Antineoplastic Agents II.
Topoisomerase inhibitors

- Podophyllotoxin from the roots of the poisonous *Podophyllum peltatum* (mayapple) has many medical uses (laxative, anthelmintic to treat worms, poison) of the native American tribes.
- Modern medicine uses it for the treatment of external condylomata acuminata (warts) caused by the human papillomavirus.
- Semisynthetic derivatives are antineoplastics.
They bind to DNA – topoisomerase II complex and suspend enzyme activity in its opened state \(\rightarrow\) DNA strand breaks.
Etoposide, Tenoposide

- Etoposide: testicle tumors, small cell lung carcinoma, Hodgkin- and non-Hodgkin lymphoma
- Teniposide: acute lymphoid leukemia
DNA intercalators

Intercalation - reversible inclusion of a molecule in the space between two adjacent base pairs helding by secondary bonds (mostly polycyclic, aromatic, and planar molecules)
Camptothecin derivatives

- Alkaloid camptothecin from the *Camptotheca acuminata* tree (China)
- Anticancer properties discovered in 1958
- Too much toxic side effects and very low solubility → semisynthetic derivatives.
**Irinotecan, topotecan**

Increasing solubility by substitution

Camptothecin  
(Camptotheca acuminata)

Irinotecan

Topotecan

Active agent
The intercalated molecule inhibits the function of topoisomerase-I enzyme.

Topotecan: small cell lung carcinoma, metastatic ovarian carcinoma.

Irinotecan: colorectal carcinoma in combination with 5-fluorouracil or cisplatin.
Bleomycin

- From *Streptomyces vermicullus* (H. Umezawa et al., 1966)
- Isolated as Cu-complex.
- The A₂ component is marketed.

\[ \text{Bleomycin A}_2: R = \text{CH}_2\text{S}^{+}\text{CH}_3 \]
\[ \text{Bleomycin B}_2: R = \text{CH}_2\text{CH}_2\text{NH} \text{CH}_2\text{NH}_2 \]

\[ \alpha\text{-D-mannopyranosyl-} \alpha\text{-L-gulose} \]
The molecule is anchored, intercalated into the DNA by the flat thiazol rings.

Active form is the Fe\(^{2+}\) chelate, the sixth position is occupied by an O\(_2\) molecule. The Fe-O chelate decomposes to yield reactive superoxide radicals, which cleave the nearby DNA strand.
Anthracycline glycoside antibiotics

- Daunorubicin, Cassinelli & Orezzi (1963), *Streptomyces peucetius*
- Doxorubicin (1969)
- Carubicin (1973) (*Actinomadura carminata*)
Chemical conversion of daunorubicin to doxorubicin
<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daunorubicin</strong></td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Hematological tumors: acute lymphocytic and granulocytic leukemia. Cardiotoxic.</td>
</tr>
<tr>
<td><em>+ + Daunomycinium chloratum</em></td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Malignant lymphomas, solid carcinomas (breast, lung, ovaries) and sarcomas, certain leukemias. Cardiotoxic.</td>
</tr>
<tr>
<td><strong>Epirubicin</strong></td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Similar to doxorubicin with less cardiotoxicity.</td>
</tr>
<tr>
<td><em>+ + Epirubicinium chloratum</em></td>
<td><img src="image4.png" alt="Structure" /></td>
<td>Myeloid leukemia and breast cancer. Somewhat less cardiotoxicity.</td>
</tr>
<tr>
<td><strong>Idarubicin</strong></td>
<td><img src="image5.png" alt="Structure" /></td>
<td>- Myeloid leukemia and breast cancer. Somewhat less cardiotoxicity.</td>
</tr>
</tbody>
</table>
Epirubicin (Farmorubicin)

Changing the configuration of OH at C-4’ reduces toxicity (faster elimination)
Mode of action

- The planar aromatic ring system is intercalated between the nucleosides, the polar sugar moiety fixes the molecule with H-bonds.
- Hindrance of topoisomerase II enzyme from normal functioning.
Adverse effects, toxicity

- Bone marrow depression, leucopenia
- Cumulative, long term cardiotoxicity is dose limiting factor.
- Reduction of the quinone moiety of the ring system leads to semiquinone radical anion, which converts O₂ to superoxide anion in the presence of Fe²⁺ → oxydative stress of the myocardium (lipid peroxydation)

- Toxic effect can be reduced with prior administration of dexrazoxane → complexation of Fe²⁺.

- Purple compounds, stain urine.
Anthracycline toxicity

- **Quinone**
  - \[ \text{O} - \text{O} - \text{OH} - \text{OH} - \text{O-glikozid} \]

- **Semiquinone radical anion**
  - \[ \text{O} - \cdot \text{OH} - \text{O-glikozid} \]

- **Iron(II)-complex**
  - \[ \text{Fe(II)} \]

- **Lipid peroxidation**
  - \[ \text{O}_2^- \]

- **Irreversible damage of the myocardium**
  - \[ \text{O}_2 \]
Mitoxantrone

- Mitoxanthroni hydrochlor.
- Novantronel® injection
- Onkotrone® injection
- Refador® injection

- Synthetic analogue of anthracycline antibiotics, strong blue stain.
- Similar mode of action and indications.
- Less cardiotoxic than anthracyclines, most disturbances are reversible.
Cytostatic agents, targeted chemotherapy

Cytostatic agents

– target biochemical processes or specific proteins, which are unique to the tumor cells or are overexpressed compared to the normal cell;
– tumorspecific;
– less toxic side effects.

→ Targeted chemotherapy

They are:

– enzyme or receptor inhibitors to block signaling pathways;
– selective carriers of cytotoxic agents.
## Potential targets and tools of selective chemotherapy

- **Monoclonal antibodies** (targeting surface proteins to block them or used as carriers)
- **Tyrosine kinase inhibitors** (targeting signal transduction)
- **Angiogenesis inhibitors** (targeting new blood vessel development)
- **Antisense and ribozyme therapeutics** (targeting oncogenes)
- **Metalloprotease inhibitors** (targeting tissue degradation → invasiveness)
Delicately balanced system with positive and negative feedbacks and loops.

External activation via "death" receptors

Caspases

Signal transduction

Inner damages, negative signals

Promoting factors

Suppressing factors

Lack of signals

Kinases

DNA fragmentation

Apoptosis

Normal activation of genes

egfr
Types of useful receptors

- Downregulation of receptors necessary for proliferation (growth factor receptors)
  - Antagonize ligand-receptor signaling
  - Extracellular growth factor signaling mediated by the receptor tyrosine kinase is inhibited
    • EGFR (epidermal growth factor receptor, ErbB, HER)
    • VEGFR (vascular endothelial growth factor receptor).

- These receptors belong to the tyrosin kinase family of signal transmitters.
Concepts of therapeutical antibodies

Receptors and ion channels embedded into the cell membrane

Overexpressed receptors in tumor cells
Concepts of therapeutical antibodies

Immunoglobulins targeted to overexpressed receptor proteins
Monoclonal antibody production
Additional genetic engineering

Chimeric antibodies are at least 70% human
Humanized antibodies are at least 90% human
Concepts of therapeutic antibodies

- **Naked MAB**
- Radioimmunoconjugate
  - Radionuclide
- Immunocytokine
- Immunotoxin
- ADEPT
  - enzyme
  - prodrug
  - drug
- Immunoliposome
- Multistep targeting
  - Biotinylated radioactive ligand
  - streptavidin
  - Killer cell
  - Cellular immunoconjugates

**Immunoconjugates**
## Therapeutic MABs approved by FDA

<table>
<thead>
<tr>
<th>Type</th>
<th>Iso type</th>
<th>Target</th>
<th>Indication</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab (Rituxan)</td>
<td>Chimeric</td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>CD20</td>
<td>1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B-cell NHL</td>
<td></td>
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<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Humaniz.</td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>HER2/neu</td>
<td>1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metastatic breast cancer</td>
<td></td>
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<tr>
<td>Alemtuzumab (Campath)</td>
<td>Humaniz.</td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>CD52</td>
<td>2001</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CLL</td>
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<tr>
<td>Bevazizumab (Avastin)</td>
<td>Humaniz.</td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>VEGF</td>
<td>2004</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Metastatic CRC</td>
<td></td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>Chimeric</td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>EGF r.</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metastatic CRC</td>
<td></td>
</tr>
</tbody>
</table>
Rituximab (Rituxan®, Mabthera®)

- 1st therapeutic chimeric murine/human monoclonal antibody approved by FDA in 1997

- Binds to the antigen CD20, which regulates the activation process for cell cycle initiation and differentiation of pre-B and mature B lymphocytes, incl. 90% of NHL.

- Binding Rituximab to CD20 causes: autophosphorylation, activation of serine/tyrosine protein kinases $\rightarrow$ caspases, apoptosis, lysis

- Infusion related adverse effects: flu-like fever, chills/rigors, nausea, urticaria, headache, bronchospasm, angioedema and hypotension.
Trastuzumab (Herceptin®)

- HER-2/neu or ErbB2: human epidermal growth factor receptor 2. (EGFR family of tyrosine kinases).
- Overexpressed in ~25% of breast cancers.
- Humanized murine MAB. Downregulates the receptor by preventing dimerization and presumably activates killer cells too.
Trastuzumab (Herceptin®)

- Metastatic breast carcinoma:
  - Monotherapy: ~4% complete remission
  - Combination (taxan, anthracyclines): ~50%
- Adverse effects: cardiotoxicity (especially after anthracyclines).
Small molecule kinase inhibitors
Imatinib (Glivec®, Gleevec®)

- Precisely targeted small molecule against CML and GIST.
- The CML tumor cells display a characteristic chromosome anomaly.
- Blocks an abnormal protein kinase found only in the tumor cells.

GIST: Gastrointestinal stromal tumor
CML: Chronic myeloid leukemia
Philadelphia chromosome

Rowley et al. (1973)

Nowell and Hungerford (1960, Philadelphia)
Philadelphia chromosome and Bcr-Abl kinase

Bcr gene of chromosome 22

Abl gene of chromosome 9

translocation

Bcr-Abl chimeric protein

Flawy but still functioning kinase

Code of a tyrosine kinase (Abelson kinase)

Mixed but functioning code
Bcr-Abl kinase and false signals

Substrate protein, next member of the signaling pathway

Kinase

Continuous phosphorylation without being triggered

Bcr-Abl

Continuous signaling and unregulated proliferation

leukemia

normal proliferation

signal transduction

signal

ATP

ADP P

P

P

P

P

P
Bcr-Abl kinase and imatinib

Imatinib fits and blocks the ATP binding site

no signal, no proliferation

Imitanib mesylate
<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dasatinib</strong></td>
<td><img src="image" alt="Structure" /></td>
<td>BCR/ABL tyrosine kinase + chronic myeloid leukemia in case of imatinib resistance</td>
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<td>Sprycel® filmtablets</td>
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<td><strong>Nilotinib</strong></td>
<td><img src="image" alt="Structure" /></td>
<td>EGFR overexpressed pancreatic and non-small cell lung carcinoma</td>
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<td>Tasigna® filmtablets</td>
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<tr>
<td><strong>Erlotinib HCl</strong></td>
<td><img src="image" alt="Structure" /></td>
<td>Renal cell carcinoma, GIST</td>
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<td>Tarceva® filmtablets</td>
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<tr>
<td><strong>Sunitinib malate</strong></td>
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<td>Sutent® capsules</td>
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<tr>
<td></td>
<td>* HCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* malic acid</td>
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</tbody>
</table>
Imatinib (Glivec®, Gleevec®)

- 400-800 mg daily CML or metastatic GIST, good oral bioavailability
- Metabolized in the liver by cytochrome P450
  - May increase the plasma level of other drugs
- Mild hepatotoxicity, fluid retention, nausea, vomiting
Angiogenesis - Formation of nascent blood vessels

Tumor cells release angiogenic factors VEGF-A, VEGF-B etc.

Surrounding blood vessels

1-2 mm avascular tumor
VEGF = Vascular endothelial growth factor

- VEGF
  - One of the most upregulated antigens in cancer
  - Protect endothelial cells from apoptosis via activation of PKC pathways and upregulation of anti-apoptotic proteins such as Bcl-2
  - Activity mediated by tyrosine kinase receptors, VEGFR 1 and VEGFR 2
  - Functions indirectly as survival factor for tumor cells

- Inhibition of VEGF signaling:
  - Block the receptor
  - Inhibits tumor growth and metastasis
  - Deprives tumors of nutrient-providing blood vessels
VEGF

VEGF A
VEGF B

Neutralization of VEGF

VEGF receptor

kinase activity

signal transduction

gene expression

Endothelial cell

Endothelial cell proliferation and migration, Capillary formation

Angiogenesis
Bevacizumab (Avastin®): Recombinant Humanized Monoclonal Antibody to VEGF

- 93% human, 7% murine (IgGk1 construct)
- Recognizes all isoforms of VEGF
- Received accelerated FDA approval in February, 2004
Bevacizumab (Avastin ®)

- Advanced/metastatic breast or colorectal cancer in combination with 5-FU and irinotecan
- Toxicities: hypertension, thromboembolic risk26
Immun conjugates

- Targeted delivery of cytotoxic agents.
- Toxic substances are physically linked to MABs via a spacer moiety.
- Cell surface antigen must be internalized upon MAB binding.
- Drug must be released from macromolecule after cellular uptake of the conjugate.
Internalization and endocytosis of conjugates

antibody-drug conjugate

coated pit

clathrin

coated endosomal vesicles

lysosome
Trastuzumab emtansine (Kadcyla®)

Maytansine spacer

Ansamacrolide

- End of 70s: human phase II cancelled because of toxicity.

- Beginning of 90s: first experiments with derivatives attached to mab.

- 2005-7: further optimalizations, etc.

- 2013: Trastuzumab emtansine (against her2 positive metastatic breast carcinoma)
Ibritumomab-tiuxetan (Zevalin™)

- Monoclonal antibody radioimmunotherapy treatment for some forms of B cell non-Hodgkin's lymphoma.