ONCOLOGY 520
CHEMOTHERAPY

Lecturer: Dr. David Murray

March 22nd, 2012

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

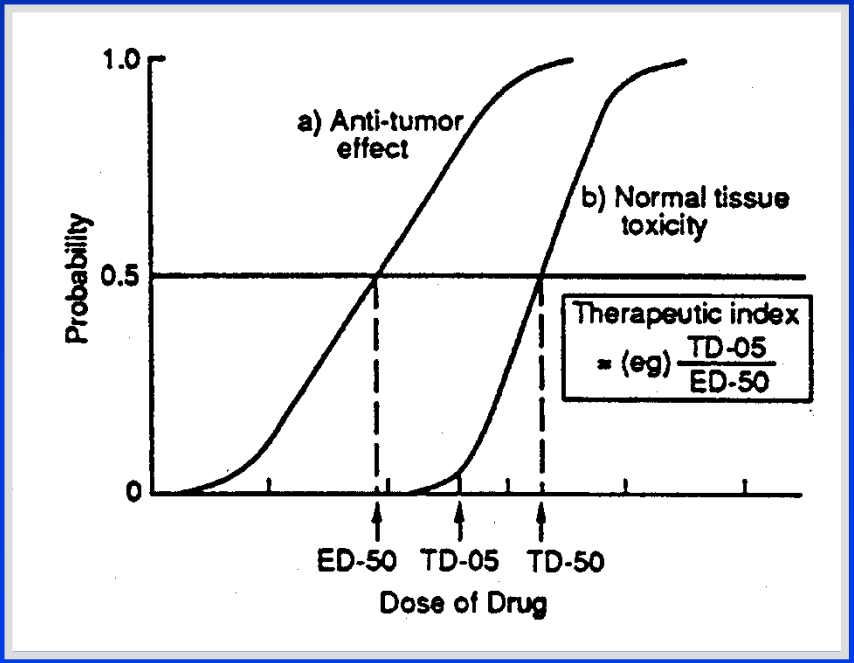
(d) in combination with other modalities (usually XRT) to improve their therapeutic effects.

Combination CT

- 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).

Therapeutic Index

- 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.



Source: IF Tannock and RP Hill, Basic Science of Oncology. 3rd Ed., McGraw-Hill, 1998.

Therapeutic Index, contd.

- 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.

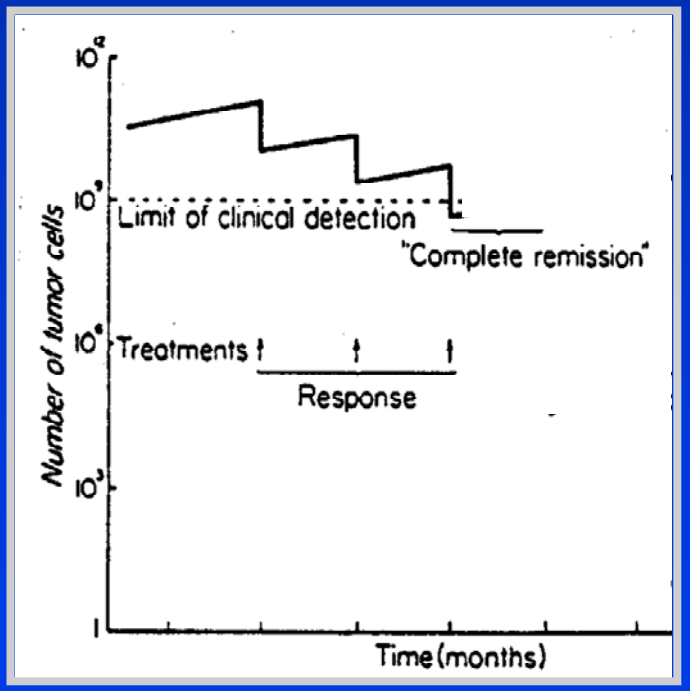
Relationship between tumor remission and cure

- For most solid tumors, the limit of clinical/radiological detection is ~1 g of tissue (~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.

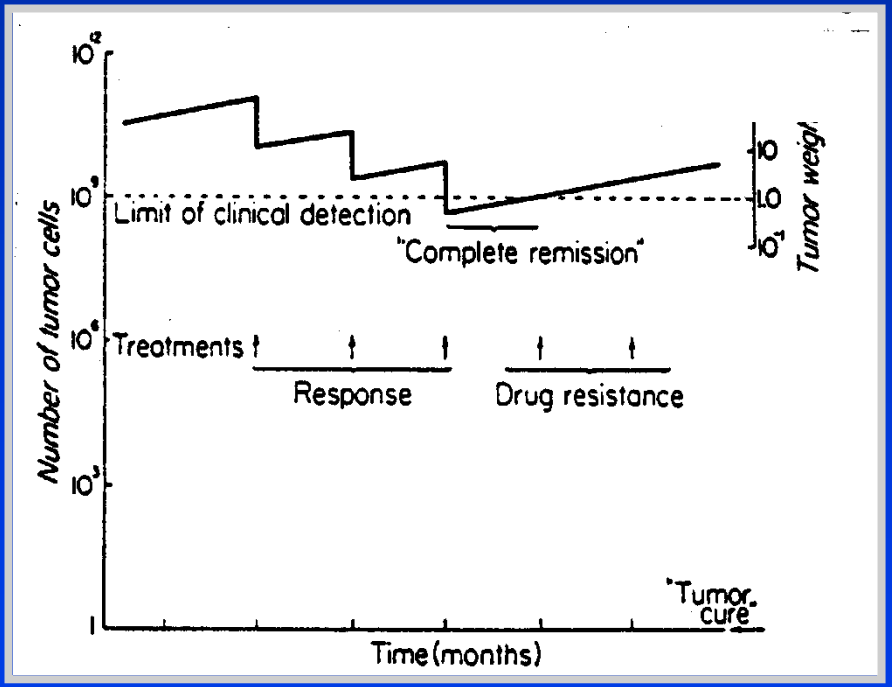


Adapted from Tannock and RP Hill, Basic Science of Oncology. 3rd Ed., McGraw-Hill, 1998.

Relationship between remission and cure, contd.

- 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.



Source: IF Tannock and RP Hill, Basic Science of Oncology. 3rd Ed., McGraw-Hill, 1998.

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.

Classes of anticancer drugs

- "Classical" anticancer drugs are grouped into several families based on their biochemical activities/origins:

ALKYLATING AGENTS

Nitrogen Mustard
Chlorambucil
Melphalan
Cyclophosphamide
Busulfan
Chloroethyl nitrosoureas:
- BCNU
- CCNU
- Methyl-CCNU

ANTIMETABOLITES

Methotrexate
5-Fluorouracil
Cytosine arabinoside
6-Thioguanine
6-Mercaptopurine
Gemcitabine

NATURAL PRODUCTS

Doxorubicin (Adriamycin)
Daunorubicin
Actinomycin C
Bleomycin
Mitomycin C
Vinblastine
Vincristine
VP-16 (Etoposide)

MISCELLANEOUS AGENTS

Cisplatin
Carboplatin
DTIC (Dacarbazine)

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:

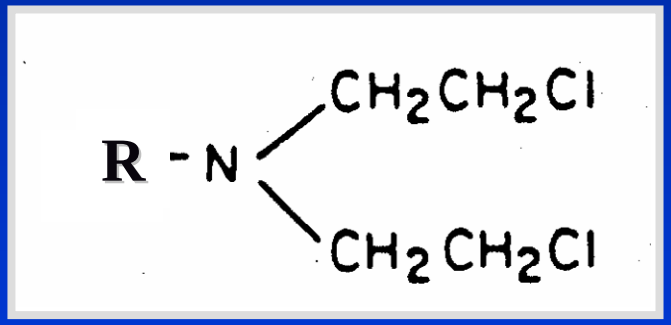
  (a) nitrogen mustards (e.g., melphalan, cyclophosphamide);

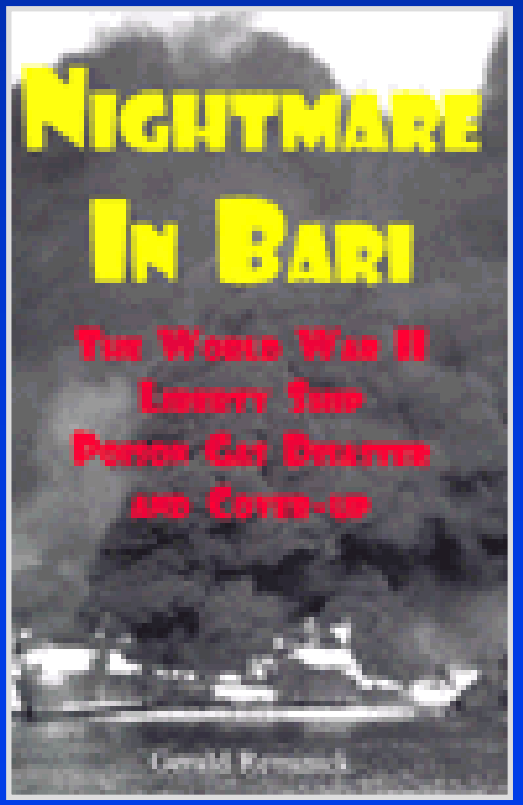
  (b) chloroethyl nitrosoureas (e.g., BCNU, CCNU, methyl-CCNU); and

  (c) methane sulfonic acid esters (e.g., busulfan).

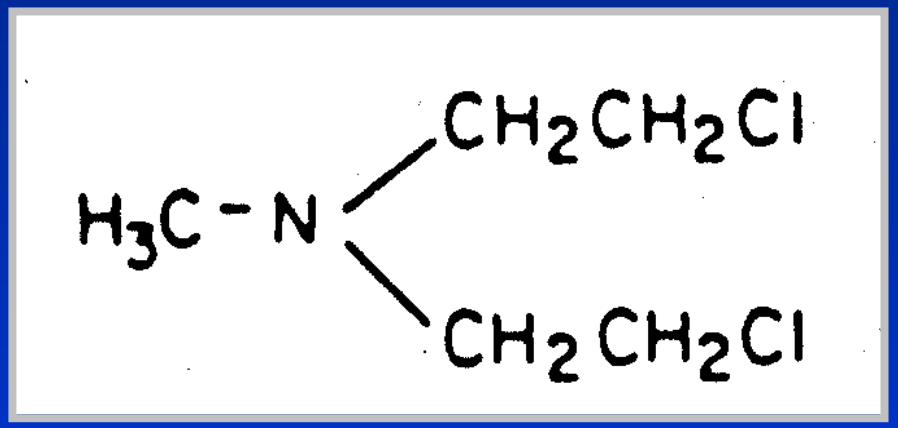
a. Nitrogen mustards

- Bifunctional drugs with 2 electrophilic **chloroethyl** ($-CH_2CH_2Cl$) groups that alkylate DNA predominantly at the electron-rich N-7 position of guanine.





Mechlorethamine (nitrogen mustard)

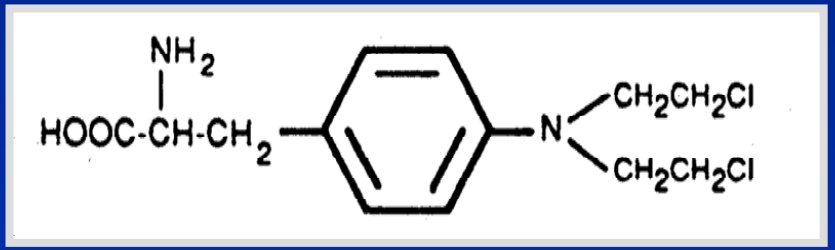


Louis Goodman and Alfred Gilman in San Francisco, 1955.

- 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 **M**echlorethamine-vincristine (**O**ncovin)-**P**rocarbazine-**P**rednisone).
- 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)

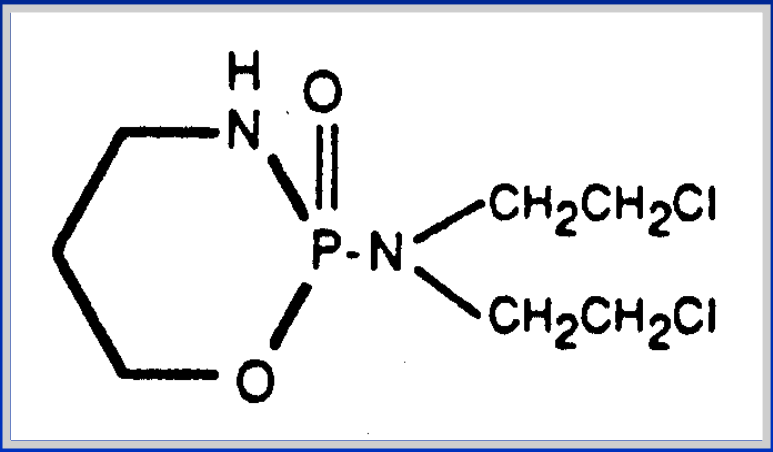
- One of several modified mustards that were synthesized in an attempt to produce a drug that would preferentially localize in a particular tumor.



- 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)

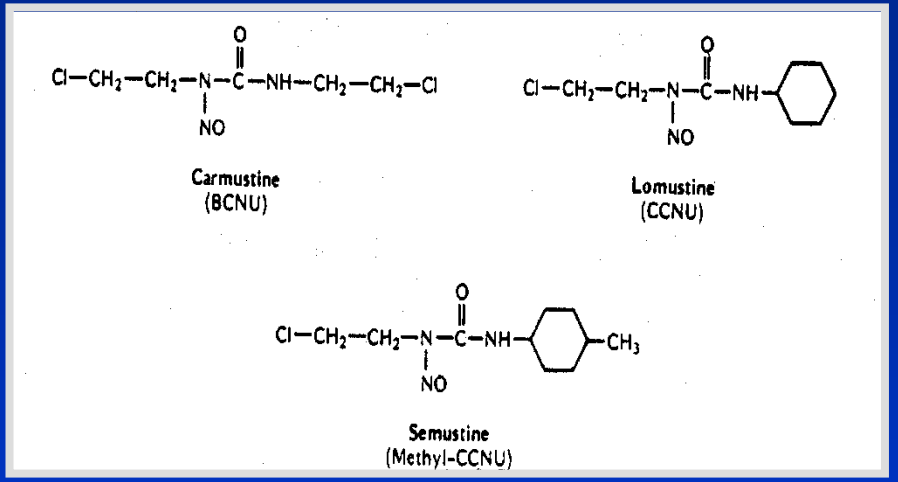
- Another "modified" mustard developed in the hope that might be preferentially activated in tumor cells. High phosphatase and phosphoramidase activity had been observed in some tumors; cleavage of the phosphamide ring might produce an active compound within the tumor cells.



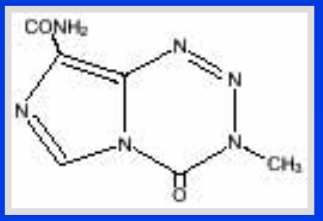
- Although such selectivity was not achieved, the drug has a wide application in CT.
- Ctx is a nontoxic pro-drug that is metabolically activated to 4-hydroxyCtx by microsomal cytochrome P-450 in the liver.
- Used to treat CLL, multiple myeloma, Hodgkin's disease, non-Hodgkin's lymphomas, ALL, Burkitt's 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 ($^{+}CH_2CH_2Cl$) 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®) for brain tumors.



Temodar

Effects of alkylating agents on mammalian cells

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.

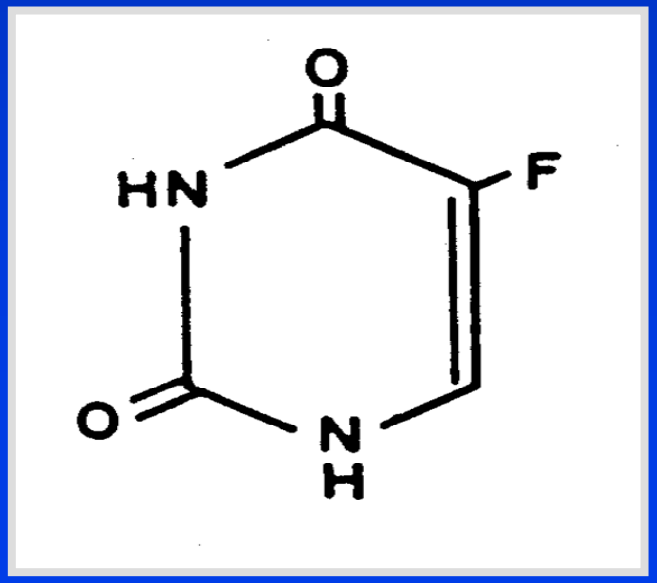
- **Mutation and transformation**: alkylating agents often cause secondary malignancies; there is increased risk if patients also receive XRT.

2. Antimetabolites

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

5-fluorouracil (5FU)

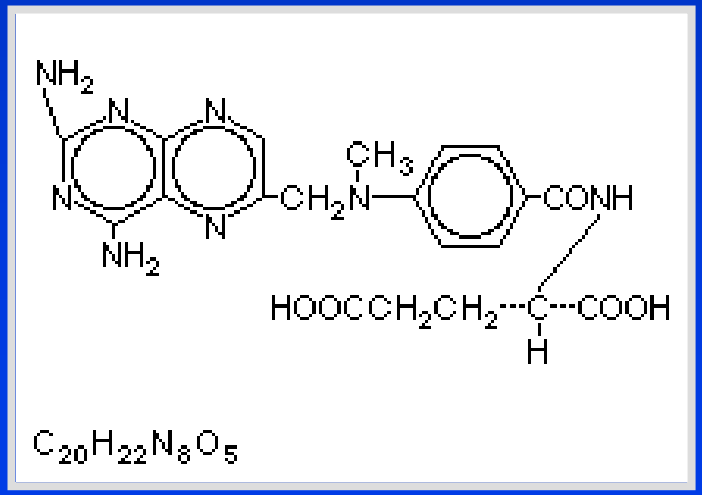
- An inhibitor of **thymidylate synthase (TS).**
- Widely used for breast and gastrointestinal (GI) cancers.
- Major toxicity is to bone marrow and mucous membranes.



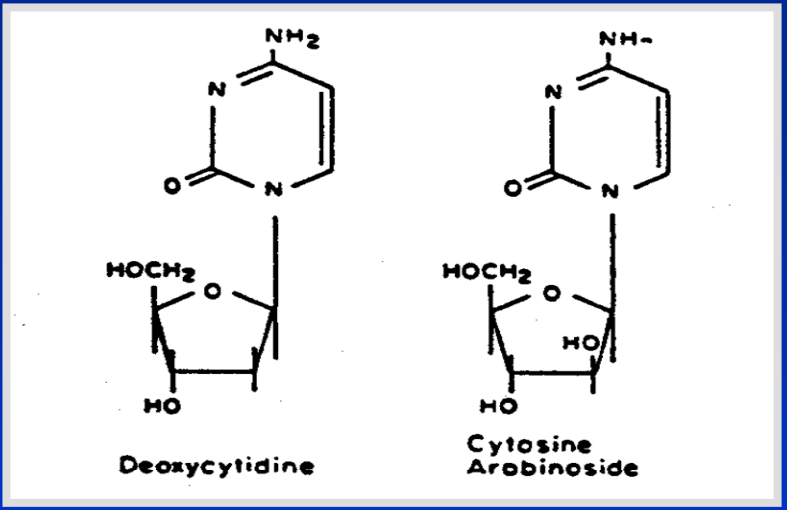
Antimetabolites, contd.

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.



Antimetabolites, contd.



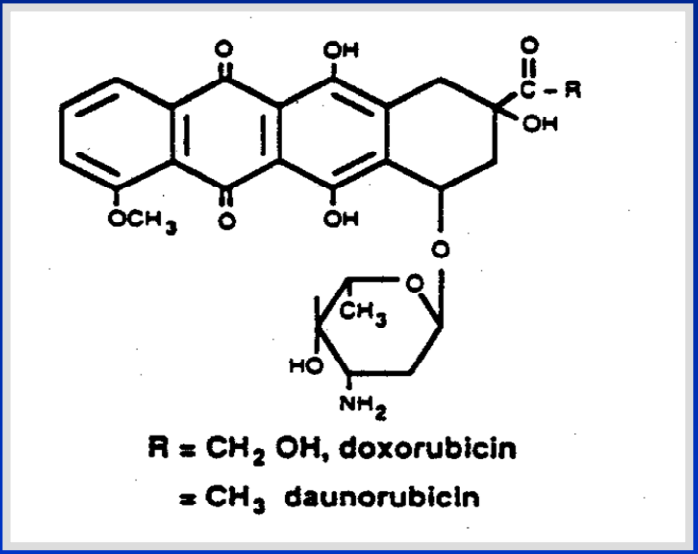
Cytosine arabinoside (Ara-C)

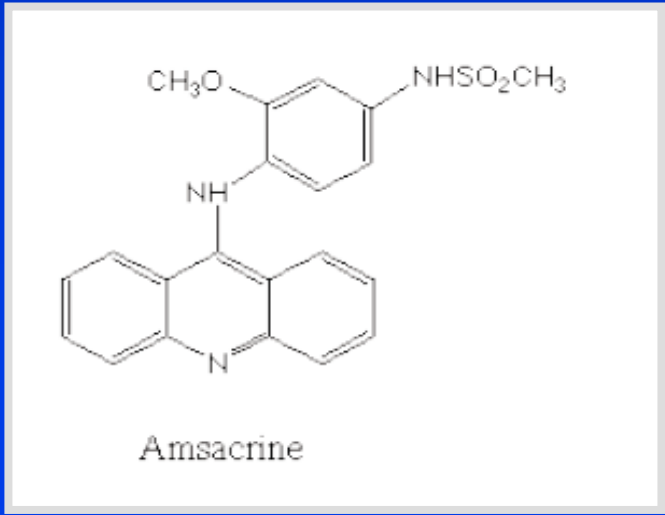
- Ara-C differs from deoxycytidine in having a β-OH group on the 2-position of the sugar, so its sugar moiety is arabinose rather than deoxyribose.
- A competitive inhibitor of **DNA polymerase**; it also **incorporates** into DNA.
- Used primarily to treat acute leukemia.
- Bone marrow toxicity is major 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.





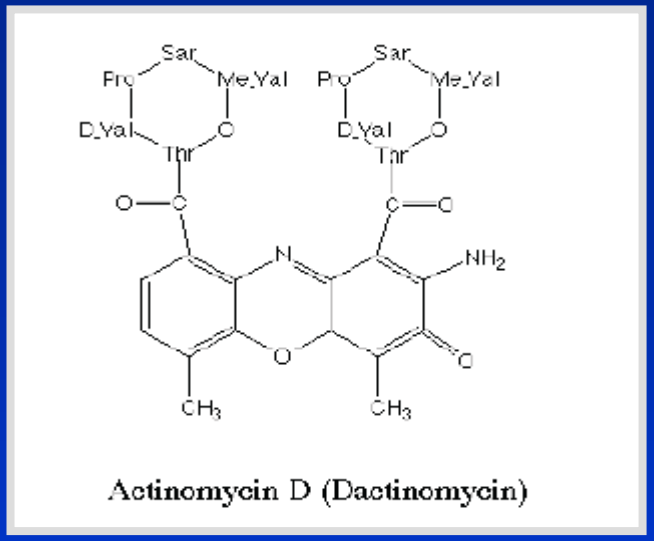
➔ Amsacrine (AMSA)

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

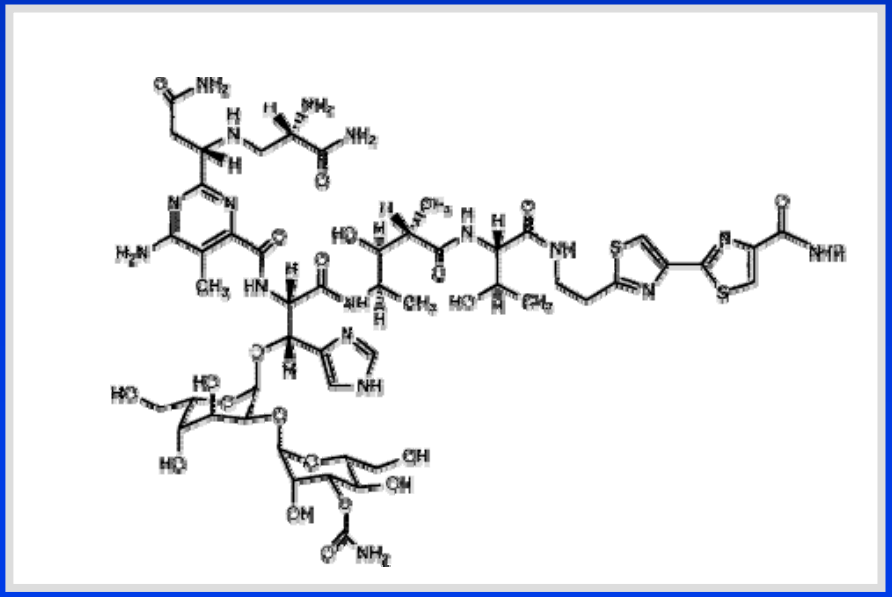
Natural products, contd.

Actinomycin D ➔

- Used mainly in the treatment of childhood tumors.



Actinomycin D (Dactinomycin)



➔Bleomycin

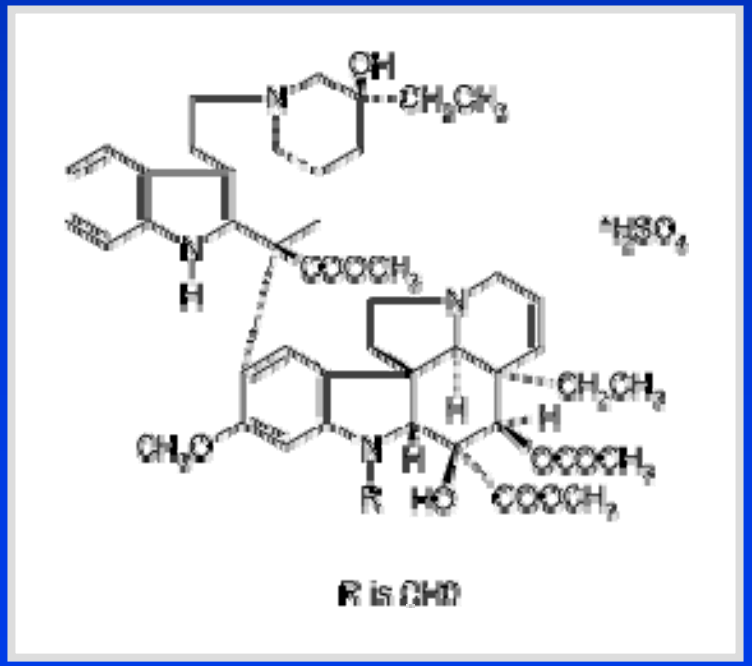
- Causes DNA strand breaks through a complex sequence of reactions that involves the binding of a bleomycin-Fe(II) complex to DNA.
- Used in combination CT for testicular cancer and lymphomas.

Natural products, contd.

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.

Vincristine

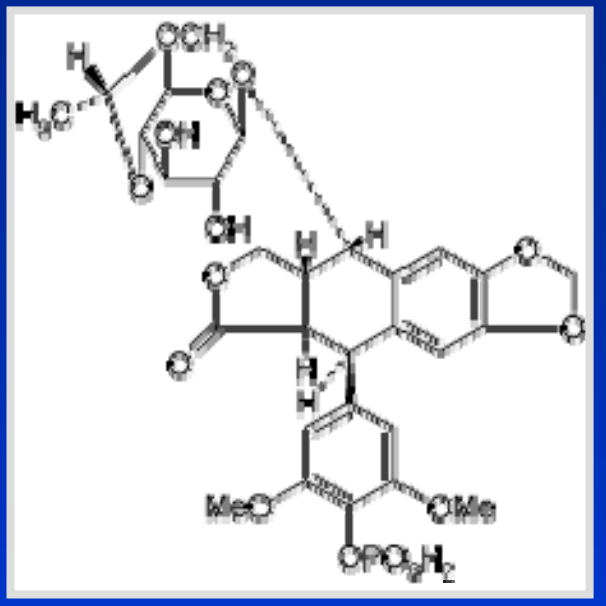


Natural products, contd.

VP-16 and VM-26

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

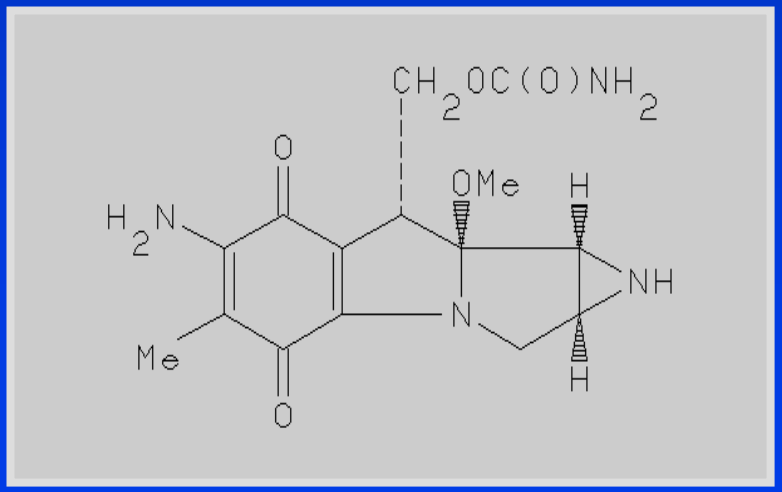
- VP-16 is used to treat small-cell lung cancer, testicular cancer, and lymphomas.



Etoposide

Mitomycin C ➔

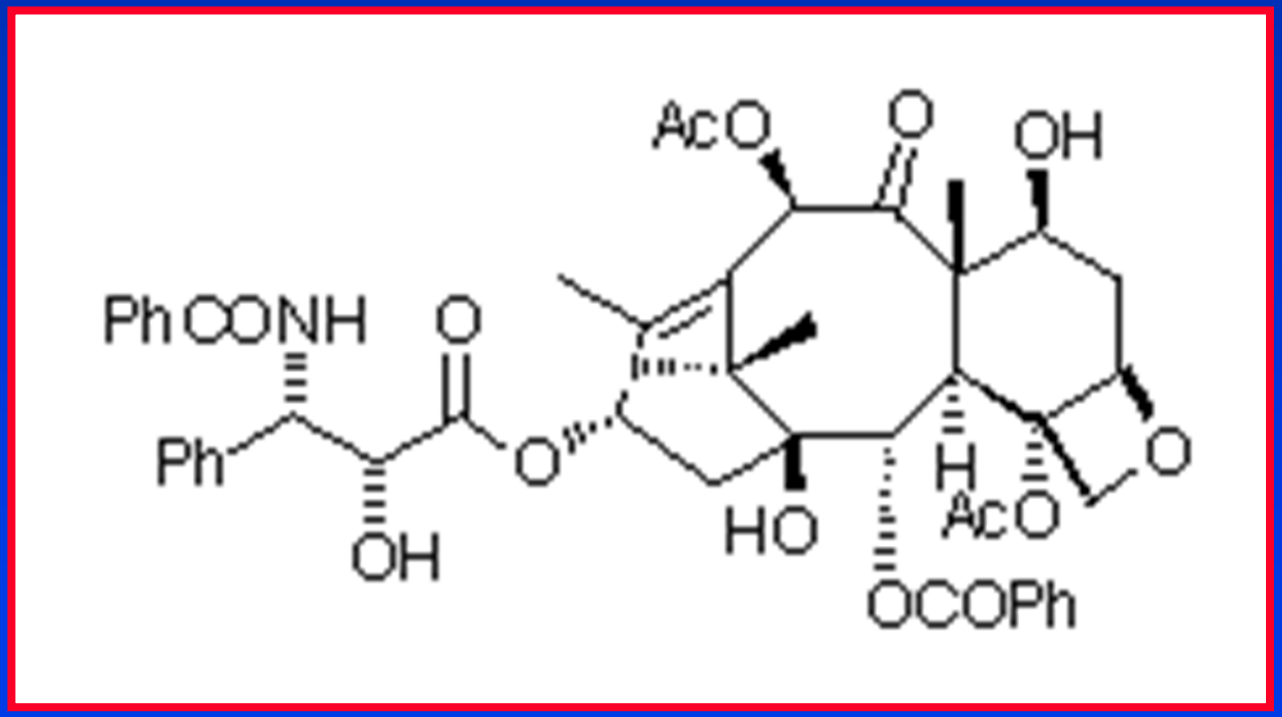
- Acts similarly to alkylating agents.
- More active against hypoxic than against aerobic cells in tissue culture.



Mitomycin C

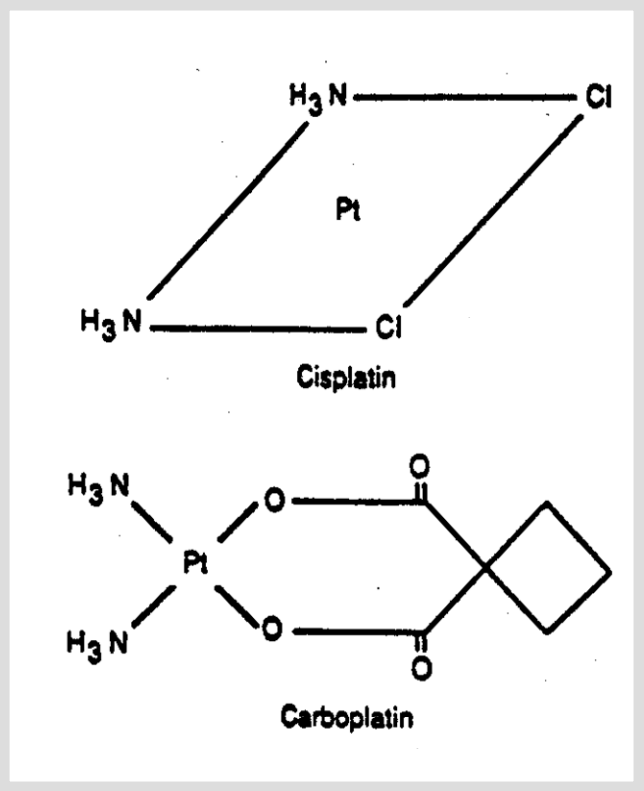
Natural products: Taxanes

- 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.



Mechanism of resistance to anticancer drugs

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.**

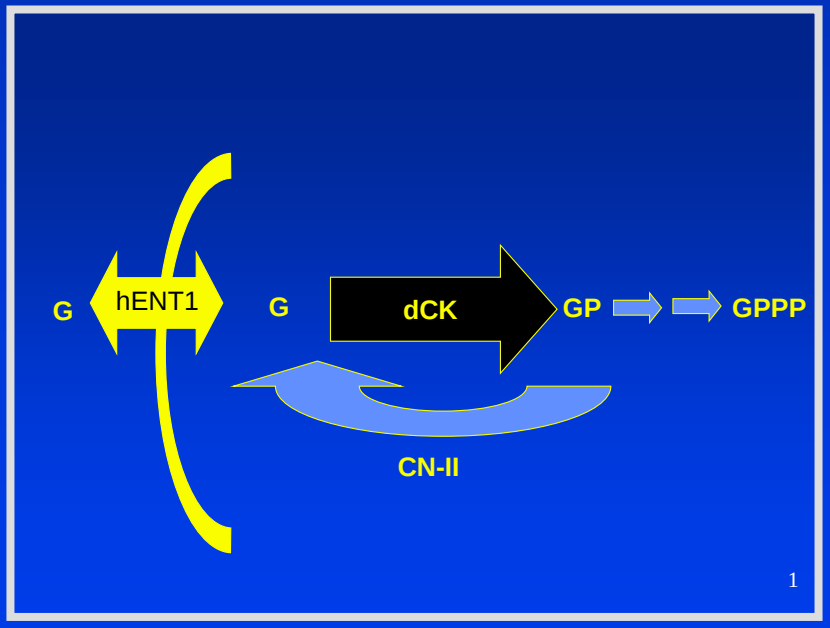
1. Transport

- 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**.

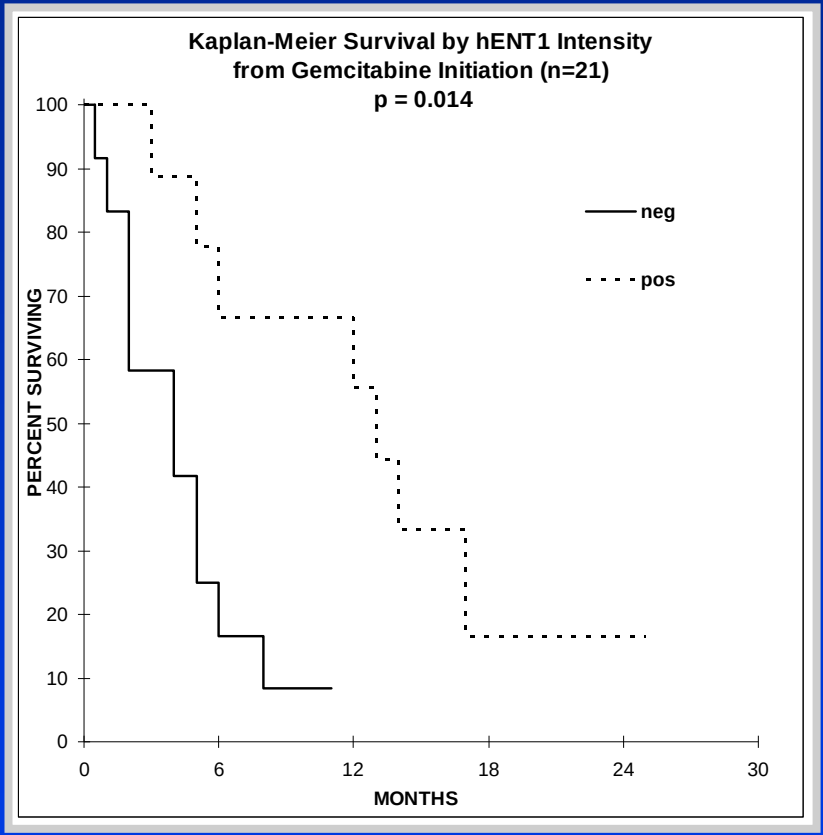
- Down-regulation of hENT1 might thus represent a mechanism of resistance to such drugs.



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.



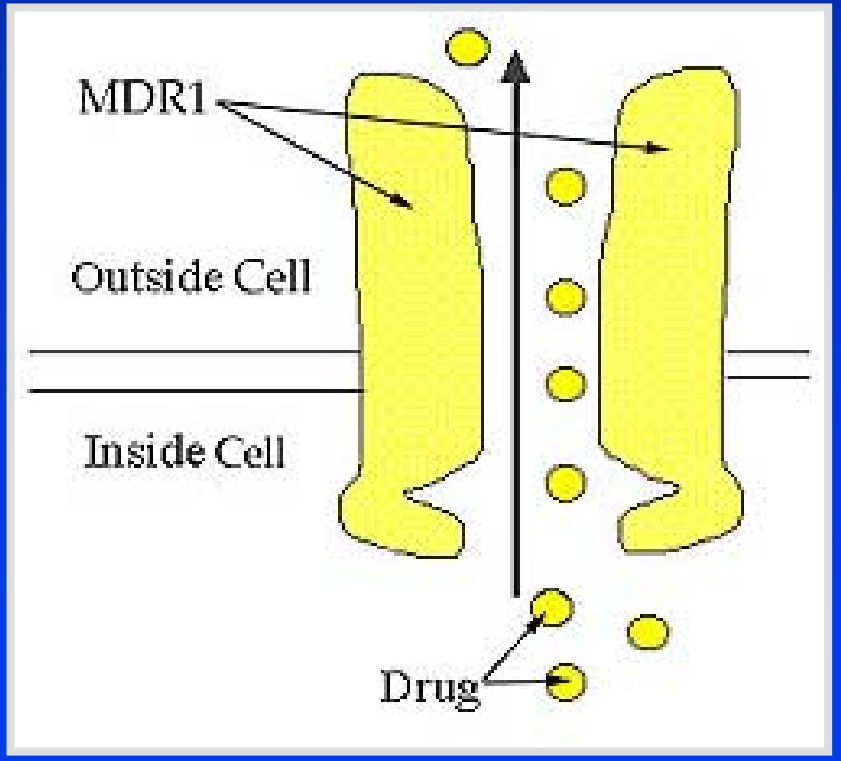
J. Spratlin, R. Sangha, D. Glubrecht, L. Dabbagh, J. Young, C. Dumontet, C. Cass, R. Lai, J. Mackey. Clin Cancer Res. 2004;10:6956-61.

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



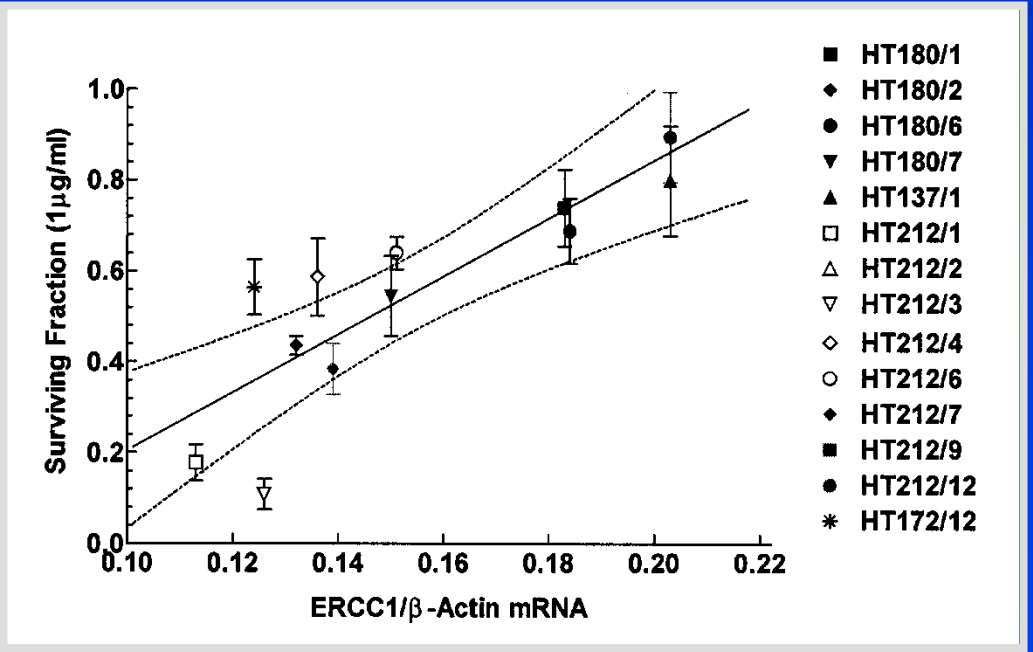
Courtesy of M. O’Leary website.

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 γ-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 *ERCC1* that mediates the repair of ISCs.



4. Tolerance to DNA damage

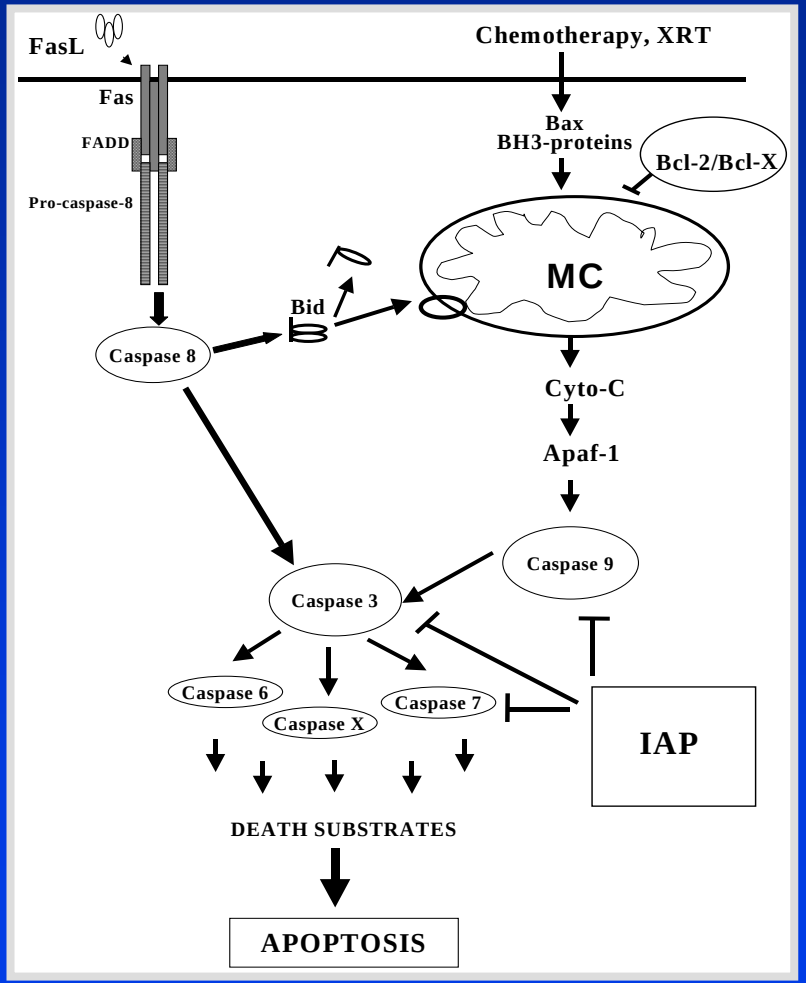
- In many studies, an increased ability of drug-resistant cells to "tolerate" DNA damage has been described which is not directly associated with increased DNA repair.

- 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.

Tolerance by blockage of apoptosis

- 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.

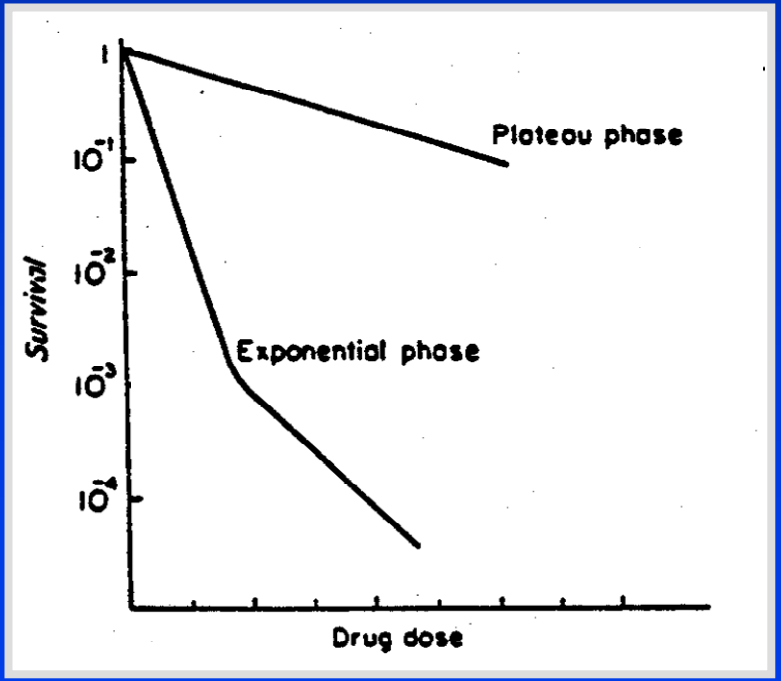


5. Drug-specific mechanisms

- Ctx: **aldehyde dehydrogenase (ALDH)** is an enzyme that inactivates the active metabolite of Ctx before it can damage DNA.
- BCNU, Temozolomide: **O^6-alkylguanine DNA alkyltransferase** is a “suicide” enzyme that removes adducts from the O^6 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**.

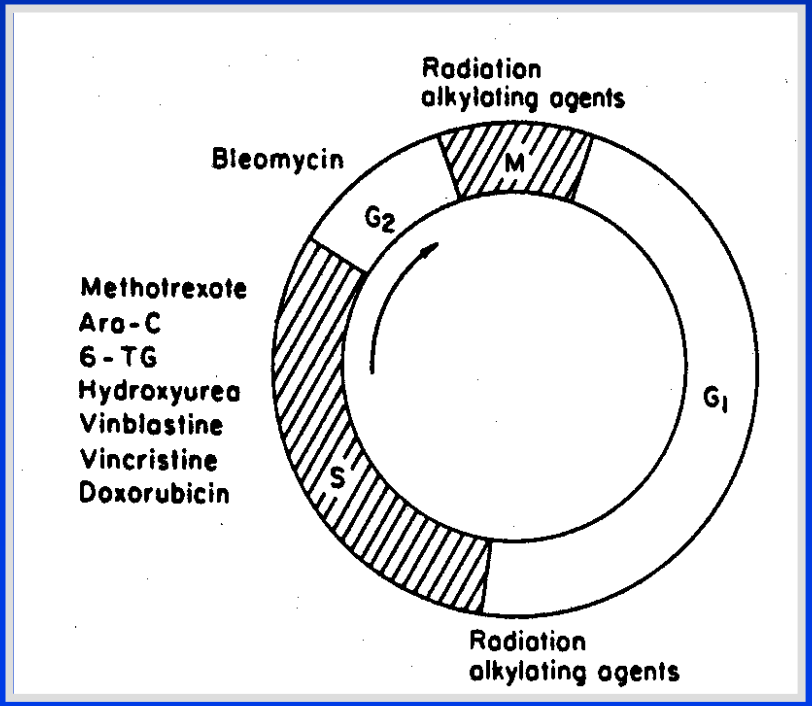
Proliferation dependency of cell killing

- 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

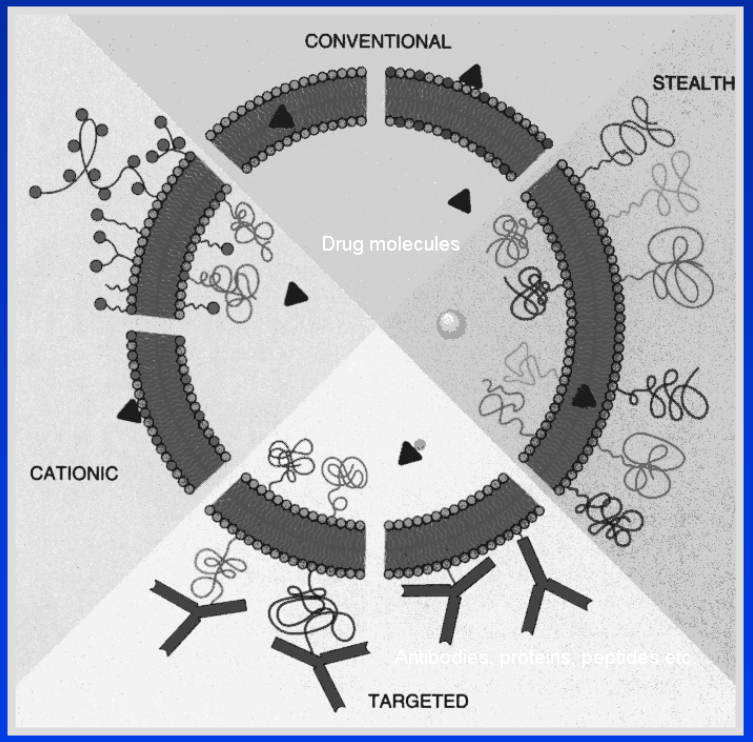
- Most drugs show a marked variation in cytotoxicity around the cell cycle.
- Alkylating agents are cell cycle phase **non**-specific (i.e., they kill cells in all phases of the cell cycle). However, they do kill differentially in the various phases, being more cytotoxic in G_2/M and at the G_1/S boundary.
- 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.



Novel approaches to CT
[1] Directed drug delivery

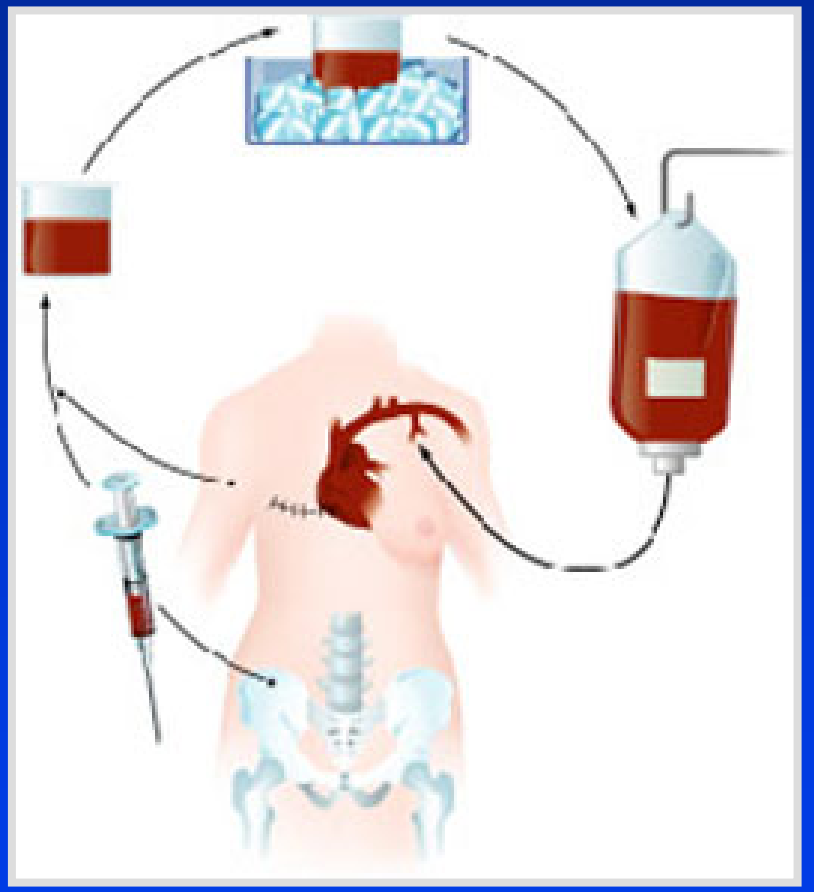
Improved CT responses might be obtained by:

- 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**).



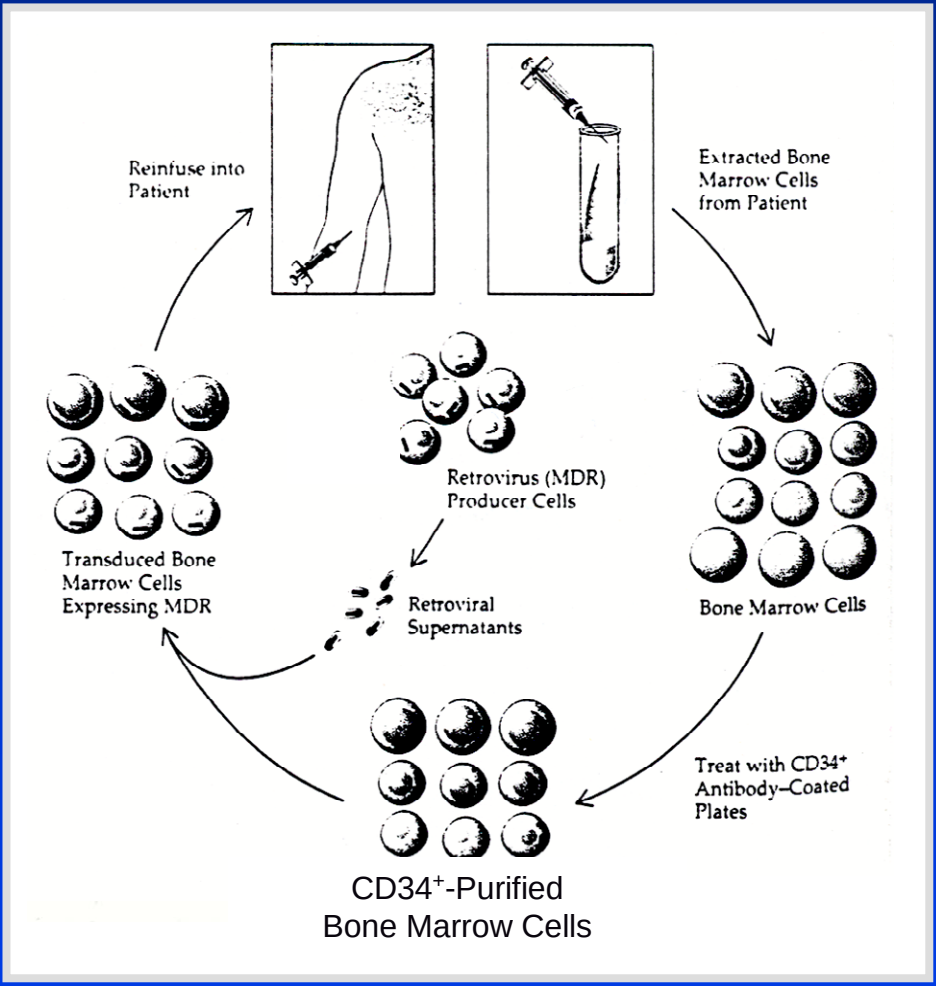
Source: Dr. L Ruth website.

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).

Bone marrow transplantation and gene therapy?

- 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*).

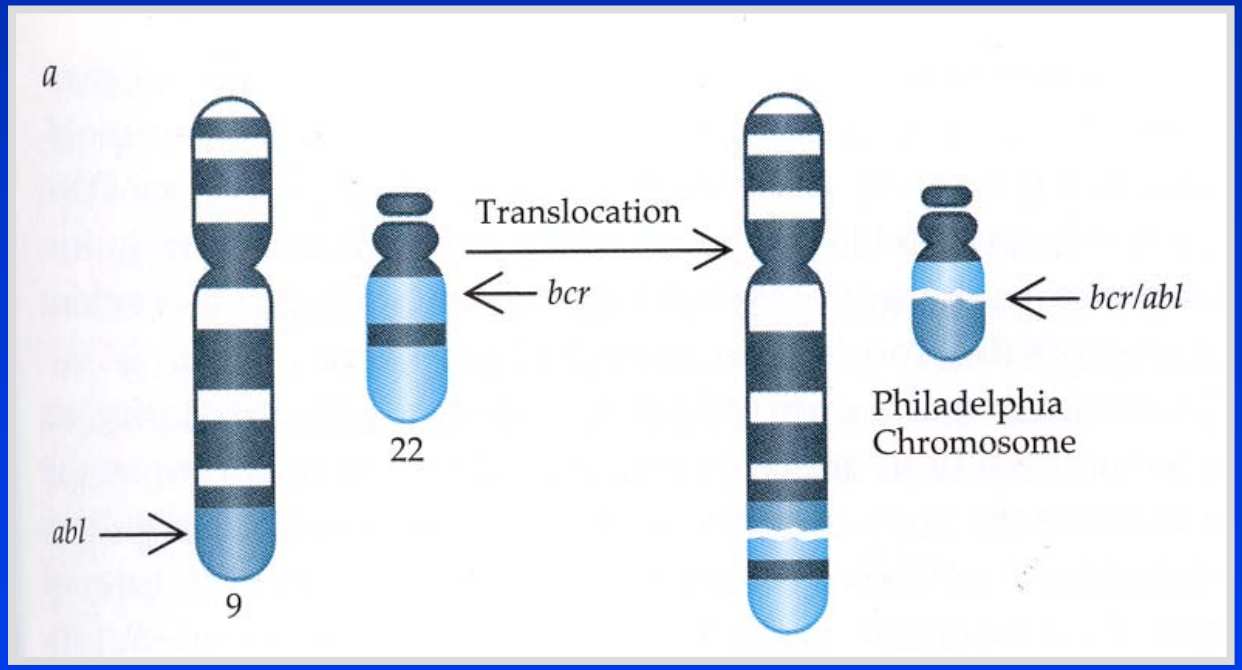


A gene therapy protocol in which the *MDR* gene is transferred *in vitro* into cytokine-treated CD34+ bone marrow precursors. Cells from this patient were infected with a recombinant RV carrying the *MDR* gene and reinfused into the patient before high-dose CT was administered

[3] The next generation: Rational CT?

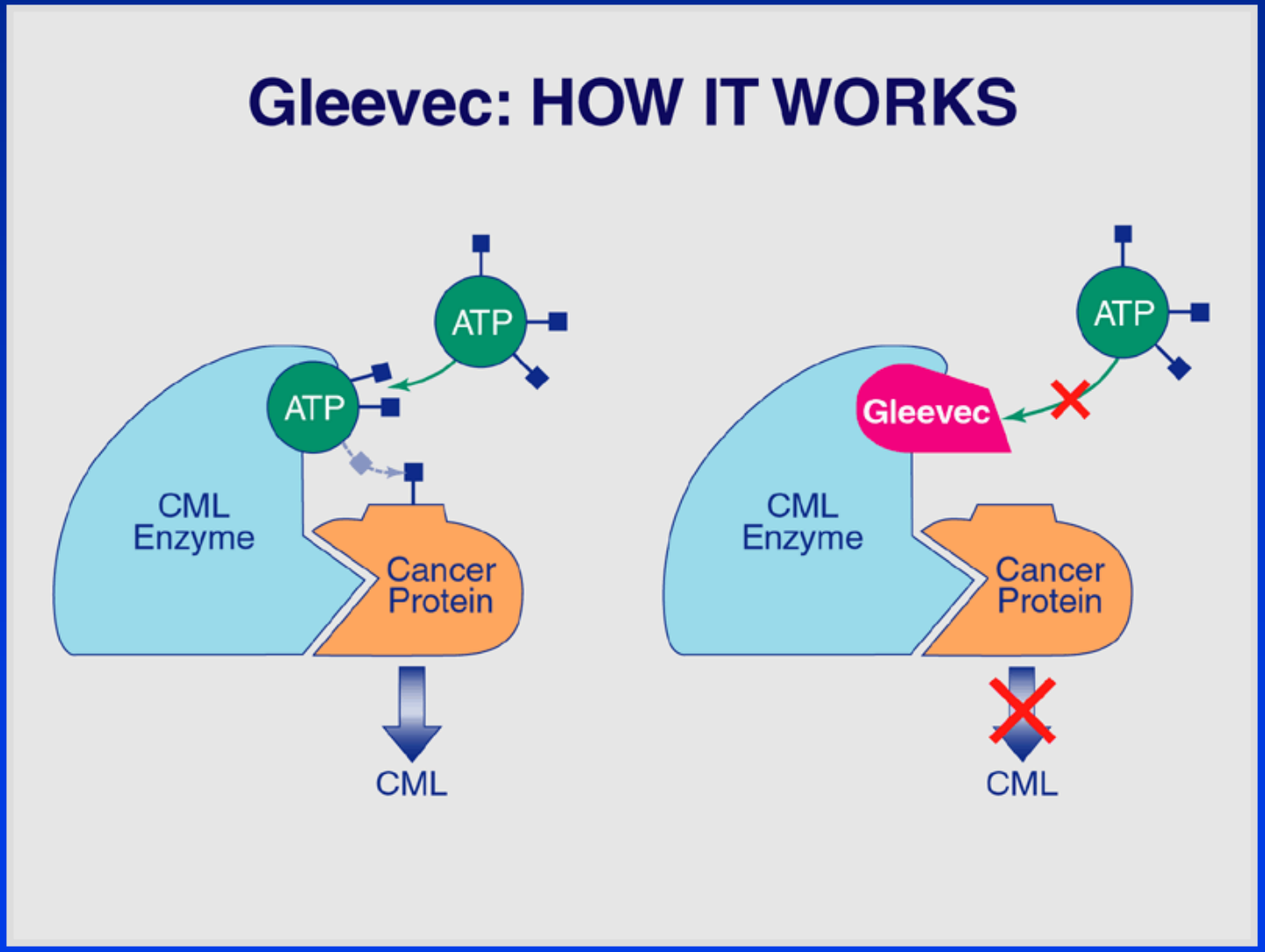
Designer drugs for the treatment of cancers with consistent molecular alterations

- Can we target molecular changes, such as the bcr-abl fusion protein tyrosine kinase (PTK) that is characteristic of CML cells?



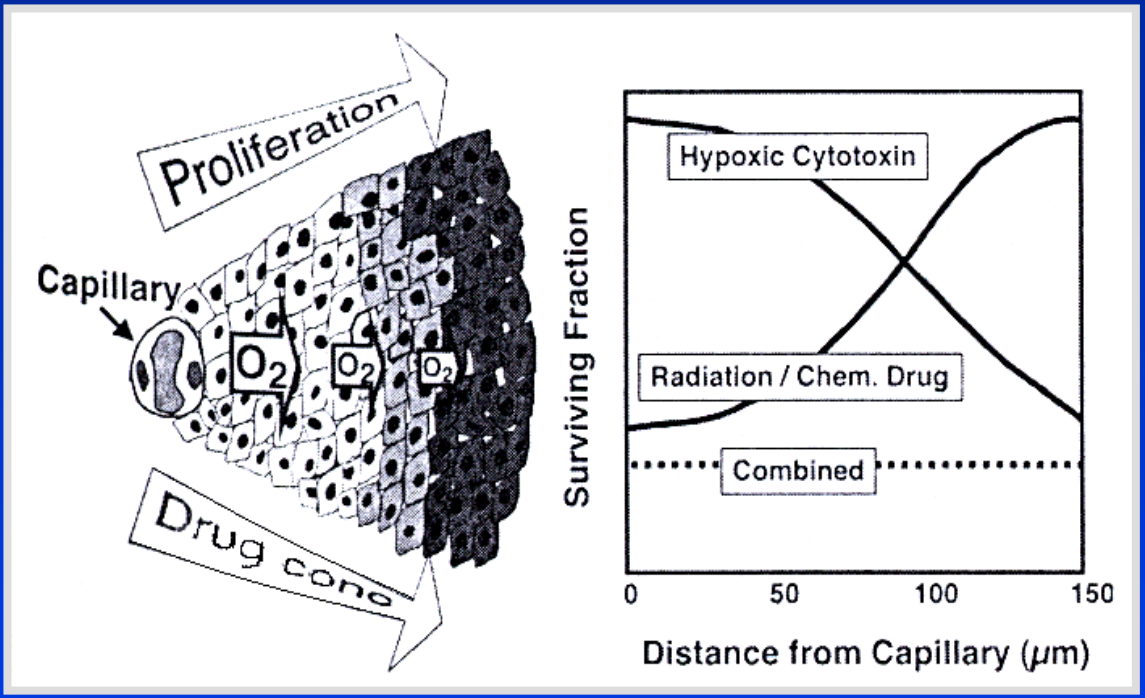
The Philadelphia chromosome

Gleevec™ (imatinib mesylate), formerly known as STI57



[4] Approaches to eradicating hypoxic tumor cells

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

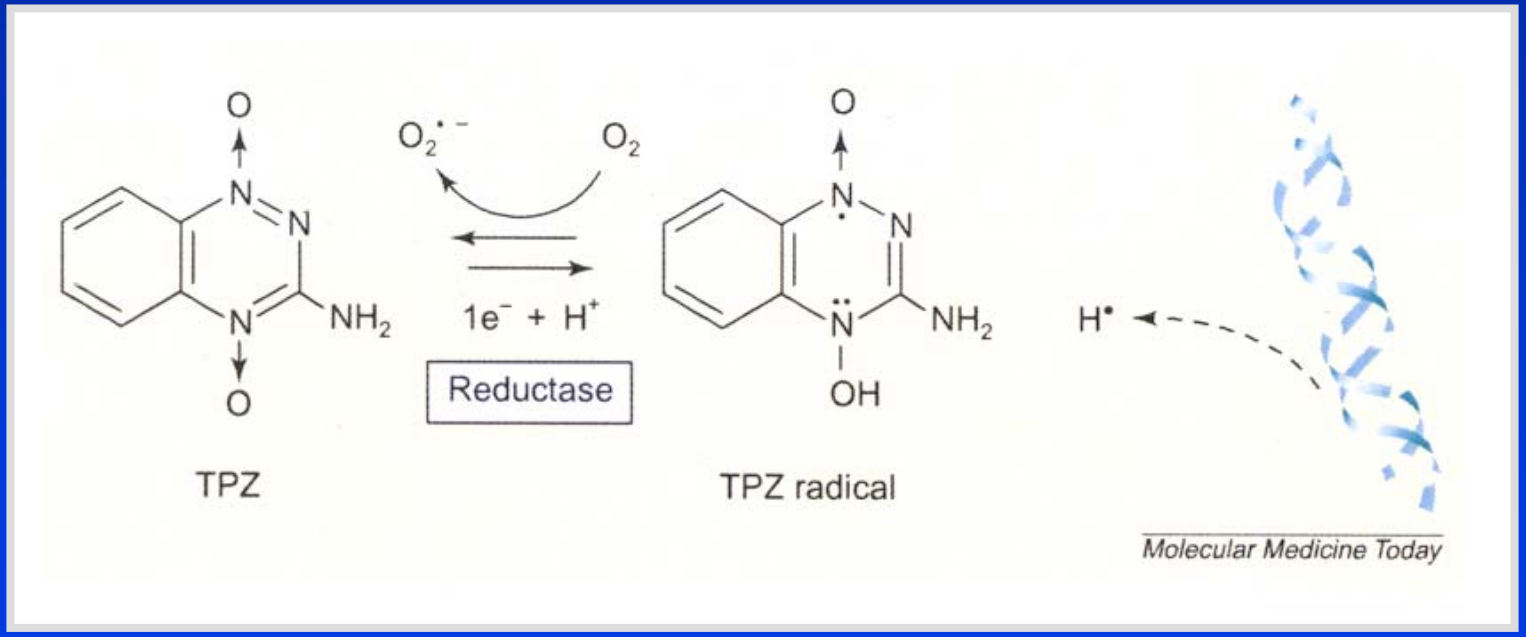


Gradient of reduced cell killing as a function of distance from the vasculature as seem in experimental tumors and spheroids.

From R Urtasun and JM Brown, Chemical Modifiers of Radiation, in "*Textbook of Radiation Oncology*". Eds. SA Leibel and TL Phillips, Saunders, 2004.

Hypoxic-cell cytotoxins: Tirapazamine

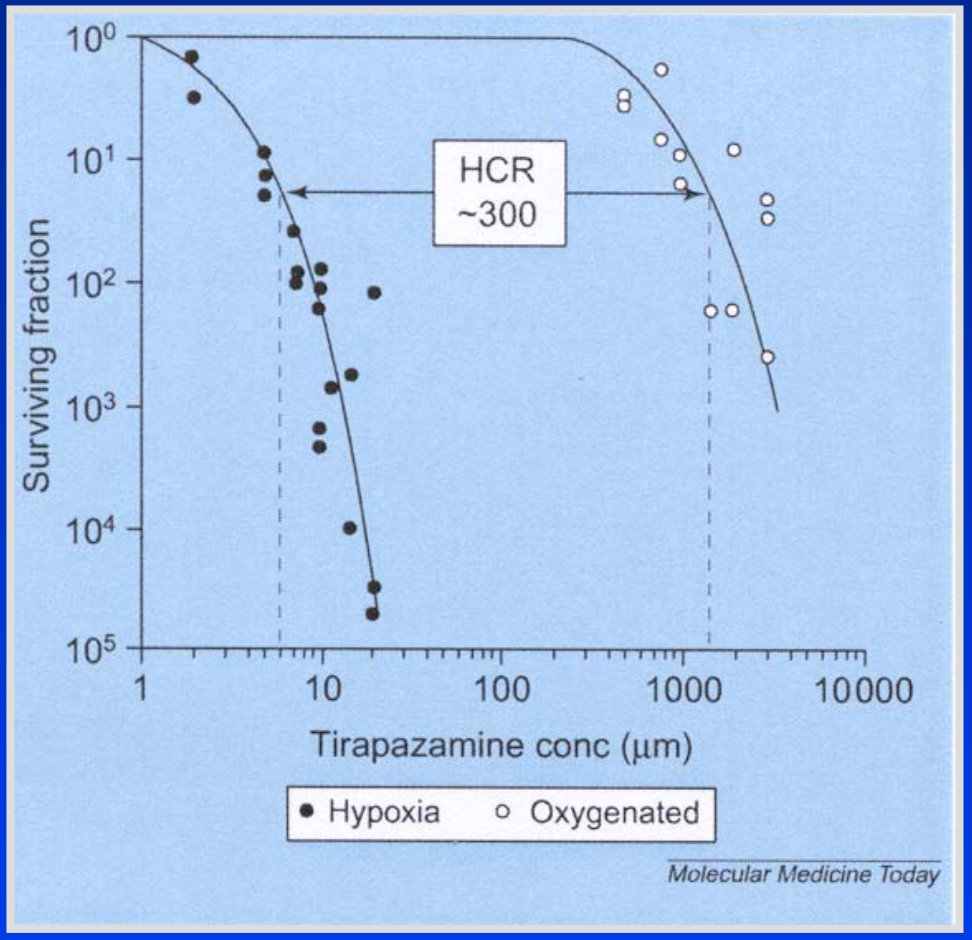
- The drug **Tirapazamine** (**TPZ**) is metabolized by cellular reductases. When oxygen is present the drug is rapidly oxidized back to the parent drug and the relatively non-toxic molecule superoxide.
- In hypoxic cells, the •TPZ radical causes DNA double-strand breaks and single-strand breaks.



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

JM Brown. *Mol Med Today* 6:157-162;2000

Tirapazamine



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

JM Brown. *Mol Med Today* 6:157-162;2000