Chemotherapy (CT): General points

- 35 CT drugs are currently available in North America; ~15 are in common use.
- Many patients receive CT at some point in their management.
- Useful symptom relief and disease arrest are often obtained.
- CT is generally used as:
  (a) the major curative modality for malignancies such as Hodgkin's disease and other lymphomas, acute childhood leukemia, and testicular cancer;
  (b) palliative treatment for many advanced cancers;
  (c) adjuvant treatment before ("neo-adjuvant") or after ("post-adjuvant") local treatment (surgery or XRT) for primary disease with the aim of eradicating occult micrometastases; or
  (d) in combination with other modalities (usually XRT) to improve their therapeutic effects.
• Improvements in CT have come largely from the use of drug combinations.

• Some drugs were combined because of a theoretical or experimental basis for expecting synergistic interaction based on mechanisms of action.

• Synergy only leads to a therapeutic benefit if the interaction between drugs is preferential/specific for the tumor.

• Important factors underlying the success of combination CT include:
  (a) ability to combine drugs at close to full tolerated doses with additive effects against tumors and less than additive toxicities to normal tissues; and
  (b) potential avoidance of the development of drug resistance (e.g., in the combined use of doxorubicin and cyclophosphamide).

• Most drugs exert dose-limiting toxicity for the bone marrow, but this is not the case for vincristine (neurotoxic), cisplatin (nephrotoxic), or bleomycin (mucositis and lung toxicity).
- All anticancer drugs have normal tissue toxicity that limits the dose that can be given.

- If a drug is to be useful, its antitumor effects must occur at lower doses than those that cause dose-limiting normal-tissue toxicity.

- **Therapeutic index** may be defined as the ratio of the doses required to produce a given probability of toxicity and antitumor effect.

- E.g., the ratio of doses that produce a 5% probability of severe toxicity and 50% probability of antitumor effect.

• Such dose-response curves have been defined only rarely for humans.

• Improving the therapeutic index is the goal of experimental CT.

• Any modification in treatment that leads to increased antitumor effect must be assessed for its effects on critical normal tissues prior to therapeutic trials.
• For most solid tumors, the limit of clinical/radiological detection is $\sim 1$ g of tissue ($\sim 10^9$ cells).

• If CT can reduce the number of malignant cells below this limit of detection, the patient is described as being in **complete clinical remission**.

• Even in a "surgically confirmed complete remission" there may be many tumor cells. Tumor **cure** requires eradication of *all* clonogenic tumor cells.

• Attaining complete remission is only a small step toward tumor cure.

As with XRT, repopulation may occur between courses, so the number of tumor cells may change with time. If each CT course kills 90% of the tumor cells and starting from a large (100 g) tumor, complete clinical remission is achieved after 3 courses. However, a further 6 to 10 courses would be required to achieve cure.

Important to continue aggressive treatment during complete remission.

Unfortunately, for most solid tumors, a drug-resistant sub-population emerges and leads to relapse.

This is the major barrier to successful CT.
Clinical resistance to CT agents

- Clinically, 2 classes of drug resistance are apparent: intrinsic and acquired. E.g., in breast cancer or small-cell cancer of the lung some patients do not respond to their initial CT (intrinsic resistance); others respond to the initial treatment, but acquired resistance to further therapy usually prevents drug treatment from being curative.

- Even if drug-resistant cells are present initially only at low frequency (e.g., 1 per $10^5$ cells), their selective advantage during treatment will lead to their rapid emergence as the dominant cell population and to relapse.

- Clinically important drug resistance is probably due to both genetic (e.g., point mutation and gene amplification) and epigenetic (e.g., changes in patterns of DNA methylation that influence gene expression) mechanisms.
“Classical” anticancer drugs are grouped into several families based on their biochemical activities/origins:

<table>
<thead>
<tr>
<th>ALKYLATING AGENTS</th>
<th>NATURAL PRODUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen Mustard</td>
<td>Doxorubicin (Adriamycin)</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Daunorubicin</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Actinomycin C</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Bleomycin</td>
</tr>
<tr>
<td>Busulfan</td>
<td>Mitomycin C</td>
</tr>
<tr>
<td>Chloroethyl nitrosoureas:</td>
<td>Vinblastine</td>
</tr>
<tr>
<td>BCNU</td>
<td>Vincristine</td>
</tr>
<tr>
<td>CCNU</td>
<td>VP-16 (Etoposide)</td>
</tr>
<tr>
<td>Methyl-CCNU</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANTIMETABOLITES</th>
<th>MISCELLANEOUS AGENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>Cisplatin</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Carboplatin</td>
</tr>
<tr>
<td>Cytosine arabinoside</td>
<td>DTIC (Dacarbazine)</td>
</tr>
<tr>
<td>6-Thioguanine</td>
<td></td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td></td>
</tr>
<tr>
<td>Gemcitabine</td>
<td></td>
</tr>
</tbody>
</table>
1. Alkylating agents

- Highly reactive electrophilic drugs that form covalent bonds with a number of electron-rich groups of biological molecules (such as amino, phosphate, sulfhydryl, and hydroxyl groups).
- Have either 1 (monofunctional) or 2 (bifunctional) electrophilic side chains.
- Major mechanism of cytotoxicity involves interaction with DNA bases.
- Bifunctional drugs can form DNA interstrand cross-links (ISCs).
- Major classes of alkylating agents include:
  (a) nitrogen mustards (e.g., melphalan, cyclophosphamide);
  (b) chloroethyl nitrosoureas (e.g., BCNU, CCNU, methyl-CCNU); and
  (c) methane sulfonic acid esters (e.g., busulfan).
a. Nitrogen mustards

- Bifunctional drugs with 2 electrophilic chloroethyl (-CH₂CH₂Cl) groups that alkylate DNA predominantly at the electron-rich N-7 position of guanine.
Mechlorethamine (nitrogen mustard)

- The first drug used for modern cancer CT, it is now used mainly to treat Hodgkin's lymphoma as part of the MOPP regimen (combined Mechlorethamine-vincristine (Oncovin)-Procarbazine-Prednisone).
- Problems with clinical use because of its high reactivity.
- Because of its rapid reaction rate, mechlorethamine must be given i.v.
- As with all alkylating agents, bone marrow function is depressed.
Melphalan (L-phenylalanine mustard)

- One of several modified mustards that were synthesized in an attempt to produce a drug that would preferentially localize in a particular tumor.

- Phenylalanine is a precursor of melanin; a phenylalanine mustard derivative might accumulate in melanomas and produce a selective toxicity.
- Although this effect was not achieved, melphalan is a very useful drug.
- The presence of the electron-withdrawing aromatic ring next to the nitrogen slows the rate of cyclization (immonium ion formation) and thus of alkylation, making melphalan much less reactive than mechlorethamine.
- This allows time for absorption and wide distribution before extensive alkylation occurs.
- It also means that the drug can be given orally.
- Melphalan crosslinks DNA in a manner similar to mechlorethamine.
- Principally used to treat multiple myeloma; also ovarian/breast tumors.
- Major toxicity is bone marrow suppression.
Cyclophosphamide (Cytoxan; Ctx)

- Another "modified" mustard developed in the hope that might be preferentially activated in tumor cells. High phosphatase and phosphoramidase activity had been observed in some tumors; cleavage of the phosphamide ring might produce an active compound within the tumor cells.

- Although such selectivity was not achieved, the drug has a wide application in CT.

- Ctx is a nontoxic pro-drug that is metabolically activated to 4-hydroxyCtx by microsomal cytochrome P-450 in the liver.

- Used to treat CLL, multiple myeloma, Hodgkin's disease, non-Hodgkin's lymphomas, ALL, Burkitt's lymphoma, and a variety of solid tumors, including Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, and carcinomas of the breast, lung, and ovary.

- Given either orally or i.v.
b. Chloroethyl nitrosoureas

- The 2-chloroethyl carbonium ion (\(^{+}\text{CH}_2\text{CH}_2\text{Cl}\)) is the alkylating moiety of these drugs.

- BCNU has 2 chloroethyl groups, whereas CCNU and methyl-CCNU are monofunctional agents. BCNU induces ISCs.

- Nitrosoureas are highly lipophilic and readily pass into the cerebrospinal fluid. For this reason they are used to treat tumors in the CNS of both primary and metastatic origin.

- Significant activity in Hodgkin's disease and melanoma and useful in 2ndry therapy of non-Hodgkin's lymphomas and lung/colorectal cancers.

- BCNU is given i.v. CCNU and methyl-CCNU are usually given orally.

- Largely replaced by Temozolomide (Temodar\(^{\text{®}}\)) for brain tumors.
Alkylating agents have a number of important biological effects, including:

- **Cell killing and apoptosis**: since the aim of CT is cure or long-term remission, the critical factor for drug efficacy is the killing of tumor stem cells.

- **Cell-cycle arrest**: cells treated with alkylating agents display a marked arrest in $G_2$ phase.

- **Mutation and transformation**: alkylating agents often cause secondary malignancies; there is increased risk if patients also receive XRT.
2. Antimetabolites

Drugs that resemble normal metabolites and that compete as substrates for enzyme activity.

5-fluorouracil (5FU)
- An inhibitor of thymidylate synthase (TS).
- Widely used for breast and gastrointestinal (GI) cancers.
- Major toxicity is to bone marrow and mucous membranes.
**Antimetabolites, contd.**

**Methotrexate**

- Competitively inhibits **dihydrofolate reductase (DHFR)**, the enzyme that converts folic acid to reduced folate cofactors (i.e., tetrahydrofolate).

- Use includes osteosarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, cutaneous T cell lymphoma, head and neck cancer, lung and breast cancers.

- Given orally or intrathecally.

- Toxicity: immunosuppressive.
Cytosine arabinoside (Ara-C)

- Ara-C differs from deoxycytidine in having a $\beta$-OH group on the 2-position of the sugar, so its sugar moiety is arabinose rather than deoxyribose.
- A competitive inhibitor of DNA polymerase; it also incorporates into DNA.
- Used primarily to treat acute leukemia.
- Bone marrow toxicity is major side effect.
- Others: gemcitabine, fludarabine, cladribine.
3. Natural products

Doxorubicin and related drugs

- Doxorubicin (Adriamycin) is a planar 4-ring anthracycline molecule linked to the sugar daunosamine. It has a wide range of clinical activity for many solid tumors and leukemia.
- Cytotoxicity is mediated by intercalation and by the induction of topoisomerase II-mediated DNA strand breaks.
- Epirubicin and daunorubicin are analogues of doxorubicin.

Amsacrine (AMSA)

- A synthetic acridine derivative that causes topoisomerase II-mediated DNA strand breaks.
- Used mainly in the treatment of acute leukemia.
**Actinomycin D**

- Used mainly in the treatment of childhood tumors.

**Bleomycin**

- Causes DNA strand breaks through a complex sequence of reactions that involves the binding of a bleomycin-Fe(II) complex to DNA.
- Used in combination CT for testicular cancer and lymphomas.
Vinca Alkaloids

- Vinblastine and Vincristine are multi-ring compounds derived from the periwinkle plant.
- They bind to tubulin, thus inhibiting its polymerization to form microtubules and disrupting the mitotic spindle.
- Vinblastine is an important drug in combination CT of testicular cancer.
- Vincristine is a mainstay of treatment for childhood leukemia.
VP-16 and VM-26

- VP-16 (etoposide) and VM-26 (teniposide) are semisynthetic glycoside derivatives of podophyllotoxin that target topoisomerase II.

- VP-16 is used to treat small-cell lung cancer, testicular cancer, and lymphomas.

Mitomycin C

- Acts similarly to alkylating agents.
- More active against hypoxic than against aerobic cells in tissue culture.
Natural products: Taxanes

- Taxanes (taxol, taxotere) stabilize microtubules.
- Widely used in the treatment of breast cancer.
4. “Platinating” agents: Cisplatin and Carboplatin

- Act by a mechanism similar to the alkylating agents.

- Cisplatin is used in drug combinations that can cure testicular cancer and palliate a variety of solid tumors.

- Carboplatin is an analogue of cisplatin that has come into clinical use.

- Newer compounds include tri-nuclear platinating agents.
Tumor cells may develop resistance to CT drugs through a number of mechanisms, including:

1. **Transport.**
2. **Glutathione and associated enzymes.**
3. **DNA repair.**
4. **Tolerance to DNA damage.**
5. **Drug-specific mechanisms.**
1. Transport

• Mutation/altered expression of a drug transport protein leading to decreased drug uptake (for agents such as melphalan and mechlorethamine that are actively transported).
Nucleoside analogs: Membrane transport proteins and drug resistance

• Drug-specific transporters such as hENT1 (human equilibrative nucleoside transporter 1) are responsible for the uptake of important anticancer nucleoside analogs, such as gemcitabine.

• Down-regulation of hENT1 might thus represent a mechanism of resistance to such drugs.

Courtesy of Dr. J. Mackey.
Nucleoside analog responsiveness and hENT1

- hENT1 immunohistochemistry on 21 consecutive patients treated with gemcitabine for palliation of advanced/recurrent pancreatic adenocarcinoma.

Overall patient survival from initiation of gemcitabine therapy in pancreatic cancer

Drugs associated with MDR and increased expression of p-glycoprotein

- The "multi-drug resistance" or MDR-associated drugs: amplification of the *mdr* genes that encode the ~170 kDa membrane P-glycoprotein (MDR1) that effects the efflux of drug from resistant cells.
- Also “multidrug resistance protein (MRP) and others.

**Actinomycin D**
- Colchicine
- **Daunorubicin**
- **Doxorubicin**
- Epirubicin
- **Etoposide (VP-16)**
- Mitoxantrone
- Puromycin
- Vinblastine
- **Vincristine**
- Vindesine

Courtesy of M. O’Leary website.
2. Glutathione and associated enzymes

- Cells can synthesize high concentrations of nucleophilic sulfhydryl compounds such as glutathione (GSH), which can react with electrophilic drugs and render them less toxic and more easily excreted.

- GSH is a tripeptide of \(\gamma\)-glutamic acid/cysteine/glycine.

- GSH synthesis can be inhibited by buthionine sulfoximine (BSO). BSO has undergone extensive clinical trials for reversing clinical drug resistance.

- Conjugation with GSH is catalyzed by glutathione S-transferases (GST), which have several isozyme forms that are encoded by multiple genes.

- Some drug-resistant cell lines have increased activity of one or more GSTs.

- Increased levels of metallothioneins, proteins rich in sulfhydryl-containing cysteine residues, are associated with resistance to alkylating agents and cisplatin.
3. DNA repair

- DNA ISCs are the major cause of cytotoxicity for the bifunctional alkylating agents and cisplatin.
- Increased ability to repair ISCs is often a factor in the development of resistance to these drugs.
- E.g., drug-resistant human tumor cells have been found to over-express a specific DNA-repair gene such as \textit{ERCC1} that mediates the repair of ISCs.
4. **Tolerance to DNA damage**

- In many studies, an increased ability of drug-resistant cells to "tolerate" DNA damage has been described which is not directly associated with increased DNA repair.

- Mechanisms of tolerance include the loss of proteins that recognize DNA damage, such as the mismatch repair (MMR) proteins and the high-mobility group (HMG)-domain family of proteins.

- Well established for cisplatin, the extension of this mechanism to other drugs is uncertain.
• Some CT agents kill tumor cells by collaborating with the cells apoptotic (suicide) program, which must be functional for the cell to be sensitive to treatment.

• Alterations in pathways that detect damage and transmit signals to the apoptotic machinery may contribute to tolerance.

• Mutations that result in the loss of factors responsible for triggering apoptosis can contribute to resistance to CT agents.
5. **Drug-specific mechanisms**

- **Ctx:** aldehyde dehydrogenase (ALDH) is an enzyme that inactivates the active metabolite of Ctx before it can damage DNA.

- **BCNU, Temozolomide:** O⁶-alkylguanine DNA alkyltransferase is a “suicide” enzyme that removes adducts from the O⁶ position of guanine.

- **Methotrexate:** amplification of the gene encoding the drug's target enzyme, dihydrofolate reductase (DHFR).

- **5FU:** amplification or over-expression of the gene encoding the drug's target enzyme, thymidylate synthase (TS).

- **AMSA, doxorubicin, VP-16 and VM-26:** decreased activity and/or presence of variant forms of topoisomerase II.
Proliferation dependency of cell killing

- Most CT agents are more cytotoxic toward rapidly proliferating than non-proliferating cells, i.e., they are "proliferation-dependent" agents.
- These include the nitrogen mustards, doxorubicin and 5FU.
- Exceptions include BCNU, CCNU and cisplatin.
- This factor is generally 1-2-fold, but Ctx has a greater (2-5-fold) specificity for cycling cells.
- Proliferative rate is therefore a major determinant of drug activity.
- For example, doxorubicin.
Cell-cycle phase specificity of CT agents

- Most drugs show a marked variation in cytotoxicity around the cell cycle.

- Alkylating agents are cell cycle phase non-specific (i.e., they kill cells in all phases of the cell cycle). However, they do kill differentially in the various phases, being more cytotoxic in G₂/M and at the G₁/S boundary.

- Antimetabolites are generally toxic only to cells that are synthesizing DNA; methotrexate and doxorubicin have maximum toxicity for S-phase cells but have some activity during the other phases.

- Surprisingly, Vincristine and Vinblastine are toxic to cells in S phase, which is presumably when formation of the mitotic spindle is initiated.
Improved CT responses might be obtained by:

- Linking drugs to antibodies or growth factors that recognize antigens or receptors on tumor cells (cf. RIT).
- Entrapment of drugs such as Doxorubicin in lipid vesicles known as **liposomes**.
- The site of localization depends on the size of the liposomes and their membrane composition.
- Liposomes might overcome drug resistance that is due to decreased membrane transport.
- Ditto nanoparticles.
[2] Use of high-dose CT through overcoming bone marrow toxicity

- CT dosage is usually limited by toxicity to bone marrow.

- Reduction of these toxic effects may be achieved by co-administration of growth factors such as G-CSF and GM-CSF.

- Bone-marrow transplantation (BMT) allows high-dose CT.

- Marrow may be derived from other histocompatible individuals (allogeneic BMT) or from the patient prior to CT (autologous BMT).

Source: Dr. L Ruth website.
Bone marrow transplantation

• Allogeneic BMT has been used successfully in the treatment of acute leukemia.

• Autologous BMT is being used in clinical trials for lymphoma patients.

• Developments in autologous BMT include novel methods for *ex vivo* chemopurging (e.g., 4-hydroperoxy-Ctx) or immunopurging (e.g., Shigella like toxin).
• Strategies aim to manipulate bone marrow stem cells *ex vivo*.

• Examples are the multidrug resistance (*MDR*) gene and the DNA repair gene O6-alkylguanine DNA alkyl transferase (*AGT*).

A gene therapy protocol in which the *MDR* gene is transferred *in vitro* into cytokine-treated CD34+ bone marrow precursors. Cells from this patient were infected with a recombinant RV carrying the *MDR* gene and reinfused into the patient before high-dose CT was administered.
Can we target molecular changes, such as the bcr-abl fusion protein tyrosine kinase (PTK) that is characteristic of CML cells?
Gleevec™ (imatinib mesylate), formerly known as STI57

Gleevec: HOW IT WORKS

Gleevec

CML Enzyme

Cancer Protein

ATP

CML

ATP

Gleevec

CML Enzyme

Cancer Protein

ATP

CML
Hypoxic cell cytotoxins (bioreductive drugs) target a different population of tumor (i.e., hypoxic) cells than XRT, resulting in a complementary pattern of cytotoxicity.

Gradient of reduced cell killing as a function of distance from the vasculature as seem in experimental tumors and spheroids.

Hypoxic-cell cytotoxins: Tirapazamine

- The drug **Tirapazamine** (TPZ) is metabolized by cellular reductases. When oxygen is present the drug is rapidly oxidized back to the parent drug and the relatively non-toxic molecule superoxide.
- In hypoxic cells, the •TPZ radical causes DNA double-strand breaks and single-strand breaks.

*Metabolism of TPZ to its active free-radical moiety causing preferential toxicity to hypoxic cells by damaging DNA.*

Killing of mouse SCCVII cells exposed for 1 h to different concentrations of TPZ under either aerated (O) or hypoxic (●) conditions. HCR = hypoxic cytotoxicity ratio.