ANTINEOPLASTICS I

REFERENCES:
Katzung Basic and Clinical Pharmacology (9th ed.) Chapter 55, pg. 898-920

CRITICAL FACTS
(if med school is a Minnesota forest with millions of trees, these are the red pines)

1. The **GOAL** is to eradicate the cancer cells without affecting normal tissues. The **REALITY** is that all cytotoxic drugs affect normal tissues as well as malignancies → aim for a favourable therapeutic index.

2. Cancer drugs can be divided into two general classes: **CELL CYCLE SPECIFIC DRUGS** (CCS; esp. plant alkaloids and antimetabolites), and **CELL CYCLE NON-SPECIFIC DRUGS** (CCNS; esp. alkylating agents and some natural products). Antineoplastic agents can also be organized according to their chemical class, mechanism of action, therapeutic use or their toxicities.

3. The **CELL KILL HYPOTHESIS** proposes that actions of CCS drugs follow first order kinetics: a given dose kills a constant **PROPORTION** of a tumor cell population (rather than a constant **NUMBER** of cells).

4. Resistance to cancer chemotherapeutic drugs is a major limitation to treatment. **PRIMARY RESISTANCE** occurs when some inherent characteristic of the cancer cells prevents the drugs from working; **ACQUIRED RESISTANCE** occurs when cancer cells become resistant during treatment. **MULTIDRUG RESISTANCE** is particularly problematic; this occurs when tumour cells become cross-resistant to a wide range of chemically dissimilar agents after exposure to a **SINGLE** (typically natural product) drug.

5. Although crosslinking agents are dependent upon proliferation, these agents **ARE NOT** cell cycle specific because the alkylation reactions that initiate cell death can occur during any phase of the cell cycle. However, primary toxicity occurs in late $G_1$ and $S$ phase (not $G_2$, $M$ or early $G_1$).
6. One major distinguishing feature among alkylating agents is the rate at which the parent drug is converted to become strong electrophiles. For some drugs (e.g., MECHLORETHAMINE), the reaction is virtually instantaneous --- these agents are given IV to minimize blistering and GI effects. Others agents (such as CYCLOPHOASPHAMIDE) must be activated in the liver and other tissues, and they can be given orally.

7. The anthracycline antibiotics DAUNORUBIN and DOXORUBICIN work by intercalating into DNA. Their major toxicity is an UNUSUAL CARDIOMYOPATHY that is often IRREVERSIBLE and related to the TOTAL DOSE of the drug.

8. The vinca alkaloids (VINCRISTINE and VINBLASTINE) and the taxanes (PACLITAXEL) are mitotic spindle poisons. Despite their structural similarity, VINCRISTINE and VINBLASTINE have significantly different toxicities (VINCRISTINE=CNS; VINBLASTINE=Bone marrow suppression).

ESSENTIAL MATERIAL FROM OTHER LECTURES:

2. DNA & Replication - Dr. Perkins, Principles.
3. Regulation of Gene Expression and Cancer, a multifactorial genetic disease – Dr. Cormier, Principles
4. Principles of Pharmacology – Dr. Knych, Principles
5. Cell injury and cell death, Neoplasia, Nomenclature - Malignancy and Metastasis - Dr. Ward, Histopathology

PRIMARY LEARNING OBJECTIVES:

1. List the potential targets and actual mechanisms of action of antineoplastic drugs. What are the strengths and weaknesses of each approach?
2. What is the cell kill hypothesis and its implications for treatment?
3. What factors can influence the success of chemotherapy in curing cancer?
4. Understand why particular classes of drugs act at specific points in the cell cycle.
5. What are the generic mechanisms for drug resistance to antineoplastics? What factors contribute to the development of resistance?
6. Why are most cancer drugs administered in combination?

7. Identify the steps in the activation process of cyclophosphamide and how this relates to its specific toxicity. What is the use of MESNA?

8. Describe the specific mechanisms of action, causes of drug resistance and toxicities associated with the drugs presented. Be able to classify these particular agents according to 1) chemical class, 2) mechanism of action, 3) cell cycle specificity, 4) therapeutic uses, and 5) major toxicities.
My Turn

A Poker Player’s Guide
To Beating Cancer

When I got sick, it was what I learned from the game that sustained me: it takes faith to trust the odds.

BY NICK KURZON

As a poker player, I’ve gotten familiar with the fluctuations of luck and the endurance of probability. That’s why I like the game. I’m trying to make sense of those rival twins—luck and probability—because they are why I’m still alive, and they are why I nearly died. I got into poker after I got cancer.

I’ve heard that some people get cancer and find religion. As much as I would have liked it if that had happened to me, I never found religion when I got sick with lymphoma two years ago. That’s not to say I didn’t pray. When I had to get a diagnostic gallium scan of my body, lying perfectly still in a tube for three hours, not knowing what future the results would bring for me and my family, I discovered that I knew the Lord’s Prayer. Repeated it over and over again, like a mantra, and it helped keep me from panicking. I prayed and I meditated and I offered a lot of deals to God if he’d let me through the woods. I found some comfort in God, but I didn’t find religion.

Instead, I found poker.

The beautiful thing about poker is that one’s fate is quantifiable. You can always measure your place in the universe by calculating the odds of any given hand. When you have a horrible run of luck, it is easy to measure just how horrible it is. The other day, in a big-pot-limit game, I lost a pile of chips when my full house got beaten by a Wall Street cowboy who drew to a bigger full house. It turns out there were four cards out of 44 remaining in the deck that could’ve helped my opponent. That’s how bad my luck was: 10 to 1. I was better than a 90 percent favorite to win the hand.

Those odds tell me that I did the right thing to make a huge bet, and my opponent made a mistake to call it. The fact that he next five years were also just over 90 percent. That’s a bit scarier than sitting pretty with a second-nut full house. With the stakes this high, even 10 to 1 is unsettling. Either I will survive or I won’t. I won’t be “91 percent alive” in five years: it’s all or nothing. For me, the word is “destiny,” not “odds.”

But that’s not how oncology works. Science doesn’t accept “destiny” as anything more than a metaphor, and it treats the fate of one patient as part of a pattern that can be observed and controlled in the aggregate. A doctor has to believe in the odds. And a patient has to believe in his doctor. That’s not always easy to do. Oncologists, by design, are almost impossible to read.

They have to be. At the card table it’s called “poker face,” but for an oncologist, keeping emotion out of the equation is a matter of life and death. At first it drove me crazy. I couldn’t figure out where my doctor was coming from. He told me what I had to do to survive and he told me that I should face well, but he didn’t show any trace of emotion. Fighting cancer was the most emotional experience of my life, but he was all business. Now I know why.

Poker players use the term “going on tilt” to describe the irrational play that usually follows an improbable loss. A “bad beat”—being victimized by standard deviation—can lead even the coolest shark to start playing wildly. When I lost all my chips on that 10-to-1 shot, I felt the blood rush to my head, so I got up and left the table. But an oncologist can’t leave, and he certainly can’t allow himself to go on tilt. He has to treat each patient by the book, even if that sometimes leads to tragic results.

And after one of those tragedies, he has to get right back to work, without showing any signs of frustration or apprehension—what poker players would call his “tells.” It is crucial that his next patient have absolute confidence in his advice.

Nowadays, I am grateful when I see my doctor putting on his poker face to tell me that the latest gallium scan results look good. Even though I want him to celebrate with me, I know that that’s not what makes for long-term success. After my one-year checkup, almost giddy with joy, I said to my doctor, “So you mean the cancer’s gone?” He nonchalantly replied, “Well, what did you expect?”

What matters most for my doctor is not what he wants to happen, but what he expects will happen over the long term and how he takes advantage of that to get what he wants.

Because most of all, working with the odds requires a lot of faith. Enough faith to know that even if your full house gets beaten by a long shot, it’s right to play the hand the same way next time. And nine times out of 10, things will work out great. As I get better at poker, I am discovering such faith, and it reassures me and offers a sense of control over a sometimes capricious universe. Poker helps me realize how predictable it is to be as lucky as I am.

KURZON is a documentary filmmaker.
REVIEW OF THE PROBLEM

Distinguishing characteristics of cancer

- genetic or epigenetic disease
- characterized by rapid cell growth
- derived from normal tissue
- loss of differentiation
- metastasis to other tissues

Non-evidence-based Medicine

- New treatments can make historical survival rates obsolete
- Do we know what type a cancer is?  → targeted therapy and genomics
- “It’s all or nothing”

GENERAL CONCEPTS

Goals for Pharmacologic Therapy

- Cure
- Cure in adjuvant setting
- Palliation
- Create a chronic disease?

Targets for Antineoplastic Drugs

1. Rapid cell growth
2. Cell surface markers (antibodies)
3. Lack of immune system responses
4. Defective genes
5. Angiogenesis
Tissues Affected by Drugs that Target Rapid Cell Growth

- Bone marrow
- Skin
- Fetus
- GI mucosa
- Hair follicles
- Cancer cells

Cell Cycle

Cancer drugs can be divided into two general classes: **CELL CYCLE SPECIFIC DRUGS** (CCS; esp. plant alkaloids and antimetabolites), and **CELL CYCLE NON-SPECIFIC DRUGS** (CCNS; esp. alkylating agents and some natural products).

**CYCLE SPECIFIC DRUGS**
- Only proliferating cells killed
- Schedule dependent (duration rather than dose)

**CELL CYCLE NON-SPECIFIC DRUGS**
- Both proliferating and non-proliferating cells killed
- Dose dependent (total dose rather than schedule)
Growth rate = growth fraction X doubling time - apoptosis rate

The **CELL KILL HYPOTHESIS** proposes that actions of CCS drugs follow first order kinetics: a given dose kills a constant **PROPORTION** of a tumor cell population (rather than a constant **NUMBER** of cells).

**Factors affecting outcome**

**CANCER**
- Cancer type
- Stage
- Growth fraction
  - (% of cells in cycle)
- Rate of replication
  - (doubling time)
- Resistance

**HOST**
- Performance status
- Marrow capacity
- Renal function
- Liver function
- Age
- Compliance

**Drug Resistance**

Mechanisms:
1. Increased DNA repair
2. Formation of trapping agents
3. Changes in target enzymes
4. Decreased activation of prodrugs
5. Increased inactivation
6. Decreased drug accumulation

Contributing causes:
1. Poor drug distribution
2. Tumor cells not in cycle
3. Sanctuary sites
4. Heterogeneity of tumor cells
   - clonal selection
Resistance

Resistance to cancer chemotherapeutic drugs is a major limitation to treatment. PRIMARY RESISTANCE occurs when some inherent characteristic of the cancer cells prevents the drugs from working; ACQUIRED RESISTANCE occurs when cancer cells become resistant as a result of treatment. MULTIDRUG RESISTANCE is particularly problematic; this occurs when tumor cells become cross-resistant to a wide range of chemically dissimilar agents after exposure to a SINGLE (typically natural product) drug.

- increased expression of:
  1) mdr-1 gene $\rightarrow$ ↑ levels of P-glycoprotein
  2) multidrug resistance proteins 1 (MRP1-9)
  3) lung resistance protein (LRP)
- Ca$^{2+}$ blockers (e.g. verapamil) can reverse this resistance

Combination Chemotherapy

STRATEGIES
- different mechanisms of action
- active in different stages of cell cycle
- different toxicities

RESULTING IN:
- decreased development of resistance
- synergistic effects
- decreased toxic effects
DRUGS YOU NEED TO KNOW  
(today's drugs are capitalized; prototypes are underlined)

**ALKYLATING AGENTS**  
(blue cards)
- BUSULFAN
- CARMUSTINE (BCNU)
- CYCLOPHOSPHAMIDE
- DACARBAZINE
- LOMUSTINE (CCNU)
- MECHLORETHAMINE
- MELPHALAN
- THIOTEPA

**NATURAL PRODUCTS**  
(green cards)
- BLEOMYCIN
- DACTINOMYCIN
- DAUNORUBICIN
- DOXORUBICIN
- ETOPOSIDE (VP-16)
- IRINOTECAN
- MITOMYCIN C
- PACLITAXEL
- VINBLASTINE
- VINCristINE

**MISCELLANEOUS**  
(white cards)
- Angiostatin
- AMSACRINE
- L-asparaginase
- Bortezomib
- CARBOPLATIN
- CISPLATIN
- Erlotinib
- Gefitinib
- Hydroxyurea
- Imatinib
- Pentostatin
- PROCARBAZINE
- Thalidomide

**ANTIMETABOLITES**
- Azathioprine
- 5-fluorouracil
- 6-thioguanine
- 6-mecaptopurine
- Cytarabine (ara-c)
- Gemcitabine
- Methotrexate

**IMMUNOTHERAPY**
- Alemtuzumab
- Aminoglutethimide
- Bevacizumab
- Cetuximab
- Cyclosporine
- Dexamethasone
- Edrecolomab
- Gemtuzumab
- Ibritumomab
- Interferon α
- Interleukin 2
- Interleukin-12
- Prednisone
- Rituximab
- Tacrolimus (fk506)
- Tositumomab
- Trastuzumab
- Tumour necrosis factor α

**HORMONES and RELATED AGENTS**
- Aminoglutethimide
- Anastrozole
- Exemestane
- Flutamide
- Letrozole
- Goserelin
- Leuprolide
- Letrozole
- Tamoxifen

**SUPPORTING AGENTS**
- Allopurinol
- Erythropoietin
- Filgrastim
- Interleukin 11
- Leucovorin
- MESNA
- Sargramostim (GM-CSF)
Mechanisms of Action of Antineoplastic Drugs

1. Prevent DNA synthesis
2. Disrupt DNA, prevent DNA repair, and/or prevent RNA synthesis
3. Interrupt mitosis
4. Interfere with protein or hormone synthesis
5. Immunotherapy
6. Prevent angiogenesis and/or formation of metastases

DRUGS THAT DISRUPT DNA

General Categories

I. Drugs that crosslink DNA (alkylating agents)
II. Drugs that intercalate or form adducts with DNA
III. Drugs that cause strand breaks
I. CROSSLINKING AGENTS
(ALKYLATING AGENTS, MITOMYCIN C and PLATINS)

Types
- Nitrogen mustards: CYCLOPHOSPHAMIDE, MECHLORETHAMINE, MELPHALAN
- Ethylenimines: THIOTEPA
- Alkyl sulfonates: BUSULFAN
- Nitrosoureas: CARMUSTINE (BCNU), LOMUSTINE (CCNU)
- Triazenes: DACARBAZINE
- Others: CARBOPLATIN, CISPLATIN, MITOMYCIN C

Mechanism of Action
(alkylating agents)

- Activation → Nucleophilic Attack → DNA Damage

Although crosslinking agents are dependent upon proliferation, these agents ARE NOT cell cycle specific because the alkylation reactions that initiate cell death can occur during any phase of the cell cycle. However, primary toxicity occurs in late G1 and S phase (not G2, M or early G1).

- Crosslinking of DNA
- Miscoding of DNA (G-T pairing)
- DNA strand breakage (depurination)

1) DNA damage normally activates a cell cycle checkpoint that is dependent on the p53 gene and 2) 50% of human cancers have a mutated or absent p53 gene ⇒ cancer cells cannot repair the DNA alkylation or undergo apoptosis, making these drugs more effective in those types of cancers.
Structure/Activity Relationships

One major distinguishing feature among alkylating agents is the rate at which the parent drug is converted to become strong electrophiles. For some drugs (e.g., **MECHLORETHAMINE**), the reaction is virtually instantaneous --- these agents are given IV to minimize blistering and GI effects. Others agents (such as **CYCLOPHOSPHAMIDE**) must be activated in the liver and other tissues, and they can be given orally.

Activation of **CYCLOPHOSPHAMIDE**
Toxicity

Acute:
1. nausea and vomiting
2. strong vesicant properties

Delayed:
1. moderate to severe myelosuppression (peaks at 6-10 days; recovery in 14-21 days)
2. immunosuppression
3. gonadal failure
4. carcingogenesis (leukemia)
5. teratogenesis
6. mutagenesis

Resistance

1. decreased permeation
2. increased production of nucleophilic substances (e.g. glutathione)
3. increased activity of DNA repair enzymes (e.g. guanine O6-alkyl transferase)
4. increased rates of metabolism

II. DRUGS THAT INTERCALATE OR FORM ADDUCTS WITH DNA
(Antibiotics: DACTINOMYCIN, DAUNORUBICIN, DOXORUBICIN)

The anthracycline antibiotics DAUNORUBIN and DOXORUBICIN work by intercalating into DNA. Their major toxicity is an UNUSUAL CARDIOMYOPATHY that is often IRREVERSIBLE and related to the TOTAL DOSE of the drug.
Mechanism of Action

- Cell cycle non-specific, but maximal toxicity in S phase (at low concentrations will pass through S and die in G₂)
- **intercalate with DNA** (primary mechanism)

Several potential cytotoxic actions:
- single- and double-strand breaks (mutagenic), either via topoisomerase II or by free radical generation
- sister chromatid exchange (carcinogenic)
- inhibition of topoisomerase II
- in the presence of NADPH, they react with cytochrome P450 reductase to form superoxide anion radicals including both H₂O₂ and hydroxyl radicals
- interact with cell membranes to alter fluidity and ion transport

Pharmacokinetics

- usually administered IV (vesicant)
- rapid clearance from plasma
- eliminated by metabolic conversion (dependent on adequate liver function)

Toxicity

- **UNUSUAL CARDIOMYOPATHY** that is often **IRREVERSIBLE** and related to the TOTAL DOSE of the drug – due to increased production of free radicals within the myocardium – can be partially reduced by using exogenous antioxidants to boost host defenses (such as superoxide dismutase and catalase)

  Acute:  
  1) ST-T-wave alterations and arrhythmias
  2) nausea, red urine, extravasation → necrosis
Delayed: 1) congestive heart failure that is unresponsive to digitalis  
(may occur years after treatment)  
2) myelosuppression is a major dose-limiting complication  
3) stomatitis, GI disturbances and alopecia are common  
4) “radiation recall reaction” – erythema and desquamation of the skin at sites of prior radiation therapy

Resistance
1. acceleration of the efflux of anthracyclines (P-glycoprotein)  
2. increased glutathione peroxidase activity  
3. decreased topoisomerase II activity

III. DRUGS THAT CAUSE STRAND BREAKS and/or PREVENT DNA REPLICATION (some are TOPOISOMERASE INHIBITORS)

Types
1. Antibiotic: BLEOMYCIN  
2. Plant alkaloids: ETOPOSIDE (epipodophyllotoxin), IRINOTECAN (camptothecin)  
3. Miscellaneous: AMSACRINE, PROCARBAZINE

Mechanisms of Action
1. BLEOMYCIN
   • mixture of 2 glycopeptides (BLEOMYCIN A2 and B2)  
   • unique mechanism – bleomycin binds to DNA through its amino-terminal peptide, and generates free radicals that cut the DNA  
   • attacks DNA but not RNA  
   • accumulation of cells in G2 phase that have chromosomal aberrations (chromatid breaks, gaps, fragments and translocations)
2. **ETOPOSIDE** and **IRINOTECAN**
   - **ETOPOSIDE** forms a complex with topoisomerase II and DNA that results in double-strand breaks – remains bound to enzyme so repair cannot occur
   - **IRINOTECAN** is a prodrug that is converted to a potent inhibitor of topoisomerase I
   - cell cycle specific – (S and G₂ phases)

3. Miscellaneous
   - **AMSA RINE**
     - causes the formation of forms topoisomerase II-DNA complexes that are trapped at the breaking-sealing stage
   - **PROCARBAZINE**
     - unknown mechanism - inhibits DNA, RNA and protein synthesis- also causes DNA strand scission
     - must undergo metabolic activation (either spontaneously or via cytochrome P450 system)

**Resistance**

**BLEOMYCIN**
1. increased hydrolase activity
2. enhanced capacity to repair DNA

**ETOPOSIDE**
1. amplification of transporter (P-glycoprotein)
2. mutation or decreased expression of topoisomerase II
3. mutation of p53 tumor suppressor gene (required for apoptosis)
DRUGS THAT INTERRUPT MITOSIS

General Categories

I. Vinca alkaloids: **VINCRISTINE, VINBLASTINE**
II. Taxanes: **PACLITAXEL**

I. **VINCA ALKALOIDS**

Mechanism of Action

- cell-cycle-specific – cell division is arrested in **metaphase**
- bind to tubulin at the forming end and **TERMINATE** assembly
  (induce formation of structures other than microtubules)
The vinca alkaloids (VINCRISTINE and VINBLASTINE) and the taxanes (PACLITAXEL) are mitotic spindle poisons. Despite their structural similarity, VINCRISTINE and VINBLASTINE have significantly different toxicities.

Structure/Activity Relationships

- minor alterations in structure can result in significant changes in toxicity and anti-tumour activity (some related alkaloids are without activity)

Resistance

cross reactivity is not absolute
1. due to increased expression of P-glycoprotein
2. mutations to tubulin that prevent drug binding

Toxicity

Mnemonic: VINCRISTINE - CNS toxicity
VINBLASTINE - bone marrow suppression.

Acute: Nausea and vomiting (VINBLASTINE ONLY)
Delayed: Alopecia, bone marrow suppression (greater for VINBLASTINE), loss of reflexes, constipation

VINCRISTINE also produces muscle weakness, peripheral neuritis, paralytic ileus

Neurotoxicity is prominent because tubulin is essential for the structure and function of axons. Depression of deep tendon reflexes occurs within 2-3 weeks in 100% of patients, followed by severe paresthesias and mild to moderate sensory loss (indication to decrease dose). VINCRISTINE has more CNS effects because it is more lipid soluble.
II. PACLITAXEL

Mechanism of Action

- cell-cycle-specific (arrest in mitosis)
- **ENHANCES** microtubule assembly without the involvement of microtubule-associated proteins or guanosine triphosphate, and prevents disassembly
- significant activity in ovarian and advanced breast cancer

Example questions:  A 56-year-old man with non-Hodgkin’s lymphoma underwent a successful course of therapy with the CHOP regimen.

1. Which of the following classes of anticancer drugs is cell cycle-specific (CCS) and used in the CHOP regimen?
   A) Alkylating agents  
   B) Vinca alkaloids  
   C) Antimetabolites  
   D) Glucocorticoids  
   E) Plant alkaloids

2. During the second course of treatment, this patient developed hemorrhagic cystitis. The most likely causative agent is:
   A) Bleomycin  
   B) Cyclophosphamide  
   C) Doxorubicin  
   D) Prednisone  
   E) Vincristine