expression levels greater than the mean: red, below the mean: blue.

Expression levels of predictive 50 genes most highly correlated with the ALL-AML in independent dataset.

**Independent Set**
- 35 patients

**Expressed in ALL**
- 20 genes

**Expressed in AML**
- 20 genes

**Expression levels greater than the mean: red, below the mean: blue.**