PHAR 509: Exam I Lecture Review

“The story of cancer is the story of patients who struggle and survive, moving from one embankment of illness to another. Resilience, inventiveness, and survivorship – qualities often ascribed to great physicians – are reflected qualities, emanating first from those who struggle with illness and only then mirrored by those who treat them. If the history of medicine is told through the stories of doctors, it is because their contributions stand in place of the more substantive heroism of their patients.”

-Siddhartha Mukherjee, The Emperor of All Maladies

(1/16) Cuellar Lecture: Introduction to Oncology²

Everyone is at risk for developing cancer. Population data highlights gender-specific trends and mortality rates by cancer type. The impact of cancer in 2019 is estimated in the table below. The leading cause of mortality among cancer types is lung cancer. This is because, while most cancer types have screening techniques and tests for early detection, lung cancer evades recognition until it is too late. Here are some gender-specific differences

- Incidence: Highest = sex organ (prostate/breast) followed by lung
- Mortality: Highest = lung followed by sex organ (prostate/breast)

### Epidemiology

<table>
<thead>
<tr>
<th>2019 Estimated US Cancer Trends: Incidence and Mortality Rates by Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Incidence</td>
<td>Cancer Mortality</td>
<td>Cancer Incidence</td>
</tr>
<tr>
<td>Prostate (20%)</td>
<td>Lung + Bronchus (24%)</td>
<td>Breast (30%)</td>
</tr>
<tr>
<td>Lung (13%)</td>
<td>Prostate (10%)</td>
<td>Lung (13%)</td>
</tr>
<tr>
<td>Colon &amp; Rectum (9%)</td>
<td>Colon &amp; Rectum (9%)</td>
<td>Colon &amp; Rectum (8%)</td>
</tr>
<tr>
<td>Melanoma of Skin (7%)</td>
<td>Pancreas (7%)</td>
<td>Uterine corpus (7%)</td>
</tr>
</tbody>
</table>

### Etiology = Multifactorial

The list of contributing factors to cancer development is endless, but can be summarized by category:

- Occupation: ionizing radiation, UV light
- Environmental: Asbestos, benzene
- Infection: hepatitis B, papillomavirus, Epstein Barr
- Lifestyle: diet, alcohol, tobacco,
- Heredity: breast, colon
- Drugs/Hormones: estrogens, chemotherapy

### Pathophysiology

According to the American Cancer Society (ACS), cancer is a group of diseases characterized by uncontrolled growth and the spread of abnormal cells. If not contained, this spread can result in death. The heterogeneity of each cancer’s genetic infrastructure and physical manifestations can make cancer exceptionally difficult to treat

- **Uncontrolled growth**
  - **Mutation**: Normally, cells proliferate under strict control. If damage is detected, proliferation is paused for repair by cellular machinery. If unsuccessful, the cell is targeted for self-destruction via apoptosis. These processes occur to ensure damage/mutations are not passed on to subsequent generations
  - **Selection**: Although mutations naturally occur, certain mutations confer selective advantages, such as: uncontrollable proliferation, prevention of apoptosis, heightened responses to growth signals, etc.
  - **Progression**: Unregulated, rapidly dividing cells welcome additional mutations. The summative result produces a lineage of cells with abnormal cellular division and uncontrollable growth – the primary tumor

- **Abnormal cells**
  - Cancer cells have distinct malformations which are recognizable under microscope. Cells are large and irregular in shape, distinguishable even among other cancer cells, deficient in specialized cell features, disorganized in arrangement, and with poorly defined boundaries.

- **Metastasis**
  - Metastasis is the spread of neoplastic cells from the primary tumor to distant sites, forming new tumors
  - **Intravasation**: Invasion starts when the primary tumor interacts with components of the blood or lymph. This process, referred to as intravasation, facilitates systemic transport, occurring by one of two paths:
    - Hematogenous: Transport using the circulatory vasculature
    - Lymphatic: Transport using the lymphatics
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    - Lymphatic: Transport using the lymphatics
  - **Patterns of Metastasis**: Trends for the progression of specific cancers are well-elucidated. We have found that particular cancers metastasize to predictable anatomical sites
• Knowing the pathophysiologic mechanisms of cancer helps identify opportunities for pharmacologic intervention.
• Originally published in Cell in 2000 and then later updated in 2011, Hanahan and Weinberg’s “Hallmarks of Cancer: The Next Generation” is a high impact review identifying the core pathologic features amidst the befuddling heterogeneity of cancer. Examples include the genetic insult and angiogenesis.

**Tumor Development and the Genetic Factors**

- The onset of cancer starts with genetic mutation or epigenetic chemical modifications. The majority of malignant processes have several genetic/epigenetic alterations amongst many genes. Each individual’s cancer is unique, encouraging the use of ‘tumor fingerprints’ and tumor profiling to tailor therapy to specific mutations.

- **Genetic Pathway:** Alterations or mutations in DNA affects specific genes associated with malignancy, such as proto-oncogenes, tumor suppressor genes, and DNA repair genes. Cancer-promoting mutations can occur in many other genes, however.
  - Germline mutation: Heritable – found in the original egg/sperm, can be tested for in the blood
  - Somatic mutation: Acquired – develops after birth, Found only at the site of the tumor

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function/Description</th>
<th>Mutation</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proto-oncogene</td>
<td>Essential regulators of normal cellular functions</td>
<td>Mutations develop them as oncogenes</td>
<td>BCL2, HER2,</td>
</tr>
<tr>
<td>Oncogene</td>
<td>A gene capable of causing cancer</td>
<td>Redundant mutations can arouse resistance</td>
<td>RAS</td>
</tr>
<tr>
<td>DNA Repair</td>
<td>Correct DNA replication errors prior to cell division</td>
<td>Allows DNA mutations to be heritable</td>
<td>BRCA2</td>
</tr>
<tr>
<td>Tumor suppressor</td>
<td>A natural regulator against cancer-like processes</td>
<td>Inactivation permits cellular growth and proliferation</td>
<td>BRCA1/2, p16</td>
</tr>
</tbody>
</table>

  - **Total Mutational Burden (TMB):** Routing anticancer therapy has recently taken on a new approach of assigning treatment based on the TMB. This is an individual’s collective sum of mutations, including point mutations, deletions, insertions, translocations, and amplifications.
  
  - **Cytogenetics:** Cytogenetics is the study of chromosomes, which is particularly useful in cancer therapy. Recognizing a particular individual’s genetic mutations can assign the disease category and offer prognostic data and suggested treatment options. For instance, an individual with chronic myelogenous leukemia (CML) identified to have the Philadelphia Chromosome, a t(9;22) translocation, suggests a good prognosis and imatinib as the opportune chronic suppressive therapy.

  - **Epigenetic Pathway:** Chemical, topological modifications of chromatin leads to alterations in gene expression, potentially upregulating or repressing genes involved with cell cycle control, angiogenesis, cell migration, and cellular responses to DNA damage.
    - Features: These changes are reversible – meaning they can be turned on and off.
    - Examples of chemical modifications include histone methylation, demethylation, and acetylation. Most pronounced are the repressive qualities of methylation at specific DNA sequences. Hypermethylation of CpG islands effectively prohibits transcription, potentially silencing tumor suppressor genes.

**Tumor growth and Angiogenesis**

- Angiogenesis is the formation of new blood vessels. When the tumor grows beyond its nutritional capacity, malignant cells and the surrounding tissues secrete growth factors to stimulate the formation of new blood vessels to deliver oxygen and nutrients to the cancer cells.

- VEGF-Inhibitors: Vascular endothelial growth factor (VEGF) is the primary angiogenic growth factor. Anticancer drugs have been developed to block the activity of VEGF, such as the antibody-based drug bevacizumab (Avastin).

**Treatment Modalities**

- Chemotherapy
  - Cytotoxic chemotherapy is the earliest treatment strategy that works by killing rapidly dividing cells via chemical interactions with DNA itself. Whilst this sounds ideal and specific to cancer cells, there are healthy host cell types that undergo rapid division, such as hair, skin, and endothelial organ linings. Thus, some host cells are sensitive to the effects of chemotherapy, resulting in adverse consequences
    - Neutropenia: Neutrophils have a 1/2 of 8 hours, making them inadvertent targets of chemotherapy. Consequently, recipients of non-specific chemotherapy are often rendered neutropenic and at-risk of infection.
  - Classic chemotherapy targets the relationship between apoptosis and mechanisms of the cell cycle
    - G1: Steroids, tamoxifen, asparaginase
    - S: Antimetabolites, steroids, camptothecins
    - G2: Bleomycin, epipodphyllotoxins, camptothecins
    - M: Vinca alkaloids, taxanes
Combination Chemotherapy: Based on the Goldie-Coldman hypothesis, all tumors inherently develop resistance through mutations occurring at a rate of every $10^7$–$10^8$ cells. Therefore, the maximal chance of cure involves the use of multiple chemotherapy agents with non-overlapping toxicity profiles at consistent intervals. By this approach, combination chemotherapy theoretically can overcome resistance
- Toxicties of concern: myelosuppression, nephrotoxicity, GI toxicity/mucositis
- Advantages: Maximal cell kill within acceptable toxicity limits. Broad coverage.
- Disadvantages: Elicits multiple toxicities garnering greater patient discomfort

- Molecular-targeted Therapies
  - Targeted anticancer therapy includes drugs with focused MoAs specifically acting on well-defined targets or biological pathways, which, upon activation, cause regression or destruction of the malignant process
    - Pathways of interest: Cell growth and cell death
    - Point of attack: ECF $\leftrightarrow$ ICF interchange, cell surface receptors
    - Utilized molecules: monoclonal antibodies (mAb), small molecule inhibitors (TK, MK, prot)

(1/18) Cuellar Lecture: Principles of Oncology

Staging of Cancer
Knowing the patient’s extent of disease has great utility and serves many benefits. Some of staging’s impacts include: assigning definitive therapy (or treatment, in general), prognosis, monitoring treatment response, and standardization of terminology for clinical research and academic purposes

- Tools for staging: X-Ray, CT, MRI, Bone Scan, Ultrasound, Tumor markers (CEA, CA-125, AFP, PSA)
  - PET Scan: Highlights glucose metabolism. Normally, the brain and bladder are always lit up
- Solid Tumor (ST) Staging
  - American Joint Committee for Cancer (AJCC): Roman numeral scoring system assigning severity based on lymph node involvement and metastasis. Whereas a N-III suggests local-regional spread (at local lymph nodes), stage IV signifies distant involvement/metastases
  - TNM Classification ($T$,$N,M$): Generalities are used in this system, as each cancer type may be specific
    - $T_{1-4} =$ Initial Tumor size. Location-specific, is it resectable?
    - $N_{1-4} =$ Nodal involvement and spread of disease ($N_0 =$ none, $N_1 = 1$, etc) More = worse
    - $M_{1-0} =$ Metastasis – advanced disease spreading, Yes or No. When cancer has metastasized, local option (surgery and radiation) are no longer available – systemic therapy is required
- Hematologic Malignancies (HM)
  - By the nature of these cancers, different staging systems are used. Roman numeral stages are assigned based on cytogentic, mutations, blood counts, presence or absence of symptoms, etc. This applies to leukemia, lymphoma, and both Hodgkin and non-Hodgkin lymphoma

Treatment Considerations
- Patient Demographics, Cancer-type, and Staging- Discussed above.
- Medical Conditions: comorbidities, baseline organ function, performance status (scales)
  - Baseline organ function: Critical for treatment assignment and monitoring. Doxorubicin is known to cause HF, and therefore should be avoided in those with baseline EF < 50%. Cisplatin, hella nephrotoxic, should not be used in patients with X degree of renal impairment.
  - Performance Scores: ECOG and Karnosky scales
- Risk versus Benefit: Younger, healthier patients are more capable of handling aggressive treatment compared to deconditioned, poor candidates. It is important to consider the possibility of extending life and the quality of life that is extended while weighing the side effects

Performance Status
Performance status is a global assessment of the patient’s ability to be ambulatory, complete ADLs, and take care of themselves. This is important to keep track of, starting at baseline, for purposes of measuring improvement and monitoring safety. It is also a key parameter used in clinical trials.

ECOG Performance Status

<table>
<thead>
<tr>
<th>Grade</th>
<th>ECOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, no new restrictions</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity, but able to carry out work of a light/sedentary nature</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of self-care, but unable to carry out work activities. Active &gt; 50%/day</td>
</tr>
<tr>
<td>3</td>
<td>Limited self-care capabilities, confined to bed/chair &gt; 50%/day</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled, unable to carry out self-care. Totally confined to bed or chair</td>
</tr>
<tr>
<td>5</td>
<td>Dead.</td>
</tr>
</tbody>
</table>
Goals and Intentions for Cancer Treatment

Each patient’s treatment has unique goals for the management of cancer based on their prognosis, symptomatic burden, age, and personal preferences. Collectively, goals include: cure, prolonged survival, and relief of symptoms. Though, in some patients, cure is unattainable. Thus, the intent of treatment determines the interventions and style of decisions.

- **Curative Intent**
  - Decisions: Discourage dose delays and/or dose reductions
  - Treatment: Precise and aggressive, heavily involved in toxicity management

- **Non-curative intent**: When cure is no longer the intent, the goal is to preserve QoL and prolong quality survival
  - Decision: May hold or reduce doses, even discontinuing therapy to make it easier on the patient
  - Treatment: Patient has a bigger role in deciding their therapy

Depending on the goals and intentions of treatment, different anticancer regimens are incorporated

- **Induction**: High-dose combination chemotherapy with the intent of inducing complete remission
  - Induction therapy is used with curative intent, aiming for cancer eradication

- **Consolidation**: Following complete remission via induction therapy, repeated, but less intensive, induction-like regimens are used to improve the odds of complete eradication. Common for hematologic malignancies
  - Consolidation therapy is used to prolong remission and increase the chance for cure

- **Maintenance**: A less intense regimen given after primary therapy to maintain a response
  - Maintenance therapy aims to prevent progression/relapse in patients with stable disease or partial response. It also functions to delay combination chemotherapy, as it commonly is a single-agent

- **Adjuvant**: Short course, high-dose, combination chemotherapy given after surgery or radiotherapy to address the possibility of residual tumor cells – this treatment is typically for solid tumors.
  - Adjuvant therapy is used when there is curative intent, and is usually used when cure is presumed to be near. Due to the chance of micrometastases, recurrence is prevented using adjuvant therapy

- **Neo-adjuvant**: Pre-operative chemotherapy used in solid tumors to improve chances of surgical candidacy
  - Neo-adjuvant therapy aims to shrink tumors so that patients are more reasonable surgical candidates

- **Palliative**: Provision of chemotherapy to control symptoms or prolong life in patients with low likelihood of cure

Describing a patient’s response to anticancer therapy

The oncology field is unique in its wide network of collaboration and cooperative groups. By joining forces, clinical trials can accrue patients more quickly and the discovery of cure can be expedited. The National Cancer Institute (NCI) develops standardized terminology required to be utilized by all cooperative groups. This allows for consistent evaluation and comparison among regimens across different trials

- **Disease Status**
  - **Cure**: Disease-free survival for 5 years. Patients are expected to have a life expectancy similar to that of a cancer-free individual
  - **Stable Disease**: Tumor is neither growing or shrinking
  - **Progressive Disease**: Also signaling treatment failure, progressive disease is defined by a ≥ 20% increase in the size of a lesion/tumor or development of new lesions in patients receiving treatment

- **Response Evaluation Criteria in Solid Tumors (RECIST Criteria)**: Primarily used for cytotoxic therapy and targeted agents in solid tumors
  - **Partial Response (PR)**: A ≥ 30% decrease in the sum of diameters of target lesions
  - **Complete Response (CR)**: The complete disappearance of all target lesions, and no evidence of new disease for ≥ 1 month following treatment. The CR rate is the most valuable parameter in clinical trials
  - **Overall Response Rate (ORR)**: ORR = CR + PR. Frequently used in clinical trials, but mostly valid for patients with heavy tumor burdens or heavily symptomatic patients

- **Hematologic malignancies- techniques to measure response include:**
  - bone marrow evaluation, elimination of abnormal cells, tumor marker lab values, disappearance of pleural or peritoneal effusions, improved function of the affected organs (liver, spleen)

- **Immunotherapy Response Criteria**: Since drugs targeting the immune system have different mechanisms, there are specific response criteria (irRC) used to address the unique findings of imaging studies
  - **Complete Response (irCR)**: Complete disappearance of all lesions and no new lesions, which is confirmed by a repeat assessment no less than 4 weeks from the first documented remission
  - **Partial Response (irPR)**: Decrease in tumor burden by ≥ 50% relative to baseline, confirmed by a follow-up repeat assessment no less than 4 weeks later
  - **Stable Disease (irSD)**: Not meeting criteria for irCR or irPR, while in absence of irPD
  - **Progressive Disease (irPD)**: Increased tumor burden by ≥ 25% relative to nadir/baseline, which is confirmed by repeat assessment no less than 4 weeks later
Outcome evaluation methods
- Quality of Life (QoL): Most useful in cancers without a cure. Available instruments include:
  - Functional Living Index-Cancer (FLIC)
  - Quality adjusted time without symptoms and toxicity (Q-TWIST)
- Duration of Survival
- Duration of Response: The time from the first documented response to the recurrence/progression
- Progression-free Survival (PFS): A commonly used endpoint, marking the duration of time before a patient progresses while on therapy – applicable to metastatic diseases
- Disease-free Survival (DFS): In trials of agents with curative intent, this is the best endpoint – it measures the length of time following primary treatment that a patient survives

Graphical Models
- Waterfall PLOT, Swimmers PLOT, Survival Curve

Tolerability of Therapy: Toxicities and complications are important to collect data on as they can limit the duration and intensity of treatment that a patient receives. Therefore, standardized rating scales have been developed. Common Toxicity Criteria Grading System (CTC)
- The CTC is the graded 0-5 system used to measure toxicity, with 0 = none, 5 = death.
- Graded components: Organ function, labs, n/v, mucositis, fatigue, peripheral neuropathy
- Toxicity Grade 1-2: Generally continue therapy and provide supportive care
- Toxicity Grade 3-4: Usually modify therapy, reduce dose, or discontinue therapy
- Using these grading scales helps drug information translate among clinical trials

Oncology Guidelines
Guidelines for the treatment of cancer include excerpts from primary literature as well as justifications from third-party payers to suggest the optimal treatment option based on efficacy, safety, and route of administration. Collectively, these data form the basis for the two styles of guidelines: (1) evidence-based or (2) consensus-based. Though, there is a shortage on guidelines because of the rapid evolution of evidence. Major sources include:
- American Cancer Society (ACS): Provides early detection and screening recommendations
- National Comprehensive Cancer Network (NCCN): An alliance of 21 cancer centers that produces both evidence-based and consensus-based guidelines. Some of these are for treatment, some are for screening techniques. Though they are regularly updated, registration is required. The NCCN Categories of Evidence and Consensus are important to remember:
  - Category 1: [High-level evidence], Uniform NCCN consensus that the intervention is appropriate
  - Category 2a: [Lower-evidence], Uniform NCCN consensus that the intervention is appropriate
  - Category 2b: [Lower-evidence], Uniform NCCN consensus that the intervention is appropriate
  - Category 3: [Any evidence]: Major NCCN disagreement that the intervention is appropriate
- American Society of Clinical Oncology (ASCO): Develops narrative-based descriptions of evidence to assemble multiple guidelines, though is limited in its recommendation for treatment selection. ASCO is better known for its supportive care guidelines for antiemetics, anemia, chemoprotectants, and growth factors
- European Society for Medical Oncology (ESMO): The European equivalent of ASCO, ESMO produces guidelines for multiple cancer types and supportive care. It does not require registration and updates its guidelines annually. It incorporates the traditional evidence (I-V) and recommendation (A-D) rating system
  - Level of Evidence: I = Meta-analysis, V = Case reports
  - Grades of Recommendation: A = Good, B = Evidence, consistent, C = Evidence, inconsistent, D = bad
Tumor Lysis Syndrome

- **Prevalence:** The frequency of TLS varies by tumor type. It is most often found in hematologic malignancies, such as lymphomas and leukemia, but can be found in STs such as small-cell lung cancer and breast carcinoma. Risk factors for the development of TLS include:

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Type</td>
<td>• Lymphoma: Burkitt’s, Lymphoblastic, Diffuse Large Cell</td>
</tr>
<tr>
<td></td>
<td>• Leukemia: Acute Lymphoblastic, Acute Myeloid</td>
</tr>
<tr>
<td></td>
<td>• Solid tumors with high proliferative rate (small cell lung cancer)</td>
</tr>
<tr>
<td>Disease Burden</td>
<td>• Large tumor (&gt;10cm) • Elevated LDH (&gt;2x ULN) • Elevated WBC (&gt;25k/µL)</td>
</tr>
<tr>
<td></td>
<td>• Bone marrow involvement (leukemias) • Organ infiltration</td>
</tr>
<tr>
<td>Patient-Specific</td>
<td>Major: Preexisting renal failure, oliguria, and baseline hyperuricemia (&gt; 7.5mg/dL)/gout</td>
</tr>
<tr>
<td></td>
<td>Other: HTN, dehydration (give fluids), acidic urine</td>
</tr>
</tbody>
</table>

*Elevated LDH is a sign of highly proliferative tumor*

- **Pathophysiology:** TLS describes the rapid breakdown of cancer cells and their release of intracellular contents. Symptomatic manifestations of TLS occur when these contents are released too quickly for the body to remove them, resulting in accumulation and metabolic abnormalities: Uric Acid, K+, PO4, Ca^2+. Uric Acid accumulation is due to the increased metabolism of intracellular DNA. Purines are catabolized by successive oxidation steps to uric acid. Normally, renal clearance occurs at 500mg/day. D/t its poor water solubility (pKa 5.4-5.7), uric acid may crystallize if clearance is overwhelmed.
  - Damage: Crystallization in the kidney can result in acute renal failure
  - **Hyperkalemia:** Normal ICF [K+] is 150mEq/L, whereas ECF [K+] is 3.5-5.0mEq/L. Cell lysis, resulting from chemotherapy, releases these intracellular ions to the environment
  - Damage: Cardiac arrhythmia
  - **Hyperphosphatemia:** Cell lysis releases intracellular phosphates to the environment
  - Damage: Interaction with Ca^2+ forms precipitates which can induce acute renal failure

- **Diagnosis:** Guidelines for the prevention, detection, and management of TLS are released by ASCO.
  - **Laboratory Definition:** The presence of ≥ 2 of the specified metabolic abnormalities presenting 3 days prior to therapy or 7 days following treatment initiation signifies the need for TLS tx or ppx.

<table>
<thead>
<tr>
<th>Metabolic Element</th>
<th>Point Value</th>
<th>Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid</td>
<td>≥ 8.0 mg/dL</td>
<td>25% Increase</td>
</tr>
<tr>
<td>Potassium</td>
<td>≥ 6.0 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>≥ 4.5 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>≤ 7.0 mg/dL</td>
<td>25% Decrease</td>
</tr>
</tbody>
</table>

- **Clinical Definition:** When identified via laboratory and one of the following clinical factors, dx is TLS
  - Creatinine ≥ 1.5 ULN, cardiac arrhythmia, seizure, or sudden death

- **Prior to Treatment:** Guidelines for Risk Stratification of TLS are available from the Clinical Options in Oncology website. Risk stratification helps clinicians stratify candidacy for continued treatment and identifies patients who need prophylactic treatment.

<table>
<thead>
<tr>
<th>Patient Case</th>
<th>LOW RISK</th>
<th>INTERMEDIATE RISK</th>
<th>HIGH RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>WBC &lt; 50k/µL</td>
<td>WBC 50k-100k/µL and LDH &lt; 2x ULN</td>
<td>WBC ≥ 100k/µL or LDH ≥ 2x ULN</td>
</tr>
<tr>
<td>AML</td>
<td>WBC &lt; 25k/µL</td>
<td>WBC 25k-100k/µL or LDH ≥ 2x ULN</td>
<td>WBC ≥ 100k/µL</td>
</tr>
<tr>
<td>Burkitt’s Leu/Lym</td>
<td>Early stage and LDH &lt; 2x ULN</td>
<td>Advanced or early stage and LDH ≥ 2x ULN</td>
<td></td>
</tr>
<tr>
<td>CLL (tx w/ Veneto)</td>
<td>All lymph nodes are &lt; 5cm and ALC &lt; 25k/µL</td>
<td>≥ 1 LN 5-10cm or ALC ≥ 25k/µL</td>
<td>≥ 1 LN 5-10cm or ALC ≥ 25k/µL</td>
</tr>
<tr>
<td>DLBCL</td>
<td>-</td>
<td>LDH ≥ 2x ULN and nonbulky disease</td>
<td>LDH ≥ 2x ULN and bulky disease</td>
</tr>
<tr>
<td>Indolent Lymphomas</td>
<td>LDH &lt; ULN</td>
<td>LDH ≥ ULN</td>
<td>-</td>
</tr>
</tbody>
</table>

"(1/23) Haaf Lecture: Select Oncological Emergencies"
Management of Tumor Lysis Syndrome

- ASCO’s Risk-Based Algorithm for TLS: Interventions are described in the following section
  o Low Risk: Clinical judgement + monitoring. Specifically at UIH, IV fluids + allopurinol are used
  o Intermediate Risk: IV fluids + allopurinol. Clinician-specific use of rasburicase in certain pediatric patients, but otherwise rasburicase is on hold for when hyperuricemia develops
  o High Risk: Hydration + rasburicase


• TLS Prophylaxis and Treatment
  - Non-Pharmacologic Interventions
    o Hydration: Aggressive IV fluids (125mL/h) and enhancement of urinary output of uric acid is essential for the prevention and management of TLS. Hydration and diuresis should be initiated as soon as possible preceding chemotherapy.
    o Alkalization: Sodium bicarbonate drips are no longer recommended for use due to the risks of precipitating xanthine and hypoxanthine as well as preventing rasburicase’s activity.
  - Allopurinol: Xanthine Oxidase Inhibitor (XOI): Allopurinol prevents the synthesis of urate. It is used at the initiation of chemotherapy in high tumor-burden malignancies, it works slowly.
    o Dosing: 300mg/m²/day or 10mg/kg/day in three divided doses (Max 800mg/day). Begin dosing 1-2 days prior to induction, continue 3-7 days following chemotherapy or normalization of TLS-based labs.
      - († Site-dependent. Doses of 300mg TID are common)
    o Renal-adjustment is required. Many tiers of dosing schemes based on CrCl
    o DDI: 5MP, thiazides, abx
    o ADR: Well-tolerated, incidence of rash and hypersensitivity
  - Rasburicase (Elitek): Recombinant Urate Oxidase (enzyme). Rasburicase catalyzes the oxidation of urate to the water-soluble, readily eliminated, allantoin. It mimics Urate Oxidase, an enzyme not found in humans (From *Aspergillus flavus*, developed in *Saccharomyces cerevisiae*)
    o Rasburicase decreases uric acid levels within 4 hours of administration
    o Dosing: FDA-approved for weight-based IV dosing, 0.2mg/kg daily x5 days. Max 5 days
    o Flat-Dosing: Off-label, but more commonly used, flat-dosing saves money without compromising efficacy. Using the two vial sizes, (1.5mg, 7.5mg), most patients’ doses are either 3mg or 7.5mg
    o Black Box Warnings [BBW]
      - Hemolysis (contraX in G6PD deficiency)
      - Enzymatic blood sample degradation (staff education)
      - Hypersensitivity
      - Methemoglobinemia

Hypercalcemia of Malignancy (HOM) and Bone Metastases

- Normal serum concentrations of Ca²⁺ hover around 9mg/dL. Any concentration can be considered acute – because if the velocity of the concentrations rise is rapid, significant symptoms can present. Moreover, in this case, symptoms would not correlate with the serum concentrations. Therefore, the acuity of the rise is most important

<table>
<thead>
<tr>
<th>Severity</th>
<th>Serum [Ca²⁺] (mg/dL)</th>
<th>Symptoms / Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>10.5-12</td>
<td>Asymptomatic, constipation, fatigue</td>
</tr>
<tr>
<td>Moderate</td>
<td>12-14</td>
<td>N/V/C, Anorexia, muscle weakness</td>
</tr>
<tr>
<td>Severe/Life-Threatening</td>
<td>14⁺</td>
<td>CV and neuromuscular dysfunction, renal failure, profound dehydration, coma</td>
</tr>
</tbody>
</table>

• HOM Treatment
  - Hydration and Diuresis: Saline fluid therapy should be the foundation of HOM management. Fluid delivery should target a urine output of ≥ 75mL/hour, rechecking labs intermittently. Patients with HF or renal impairment should be considered for loop diuretic therapy.
  - In the table below, symptomatic refers to notable changes on PE or EKG changes.
Drug options for HOM

- Calcitonin: The benefit of calcitonin is that it is very fast-acting compared to our other agents. However, tachyphylaxis is known to develop within 48 hours.
- Bisphosphonates
- Denosumab
- Glucocorticoids

Bone Metastases (Mets)
The bone is the 3rd most common organ affected by metastases. In solid cancers, bone mets are a common manifestation of distant relapse, accounting for 80% of the metastatic tumors in breast, lung, thyroid, kidney, and prostate cancers (BLT with a Kosher Pickel gets Mets). Bone mets may also occur in multiple myeloma (MM) and lymphoma hematologic malignancies.

- Pathophysiology: Bone mets are characterized as being osteolytic or osteoblastic. In either case, there is a relative degree of osteolytic destruction and promotion of malignant tumor cell survival. Osteoblastic activity can be remembered by: it’s a blast to put money in the bank (osteanabolic).
- Morbidity Associations: Skeletal related events (SRE, pain), pathologic fracture, spinal cord compression, hypercalcemia

Bone Mets Management
- Guidelines published by ASCO for the management of bone mets, are, like most others, derived from breast cancer management practices. Treatment options include: analgesics, radiation therapy, osteoclast inhibitors (bisphosphonates, denosumab), bone-targeted radiopharmaceutical therapy (223Ra, Sm-153 lexironam, 89Sr), systemic anticancer therapy, surgery
- Discussing a few of the available pharmacologic options
  - Bisphosphate
    - Pamidronate: 60-90mg IVPB, admin over 2 hours
    - Zolendronic Acid (ZLN): 3-4mg IVPB, admin over 15min
  - Denosumab: A RANKL-Inhibitor that prevents the formation and survival of osteoclasts
    - Administration: Bring to room temperature (allow to stand for 15-30mins), avoid vigorous shaking, may have some particulates, but do not use if cloudy or discolored (normally clear or pale yellow), administer to upper arm, thigh, or abdomen, no dose adjustments for renal impair
    - Bone Mets Dose: 120mg SC q4w
    - HOM Dose: 120mg SC q4w during 1st month, then give additional 120mg on days 8 and 15.
    - ADRs: Hypocalcemia (monitor Ca²⁺, Phosphorous, Mg²⁺, and correct prior to therapy), osteonecrosis of the Jaw (ONJ), patients with renal impairment may develop PTH
  - Radium 223 (Xofigo)
    - MoA: An α-particle-emitting isotope, which targets bone metastases. By mimicking Ca²⁺ in areas of increased bone turnover, Radium 223 can induce dsDNA breaks in adjacent cells, conferring an antitumor effect on the bone mets.
    - Dosing: Males: IV 55kBq/kg (~1.49 microcurie/kg) q4w x 6 doses
    - ADR: Myelosuppression, dehydration, secondary malignancies

(1/25) Haaf Lecture: Neutropenia and Thrombocytopenia
Hematopoiesis is the process by which pluripotent stem cells differentiate among the myeloid and lymphoid lineages to form the blood cellular components. For Hematopoietic Stem Cells (HSC) committed to the myeloid lineages, the common myeloid progenitor (CMP) differentiates within the bone marrow to the three main blood cell lineages: Leukocytes (WBC), Erythrocytes (RBC), and Platelets. Lifespans are ~ 12, 12, 120.

- Leukocytes (WBCs, granulocytes, monocytes, lymphocytes)
  - Neutrophils, of which are granulocytes, have a lifespan of 12 hours, they are considered to be immature WBC.
  - Deficiency: Leukopenia, or Neutropenia
- Platelets (From the Megakaryocyte): Lifespan = 10-14 days (12 days)
  - Deficiency: Thrombocytopenia
- Erythrocytes (RBC): Lifespan = 120 days
  - Deficiency: Anemia

Knowing the lifespans of these myeloid cells is important for determining eligibility for ongoing chemotherapy.
Myelosuppression: Since many anticancer agents indiscriminately target fast-growing cells, off-target effects can significantly reduce bone marrow activity, resulting in myelosuppression. The results of which are specific to each cell line’s deficiency. By virtue of their brief lifespan, neutrophils are affected first by myelosuppressive complications. The degree and severity of a patient’s myelosuppression is affected by their underlying bone marrow function (Hx of suppression), kinetics of their peripheral bone marrow function, type and RoA for chemotherapy, and patient-specific variations in PK.

- RBC → Anemia, presenting with hypoxia and fatigue
- WBC → Neutropenia, resulting in increased risk for infection
- Pt → Thrombocytopenia, causing increased risk for spontaneous and prolonged bleeding events.

While myelosuppression may in some cases represent an adverse effect, in many others it is the goal of cancer therapy.

- Advantages: Positive predictive outcomes – it may be a measure of a therapy’s efficacy. In many cases, neutropenia is a surrogate marker for the pharmacodynamic effects of chemotherapy, both in HM and ST.
- Disadvantages: Negative treatment outcomes (decreased palliation and curative ability), increased costs

Neutrophils (Also referred to as polymorphonuclear cells – PMNs)
A granulocyte descendant of the myeloid cell line, neutrophils are a type of WBC with membrane-enclosed granules. Because of their band-like and segmented nuclei, the phrase ‘Segs and Bands’ has been developed and refers to the neutrophil count (ANC). Neutrophils play a major role in the immune system.

- **Chemotherapy Cycle and the Nadir:** The nadir refers to the lowest blood cell counts reached during a chemotherapy cycle, usually detected via laboratory measures of ANC or [Plt]. Nadir onset is typically around 10-14 days following chemotherapy, taking up to 21 to 28 days for recovery.
  - Exceptions: Some chemotherapies, especially among patients receiving HSCTs, produce a delayed nadir at 28 to 42 days. This includes mitomycin C and the nitrosoureas (carmustine, bischloroethylnitrosourea (BCNU), and lomustine (CCNU)).

- **Absolute Neutrophil Count (ANC):** \[\left\{\left(\%\text{segs} + \%\text{bands}\right) \times \text{total WBC}\right\} ÷ 100\]
  - Neutropenia: The risks inherent to neutropenia include life-threatening infections. This risk is worsened by steep velocities of ANC decline and prolonged durations of neutropenia. CTCAEv3.0 Grading for Neutropenia are shown below. Patients with grades 3 or 4 should be hospitalized.
    - Grade 1: ANC 1500 cells/µL – Lower limit of normal (Cutoff for decisions to proceed with tx)
    - Grade 2: ANC 1000 cells/µL – < 1500 cells/µL
    - Grade 3: ANC 500 cells/µL – < 1000 cells/µL (Severe Neutropenia*)
    - Grade 4: ANC < 500 cells/µL (Severe Neutropenia)
    - Grade 5: Dead
  - Etiology: Chemotherapy is not the only cause of neutropenia. There is also congenital (born with bad bone marrow) and distinct forms of acquired neutropenia (infection, cancer, drugs (clozapine, SSZ))

- **Neutrophil Myelosuppression and Decreased Survival**
  - Chemotherapy-induced neutropenia is known to decrease survival. While this may be due to the onset of febrile neutropenia and associated infections, it can also restrict cancer therapy to lower doses, thereby reducing the chances of successful anticancer therapy.

Febrile Neutropenia (FN): FN is a common oncologic emergency with significant morbidity and mortality risks. Patients need to be counseled to have and use an oral thermometer. If they detect a fever, they should present themselves to the ER. Most initial FN events present following the first cycle of chemotherapy.

- **Diagnosis:** ANC < 500 cells/mm³ and fever (single T > 38.3°C/101°F, or 1 hour of 38°C/100.4°F)
- **FN Risk Assessment:** A patient’s risk for developing FN is determined primarily by their chemotherapy regimen and patient-specific factors. NCCN lends guidance on the associated rates of risk. For HM, the risk of FN is much higher, hence most patients receive MGF. Risk of FN increases for every day of severe neutropenia endured
  - Patient-specific: Age ≥ 65yo, poor performance status, preexisting neutropenia
  - Treatment-related: previous chemotherapy, previous radiation therapy
  - Cancer-related: Bone marrow involvement with tumor or preexisting neutropenia
  - Comorbidities: Infection, recent surgery, poor renal function, liver dysfunction (Bili), HIV-infection
- **Prevention Strategies** – for the management of chemotherapy-induced neutropenia
  - Chemotherapy dose-reduction/delay: Some clinicians opt to postpone chemotherapy until ANC > 1500 cell/mm³, and others use the event as a reason to reduce the dose for the next cycle of chemotherapy
  - Antibiotics: Prophylactic use of antibiotic increases the risks of resistance, though in some cases the risk-benefit warrants use. Prevention with abx is mostly been seen in HM, such as HSCT, but not much ST
  - Myeloid Growth Factors (MGF): filgrastim (Neupogen), peg-filgrastim (Neulasta), sargramostim
    - MoA: MGFs stimulate the proliferation and differentiation of hematopoietic progenitor cells. Formerly referred to as colony-stimulating factors (CSF), Granulocyte-CSFs specifically stimulate neutrophilic granulocytes, GM-CSF tag on eosinophils and monocytes/macrophages

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<tr>
<th>Stimulates</th>
<th>Dose</th>
<th>Onset</th>
<th>Availability</th>
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<tbody>
<tr>
<td>Filgrastim and related biologics</td>
<td>Neutrophils</td>
<td>5-10 µg/kg/day</td>
<td>2-8h. Post d/c: ANC may be ≤ 50% 2 days after d/c drug</td>
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<tr>
<td>Pegfilgrastim and related biologic - Fulphila</td>
<td>Neutrophils</td>
<td>6mg</td>
<td>72h. “Self-regulation” properties, prolonged recovery</td>
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<tr>
<td>Sargramostim</td>
<td>Neutrophils, eosinophils, macrophages</td>
<td>250 µg/m²/day</td>
<td>1-4h. Post DC: WCB return to baseline within 1 week</td>
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- **Dosing**: MGF should be *initiated* at least 24 hours after chemotherapy is complete, but sooner than 3-4 days after its completion. In addition, pegfilgrastim should not be given within 14 days of the next chemotherapy session. For weight-based dosing (filgrastim), simply round to closest vial/syringe size. Filgrastim is typically administered once daily for 10-14 days, whereas pegfilgrastim requires just one injection.
  - **Primary Prophylaxis** (first): MGF for chemotherapy with a predicted rate of FN ≥ 20%
    - NCCN Guidelines: In addition, recommends considering for intermediate risk
    - UIC Neutropenia Guidelines: Similarly, intermediate risk patients are assessed for specific risk factors and may be assigned MGF

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<tr>
<th>FN Risk</th>
<th>Curative/Adjuv.</th>
<th>Prolong survival/QOL</th>
<th>Symptom Management/QOL</th>
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<td>High (≥ 20%)</td>
<td>Give MGF</td>
<td>Give MGF</td>
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<td>Interm. (10-20%)</td>
<td>Consider MGF</td>
<td>Consider MGF</td>
<td>Consider MGF</td>
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<tr>
<td>Low (&lt; 10%)</td>
<td>No MGF needed</td>
<td>No MGF needed</td>
<td>No MGF needed</td>
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- **Secondary Prophylaxis** (repeat): MGF for patients who have previously experienced a neutropenic complication when receiving chemotherapy without MGF, or when the necessary dose reduction would significantly affect DFS or overall survival (OS)
- **Treatment**: For patients who currently have FN, the decision to use MGFs is controversial, as it shortens hospitalization, shortens abx therapy, shortens Grade 4 neutropenia duration, but provides no OS benefit. The NCCN does recognize a few populations that are indicated to receive MGFs while neutropenic, including:
  - sepsis syndrome, age ≥ 65yo, severe neutropenia (ANC < 100), neutropenia expected to last >10d, pneumonia, invasive fungal infection, fever develops during hospitalization, prior episode of febrile neutropenia

- **Pegfilgrastim’s Self-Regulation**: Pegfilgrastim’s (Neulasta) delayed and prolonged effects in promoting the proliferation of neutrophils is thought to be related to its pegylation and metabolism. As neutrophil cell count increases, it becomes more readily metabolized.
  - Neulasta OnPro Kit: An auto-injector device that is attached to the body, the Neulasta OnPro provides a subcutaneous dose of pegfilgrastim delivered over 45 minutes, initiating 27h following attachment. Once the clinician loads the dose into the device, it must be attached within 3 minutes to intact, non-irritated skin on the back of the arm or the abdomen. If desired, physician can manually start device 24h later.
  - Counseling: Do not expose to direct sunlight – keep under clothing. Avoid putting pressure on device. Keep at least 4 inches away from electrical equipment, and keep dry. Do not reapply if injector falls off.

- **ADRs of MGFs**
  - Bone-pain: Treatment with APAP or IBU may mask fever – not good.
  - Flu-like symptoms: fever, fatigue, diarrhea
  - Injection-site reactions, bruising, rash
- Rare: dyspnea, peripheral edema, sickle cell crisis (SCC, for SCD patients)
  - Due to potential risks in provoking myeloid cancers, MGFs should not be given in patients with malignancies myeloid in origin.

**Thrombocytopenia**
Platelets, derived via megakaryocytes, are small cytoplasmic bodies without nuclei, organelles, or ribosomes. They serve to coagulate the blood, forming clots to prevent excess bleeding. Like neutrophils, they have a short life-span (10-14days).

- **Etiology:** There are two general causes for platelet deficiency are decreased production and increased destruction
  - Decreased Production due to… viral infection, chemotherapy or radiation therapy, congenital or acquired bone marrow aplasia, alcohol toxicity, Vitamin B₁₂ or folate deficiency, drug-induced
  - Increased Destruction due to… idiopathic thrombocytopenia purpura (ITP), disseminated intravascular coagulation (DIC), drug-induced

- **Diagnosis:** [Plt] < 150,000 cells/μL

  **CTC Grading Criteria for Thrombocytopenia**
  - Grade 1: [75,000/μL to LLN]
  - Grade 2: [50,000 to 75,000/μL]
  - Grade 3: [25,000 to 50,000/μL]
  - Grade 4: Less than 25,000/μL
  - Grade 5: Death

  **Signs and Symptoms**
  - Asymptomatic (usually discovered via CBC)
  - Symptomatic Bleeding (cutaneous, mucosal)
  - Spontaneous bleed may occur when [Plt] < 20k

**Management of Chemotherapy-Induced Thrombocytopenia**
- *If induced by underlying disease, disease-specific therapy should be attempted first*
- Platelet Transfusion: Indicated for patients with profound thrombocytopenia or symptoms
- Pharmacologic Treatment: Formerly, Oprelvekin was an option.
- ASCO Guidelines lend advice on when to provide a platelet transfusion
  - HM and HSCT patients: Transfusion threshold is 10k
  - ST patients: Transfusion threshold is 10k, bleeding risk is related to the [Plt] nadir depth
  - Surgical Procedures: Transfusion threshold: 40-50k. BM biopsy threshold is 20k.
Traditional chemotherapy targets the rapidly-growing cells with cytotoxic intent. However, the human body has its own non-malignant rapid growing cells that are harmed in the process. The concept of targeted therapy centers on the idea of specificity. A specific target suggests the drug hits one molecule, acting on it to counteract the compound’s role in driving oncogenesis. In such cases, targeted therapies are cytostatic rather than cytotoxic. The ideal target would be one specific to the cancer, therefore bypassing the general aim for rapidly dividing cells. The theory is great, but few, if any, magic bullets exist.  

**Breast Cancer Targeted Therapies**  
Radical mastectomy was the indicated ‘cure’ for breast cancer for a significant period of time, leaving many women disfigured with only bleak hopes of cancer-free recovery. Today, optimal therapy of breast cancer varies depending, among other things, on the mutations present. More than 75% of breast cancers are estrogen receptor-positive, ER(+) . With the discovery of the estrogen receptor α (ERα) in 1958, target-based endocrine therapy became the ideal choice.

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<tr>
<td><strong>Estrogen Receptor α</strong></td>
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<td>Tamoxifen · Raloxifene · Fulvestrant</td>
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**Selective Estrogen Receptor Modulators (SERMs)**
- Tamoxifen was the first targeted therapy for cancer, discovered in the 1970’s. Based on its activity, tamoxifen is compatible for treating pre-menopausal women. To this day, it is still the standard of care for endocrine-dependent breast cancer. Raloxifene is another SERM. Cross-resistance is seen.
- Natural Biochemistry: When estradiol binds to ERα, it induces the formation of a transcriptional complex, in which Helix 12 is tucked in and coactivators are recruited to activate the agonist complex for transcription of proteins that promote cell survival and proliferation.
- MoA: SERMs block the effects of estradiol, through occupancy of the ERα’s active site. When tamoxifen binds, helix-12 is flopped outwards and different modulators are recruited for the formation of the transcriptional complex. Binding of corepressors facilitates formation of the antagonist complex, which silences ERα-mediated growth → Agonist in bone tissue. Antagonist in breast, epithelial, + tumor tissues.

**Selective Estrogen Receptor Degrader (SERD)**
- Fulvestrant: SERDs also target ERα, but at a different molecular position. Fulvestrant binding to ERα promotes formation of the antagonist transcriptional complex and also degrades the receptor.
  - Formulation Difficulties: Fulvestrant has poor PK parameters, including poor solubility and low bioavailability. The need for twice-monthly IM injections makes the plasma concentration difficult to control, such that fulvestrant is not dependable in its efficacy, especially factoring in a woman’s variable metabolism!
- GW5638: This compound is similar in structure to tamoxifen yet alike with fulvestrant’s degradation. Following phase-I hydroxylation, GW5638’s carboxylate group interacts with helix-11 of the ERα, inducing changes in helix 12, stabilizing the antagonist conformation while also destabilizing components of ERα. Due to induced exposure of hydrophobic surfaces to aqueous solution, ERα is quickly ubiquitinated and degraded by the 26s-proteasome ~ hence marking GW5638 as a SERD.

**Resistance Mechanisms**: Cancers can develop resistance mechanisms to targeted therapies. Researchers have shown upregulation of phosphorylation pathways to trigger proliferation without the ERα (mTOR). Similarly, upregulation of CDK4/6 production can help these tumor cells survive with estradiol. Additional mechanisms include: increased expression of growth factor receptors, upregulation of survival pathways (PI3K), modification of cell cycle regulators (CDK), altered uptake of ligand, receptor loss/mutation, increased nuclear coactivators.
- **ESR1 Mutant**: A SERM-resistant mutation. ESR1 encodes the four domains of ESRα (AF1, DBD, Hinge, and LBD). This mutant is in the activated transcriptional complex form without the presence of estradiol, shifting the equilibrium of receptors to the active conformation. This elicits a relative right-shift of SERM and SERD potency. Treatment with doubled SERDs concentrations have been shown to sufficiently overcome this resistance mechanism. This mutation completely eliminates AI efficacy.
- **Countering Resistance**: Combination therapy utilizes more than anticancer activity to overcome resistance and eliminate residual cancerous cells. Numerous clinical trials (FACT, PALOMA, MONARCH, etc) are showing improved efficacy. Combo therapy is now the first-line standard of care.

**Tyrosine Kinase Inhibitors (TKI)**
- Imatinib · Dasatinib · Nilotinib
**Leukemia Targeted Therapies**

Leukemia can be staged as acute (rapid) or chronic (insidious). There are four main types: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoblastic leukemia (CLL). Treatment of CML formerly relied upon interferon-α-based therapies, HSCT, and combination chemotherapy. Upon discovery of a specific driver for CML, targeted therapies became an option. The Philadelphia Chromosome t(9;22) encodes the tyrosine kinase fusion protein BCR-ABL. ABL1 is the nuclear tyrosine kinase, and BCR’s insertion disrupts the ABL’s regulating element SH3. Thus, BCR-ABL was found responsible for driving CML’s unregulated proliferation of WBCs, permanently turning on the phosphorylation activity. Imatinib is the first tyrosine kinase inhibitor (TKI)

- **Tyrosine Kinase Inhibitors (TKI)**
  - Imatinib: Selective inhibitor of ABL and its derivative BCR-ABL. Imatinib competitively binds at the ATP-binding site, stopping proliferation.

- **Resistance Mechanisms**
  - Modification of the target: Point mutations of BCR-ABL’s kinase domain to prevent TKI binding.
  - Overproduction of BCR-ABL

- **Countering Resistance**: By knowing the specifics about a particular mutation, drug design can overcome resistance using computational guidance and screening techniques. Dasatinib is a second-generation TKI indicated for imatinib-resistant CML, owing to its enhanced potency against BCR/ABL and other oncogenic kinases. However, patients using dasatinib are at-risk for developing secondary resistance. Another second-generation TKI is nilotinib, which was developed based on the crystal structure of ABL-imatinib. Although specificity was not accomplished, improved selectivity for BCR-ABL and its imatinib-resistant variations was achieved. As such, it has increased potency against resistant mutations relative to imatinib, and is indicated for imatinib-resistant CML. Although previously presented to us that nilotinib was specific and dasatanib was not specific, neither of them are specific.
  - Dasatinib is highly potent, at the cost of reduced selectivity (it hits many TKs!)
  - Nilotinib is potent, but not as potent – via its trade-off for increased selectivity for BCR-ABL

- **Recapitulation**: Imatinib was a breakthrough in cancer-targeting therapy because it underlined the importance for understanding the biology of a disease for developing effective anticancer therapies. Targeting the specific mutations in BCR-ABL has accordingly shown efficacy.

**Pan-TRK Inhibitors** – *Message: TKIs are not specific*

There are a lot of kinases. Kinome mapping has elucidated over 450 families of kinases. Companies capable of screening candidate kinase inhibitors develop heat-maps to identify off-targetedness. It is very challenging to develop a compound with high specificity. Many TKIs are considered ‘pan-TRK inhibitors,’ based on the plethora of kinases they affect. Thus, rather than ‘magic bullets,’ kinase inhibitors are magic buckshots.

**LMNA-NTRK1**: A chromosomal rearrangement on the same chromosome that forms a fusion kinase (TrkA) and lamin protein (LMNA). The fusion product results in a head-to-head assembly of dimers on the membrane surface, persistently engaging in phosphorylation activities of cell regulators like MAPK. It is implicated in cancer.

- Targeting TrkA1/NTRK1 fusion proteins with pan-TRK inhibitors has yielded promising results, especially for the management of colorectal cancer.
(2/1) Bruzik Lecture: DNA as a Drug Target: Alkylating Drugs

There are little to no differences between the DNA of one cell and the DNA of another. DNA carries the genetic blueprint of our being, and therefore DNA-targeting drugs are very toxic and only used in life-threatening disease, such as cancer.

**Mustards: The First Alkylators:** Weaponized mustard gas used during the Great War left surviving soldiers severely leukopenic. For the sake of cancer therapy, perhaps its exposure was not completely in vain. Introduction of the mustards to cancer chemotherapy began with the N-methylated Nitrogen mustard, mechloramine, in 1949 and continued throughout the 1950s. DNA alkylators are electrophilic DNA-modifiers that form covalent bonds with nucleophilic DNA sites, such as the nucleobases or phosphodiester groups. Such modifications can result in depurination, stand-scission, and activation of DNA repair mechanisms – all of which can result in permanent mutations. As a consequence, treatment with DNA alkylators can result in secondary cancers.

**Alkylation Reactions:** Alkylation is not the endgoal, formation of interstrand cross-links is the more effective intent

- **Nucleophilic Attack:** Electrophilic drugs are attacked by nucleophilic sites on DNA, typically N’s of nucleobases. The sites for alkylation differ in prevalence, dictated by increasing degrees of nucleophilicity
  - N-7 Guanine > N-3 Adenine > N-7 Adenine > N-3 Guanine > N-1 Adenine
  - Others: N-1 Cytosine, N-3 Cytosine, O-6 Guanine, and Phosphate groups

- **An Alkylator’s Electrophilic Carbon:** Nucleophilic attack occurs at the drug’s electrophilic carbon, which is located between the S/N of the mustard and its chlorine
  - A mustard nitrogen’s nucleophilicity is critical: Left unsubstituted, N is too nucleophilic and may lead to toxic effects. If substituted with a strong EWG (R), however, the N may be rendered poorly nucleophilic leading the drug becoming completely unreactive.
  - Neighboring group-mediated oxidation events enhance N electrophilicity by introducing steric strain in the form of a 3-membered ring, it is the RLS. (β-chloramine → aziridinium cation)
  - Some mustard nitrogens are non- or weakly-nucleophilic and need to be metabolically activated in order to render nitrogen more nucleophilic

- **Bifunctionality:** Most alkylators are bifunctional, capable of forming two adducts, thereby cross-linking dsDNA. Interstrand crosslinking halts DNA replication and induces apoptosis

- **Alkylation Types:** Pictured to the right. Each numbered bulb is the alkylating drug
  - (1) G-X-C Crosslink: caused by nitrogen mustards (cyclophosphamide)
  - (2) G-C Crosslink: caused by aziridines or epoxides (mitomycin C)
  - (3) G or C Crosslink: caused by nitrosurea (BCNU)
  - (4) G-O<sup>6</sup> Alkylation: caused by hydrazines or triazines (Procarbazine)

- **Results of DNA Alkylation**
  - **Alteration of Watson-Crick Base Pairing:** When a guanine N-7 is alkylated, the tautomer equilibrium is shifted to preference 6-OH, preventing the third H-bond between G-C pairs and promoting stronger pairing between G-T, precipitating mutations!
  - **Strand Scission:** Alkylation of guanine’s N-7 induces a mesomeric shift and (+) charge on the glycosidic N (N-3). As a result, the nucleobase is an excellent leaving group and departs. The resulting oxonium cation’s electrophilic carbon is attacked by water, forming an abasic hemi-acetal. The hemi-acetal is in equilibrium with its open-chain aldehyde conformer, which is basic. In a synchronous, concerted manner, the aldehyde abstracts a proton from the α-carbon, facilitating β-elimination and departure of the phosphate group - complete intra-strand scission.
  - **Interstrand Cross-linking:** For DNA alkylators that cross-link two strands of DNA, albeit a small conformational effect, the linkage affects DNA strand separation and introduces rigidity to nucleosome packing.
Alkylator Biochemistry and Managing Toxicities

Highly reactive alkylating drugs are unstable to hydrolysis and react to completion within minutes. At such a rate, severe toxicities can be seen. To improve stability, chemical modifications of the N/S mustard (R) group can be made. Addition of just the phenyl ring (R=H) weakens the basicity of the amine nitrogen, thereby slowing the rate of the aziridinium cation formation, however the product is insoluble.

- **Site-Directed Nitrogen Mustards**: Based on the hypothesis that melanoma cancers have high melanin content, adding melanin’s precursor, phenylalanine, as substituents to antineoplastics would selectively target the cancer. The designed product was melphalan, it failed in that regard, but it is otherwise effective and we still use it today. A similar effort was conducted with the drug estramustine, which targeted estrogen receptors. It too failed in its selectivity.

- **Cyclophosphamide**: Based on the hypothesis that cancer cells have upregulated phosphoramidases, phosphoramidate prodrugs of nitrogen mustards, such as cyclophosphamide, can selectively target cancer cells. Following CYP-mediated activation, successive ring-opening and β-elimination forms the active nitrogen mustard. In addition, β-elimination produces a second product, acrolein, a toxic Michael acceptor. During cyclophosphamide therapy, mesna is giving to manage accumulation of this toxic metabolite by interacting with acrolein’s terminal double bond, otherwise, it can be a dose-limiting toxicity.

- **Methanesulfonates**: In contrast with nitrogen mustards, methanesulfonates, like busulfan, react by an S_N2 mechanism, whereupon the reaction rate depends on the concentration of both the drug and the nucleophile. Also, their 3-membered rings of torsion is the bis-epoxide.

- **Ethlenimines**: Capitalizing on the fact aziridine is a potent electrophile, the ethlenimines incorporate at least two aziridine residues to confer antitumor activity and involve EWG to lower aziridine’s pKa for PK stability. One such example is thiotepa. Depending on the strength of the EWG, the aziridine moiety may be attacked by the DNA nucleophiles directly, or it may require protonation first.

- **Nitrosoureas**: For this class, the prototype compound was N-methyl-N-nitrosourea (MNU) discovered in 1959. Investigated for its antitumor activity in brain tissue, replacement of the methyl group with a β-chloroethyl residue heightened its activity while still permitting passage across the BBB. This led to the development of the popular alkylating drugs indicated for brain tumor, CCNU (lomustine) and BCNU (carmustine).
  - Mechanism: Nitrosoureas produce diazo-methanes, which diverge among 2 pathways
  - (1) Form alkyl isocyanate, which carbamoylates proteins. Although this is not antitumor activity, it can inactivate DNA repair proteins, thereby enhancing the effect
  - (2) Form diazo-derivative, which collapses to the potent electrophile: diazonium cation. The diazonium cation is responsible for the alkylation of DNA at guanine’s O-6. Thereafter, intramolecular alkylation proceeds with N-5’s attack and formation of a cyclic adduct, which is then primed for attack by the N-4 cytosine residue, establishing the two-carbon internstrad link

- **Triazines**: Temozolomide is the only brain tumor drug administered orally. It functions similar to another class member, dacarbazine (formation of MTIC intermediate). It looks like a fake purine.

Platinum Antineoplastic Agents

Platinum-based antineoplastics are not considered to be alkylators, rather they are coordination compounds. Their valence electron shells result in their square planar structure, wherein its neutral charge facilitates transmembrane transport. For the antineoplastic agents, two displaceable leaving groups are in cis-orientation, and the nitrogen groups facilitate initial contact with the DNA as well as stabilize nucleobase interactions. Cisplatin was one of the first planar platinum coordination compounds. Further development has shown that the bonds to platinum should be neither too labile (otherwise toxic!) or too strong (otherwise ineffective!)

- Mechanism: Covalent bonding forms an intrastrand crosslink at dGpG-N-7s, so at runs of GG. This distortional crosslink inhibits transcription (RNA polymerase stalls) and results in apoptosis.
- SAR: Reduced Toxicity: Alter nondisplaceable residues to ones that are more easily excreted. For instance, cisplatin is more nephrotoxic than carboplatin
- SAR: Improved Activity: Bulkier groups result in greater conformational distortion, therefore more effective blockade of replication. Modification of the nondisplaceable residues can help alleviate drug resistance.
Antimetabolites are chemicals that inhibit the action of metabolites and the processes associated with normal metabolism. Some antimetabolite compounds interfere with DNA synthesis, whether by (1) inhibiting the production of dNTPs or through mimicry (2) to become incorporated into the replicating DNA strand. In either case, this has a cytotoxic effect on the cell.

dNTPs are derived from two sources: the de novo pathway and the salvage pathway. They are comprised of 3 essential elements, a nitrogenous base, a sugar, and a phosphate. They serve as the building blocks for DNA and RNA.

- **De novo**: dNTPs are newly synthesized from basic precursors
- **Salavage**: Recycle and reuse of degraded nucleotide components

**Tumors specifically rely on the de novo pathway**, making it an attractive therapeutic target.

**Pyrimidine Antagonists**: 6-mercaptopurine (6-MP, Purinethol), 6-thioguanine (6-TG, Tabloid)
- **De novo** purine biosynthesis: The precursor to purine nucleotide biosynthesis is 5-Phosphoribosyl-α-pyrophosphate, also known as PRPP. After a series of reactions in which some include the essential cofactor tetrahydrofolate (THF), inosine monophosphate (IMP) is formed. IMP represents the branch point in purine biosynthesis either toward guanine or adenine.
- **MoA**: Dual: Inducing cell cycle arrest
  - Inhibition of purine biosynthesis at several steps of de novo pathway
    - Inhibit PRPP→PRA: Both 6MP and 6TG
    - Inhibit IMP→Guanine Branch: Both 6MP and 6TG
    - Inhibit IMP→Adenine Branch: 6MP only
  - Incorporation into RNA and DNA: 6MP poses as adenine, 6TG poses as guanine
- **ADRs**: These antimetabolites are taken up predominantly by rapidly proliferating cells, which is mostly cancerous cells. However, the human body also has proliferating cells subjected to off-target toxicities in essential organs.
  - 6MP: myelosuppression, N/D, fatigue, alopecia, skin rash, bloody stool/urine, allergic reactions. Patients with TPMT and other polymorphisms are at increased risk of FN.
  - 6TG: liver toxicity, leukopenia, neutropenia, thrombocytopenia, anemia, anorexia.

**Pyrimidine Antagonists**
- cyanarabine, gemcitabine, 5-fluorouracil (5-FU), capecitabine,
- **Fluoropyrimidine analogs** (5FU, capecitabine, trifluridine): Compounds have fluorine substitutions at the 3°C
  - Capecitabine is a prodrug, converted to 5FU by successive metabolism by 3 different enzymes in different body compartments. It has the same activity as 5FU. Tumors with high expression of Thymidine phosphorylase (enzyme 3 of 3) are more sensitive to this antimetabolite d/t increased formation. Used in breast and colon cancers

**MoA (5FU)**: Dual. Cellular uptake of 5FU occurs predominantly by rapid-proliferating cells, though there still are off-target effects. Once intracellular, 5FU is transformed into various intermediate compounds, each of which affect the cell in a different way

- (1) Inhibits thymidylate synthase (thymidine synthesis): 5-FdUMP
  - Thymidylate synthase (TS) catalyzes the methylation of dUMP to dTMP in a multistep process. There are 3 essential parties for this reaction to take place: dUMP, thymidylate synthase, and the $N^5,N^{10}$-Methylenetetrahydrofolate cofactor.
  - 5dUMP is the transformation product of 5FU. Interaction of 5dUMP, thymidylate synthase, and the cofactor form a stable ternary complex that prevents the normal formation of dTMP. This effectively sequesters the enzyme and cofactor
- (2) Incorporates into growing RNA (5-FUTP) and DNA (5-FdUTP)

**ADRs**: GI tract toxicity, myelosuppression, HA, pruritus, alopecia, hand-foot syndrome, cardiotoxicity, mood
  - Warning: Onset of toxicity is rapid. Patients can quickly enter into a coma.
  - **ANTIDOTE** for 5FU/Capecitabine toxicity → Uridine triacetate

**Unique formulation**: Trifluridine(TFT)/tipiracil (TPI) – Lonsurf
  - Indicated for metastatic colorectal cancer
  - Nucleoside analog: TFT is a fluoropyrimidine that inhibits thymidylate synthase (TS) and in its triphosphate form can be incorporated into DNA. Albeit more effective than 5FU, it is unfortunately very short-lived
  - Thymidine phosphorylase inhibitor: TPI inhibits thymidine phosphorylase, effectively increasing the bioavailability of TFT.
**Cytidine Analogs** (cytarabine, gemcitabine): These compounds differ in the sugar moiety
- Cytarabine: Incorporates into DNA and prevents DNA elongation. It is useful in the treatment of leukemia.
  - **ADR:** myelosuppression, mucositis, fever, pains
- Gemcitabine: Has a dual MoA. First, gemcitabine inhibits ribonucleotise reductase – preventing conversion to dNT. Second, in its triphosphate form, it competes with dCTP for incorporation into the DNA, blocking elongation and transcription/translation activities. Gemcitabine is useful for solid cancers, including: pancreatic, breast, ovarian, lung, etc
  - **PK:** Gemcitabine has an exceedingly short half-life ($t_{1/2} = 8$min). It is quickly deaminated by deoxycytidine deaminase to dFdU, preventing effective antitumor activity.
  - **ADR:** Affects the nervous system and bone marrow, causing myelosuppression, numbness, and rash.

**Folic Acid Analogs**
Folic acid, also known as Vitamin B₉, is an essential dietary factor because it can only be acquired through the diet. In the body, it functions as a cofactor for metabolic reactions related to DNA synthesis, DNA repair, and DNA methylation. Also, it is essential for the production of healthy blood cells (deficiency marks risk for anemia!). Vegetables are rich dietary sources of folates, especially edamame, spinach, asparagus, and artichoke. Beans and lentils are also great!
- **Folate Metabolic Physiology:** Folate is converted to its active form, tetrahydrofolate (THF) by Dihydrofolate reductase (DHFR). The reduced THF molecule serves to donate methyl groups – and is a necessary cofactor in pyrimidine and purine biosynthesis.
- **Cancer therapy:** Aminopterin was the first folic acid antimetabolite, inducing the first case of remission of childhood leukemia in 1948 by Dr. Farber’s ingenuity. The development of methotrexate (MTX) lowered the toxicity profile
  - MoA: Dual – Prevention of folate utilization inhibits pyrimidine and purine biosynthesis
    - **Pyrimidine:** TS converts dUMP to dTMP using THF as an essential cofactor.
    - **Purine:** THF is involved in numerous steps of the de novo purine biosynthetic pathway prior to IMP.
  - **Methotrexate:** MTX’s primary target is DHFR, it has 1000x higher affinity for it than folate does. By preventing formation of THF, MTX inhibits both purine and pyrimidine biosynthesis, thereby halting DNA and RNA production. MTX is useful for treating cancers of the breast, skin, head, neck, and lung.
    - **ADRs:** myelosuppression, hepatotoxicity, GI toxicity, neurotoxicity (memory loss). Neuronal toxicities present after prolonged exposure to MTX. These toxicities can be reversed
      - ContraX in pregnancy
    - **Leucovorin Rescue:** Folinic acid (leucovorin) is used to treat acute MTX overdoses and rescue bone marrow and GI mucosal cells from the effects of MTX. It should be part of the total chemotherapeutic plan and only administered when the risks of continued MTX exposure exceed the benefits.
  - **Pralatrexate (Folotyn):** Similar to MTX, pralatrexate acts as an anti-folate. One distinct advantage is its accumulation in tumor cells via preferential uptake. It is used to treat refractory peripheral T-cell lymphoma
  - **Pemetrexed (Alimta):** Pemetrexed more closely resembles THF, but with carbon substituted for nitrogen at the pteridine ring and alkyl bridge sites. It is used to treat malignant pleural mesothelioma and non-small cell lung cancer in combination with cisplatin.
    - **MoA:** Triple mode of action, inhibiting three separate enzymes responsible for folate/DNA metabolism
      - TS: Prevents formation of pyrimidine nucleotides
      - DHFR: Prevents formation of both pyrimidine and purine nucleotides
      - GARFT: Prevents formation of purine nucleotides

**Mechanisms of Resistance**
- **MTX:**
  - Decreased drug accumulation (upregulation of drug pumps)
  - Changes in DHFR activity
- **6MP/6TG:**
  - Decreased activity of phosphoribosyltransferases (decrease activation of these compounds)
- **5FU:**
  - Decreased activation of 5FU
  - Increased TS activity and reduced sensitivity to the drug
- **Cytarabine:**
  - Decreased uptake
  - Decreased conversion to Ara-CTP
Fleming Lecture: Antimetabolites II

(2/8)

Methotrexate (MTX) ~ folate antimetabolite

- MoA: Major function of MTX is inhibition of DHFR, preventing the fixation of folic acid to dihydrofolate. The result of this activity is reduced production of purine and pyrimidine nucleic acids. MTX activity is cell-cycle specific and targets rapidly-dividing cells.

- Dosing:
  - Low Dose (<50mg/m²): Generally limited to ectopic pregnancy (IM), inhibits neural tube development. Intramuscular, intramuscular, and parenteral routes are each used.
  - Intermediate Dose (50-500mg/m²): Short infusions and continuous infusions are used.
  - High Dose (≥500mg/m²): IV infusions at this dose override concentration gradient limits, permitting passive diffusion of MTX across membranes. This dose is highly toxic and requires preparation.

- ADRs: The toxicities of MTX are dose- and route-dependent. More information on the second-half of this page

High Dose IV MTX: Interventions to maximize benefit and minimize risks.

- (1) Eliminate Risk Factors
  - Renal Function: Patients with low CrCl will require dose adjustments
  - Pleural Effusions/Ascites/Hematomas: Patients with fluid collection should not receive high dose MTX. Fluid reservoirs serve as MTX depots, resulting in 3rd spacing and delayed clearance.
  - Others: poor hydration, low urine output, acidic urine

- (2) Remove drug-drug interactions prior to administration
  - Avoid drugs that will compete for renal excretion (NSAIDs, probenecid, PCN)
  - Avoid nephrotoxic drugs (AGs)
  - Avoid drugs that delay clearance (PPIs)
  - Avoid drugs that displace MTX from proteins (Salicylates, sulfonamides)

- (3) Aggressively hydrate
  - Initiate NS or D5 @100-150mL/hour 6 to 12 hours before starting MTX and continue 24h post dose.

- (4) Employ alkalization measures
  - Add 100-150mEq Bicarb to each liter of hydration volume. This will facilitate MTX solubility and promote quicker, predictable, renal elimination. With hydration, this helps prevent tubular necrosis. Attempt to maintain Urine pH > 7 (Liquids unavailable? Use PO sodium bicarb q6h)

- (5) Prepare for leucovorin rescue
  - Leucovorin should be administered 24 hours after high-dose MTX, but earlier than 40 hours after.
    - Twenty-four hours after high-dose MTX, draw a serum [MTX] level and initiate leucovorin rescue
  - DOSE: 15mg/m² IV, titration is time and concentration dependent. Check q24h. Goal [MTX] < 0.05µM
    - Leucovorin is continued until achieving [MTX] target level. Adjust q24h. Tables have been developed to decide leucovorin regimens, accounting for time of MTX exposure and [MTX].
    - PO? Oral bioavailability of leucovorin is saturable, therefore it is not an option
  - MTX depletes folate metabolites. Leucovorin is a fancy, reduced form of folic acid. Its administration rescues the normal, non-malignant cells, supporting their recovery of DNA synthesis. Having leucovorin prepared helps minimize toxicities associated with myelosuppression and mucositis. If not administered within 40 hours, the normal cells may not be able to recover. TOAST.

Major Adverse Effects and Dose-Limiting Toxicities (DLT) of High-Dose MTX

- Myelosuppression: LeukopeniaDLT, thrombocytopeniaDLT
- Nephrotoxicity: Renal Tubular Necrosis: MTX and its metabolites precipitate in renal tubules. Sufficient hydration and alkalization can prevent this from occurring
- Hepatotoxicity: Transaminitis is common
- Gastrointestinal: N/V, mucositisDLT. Mucositis renders many patients severely uncomfortable and likely NPO.

MTX Overdose: Near Antidote – Glucarpidase (Voraxaze)

- MoA: Bacterial enzyme that hydrolyzes folic acid and antifolates (~chews up MTX and restores folates)
- Indication: Toxic MTX levels (> 1µM) in patients with delayed clearance d/t impaired renal function. Thus, glucarpidase is used when leucovorin rescue is insufficient or we forgot to check for pleural effusions
- Dosing: 50 units/kg IV over 5 mins. Limitations of use include normal or impaired renal function. It is highly preferable to not use Glucarpidase with on-board leucovorin, as the enzymatic activity will be wasted on interacting with leucovorin (avoid ± 2h).

Intrathecal MTX: IT MTX is administered directly into the CSF of the patient’s brain or spinal cord using an ommaya reservoir or lumbar puncture (LP), respectively. It is the pharmacist’s duty to ensure there is NO PRESERVATIVES. The toxicity risk includes arachnoiditis-induced HA (swelling of meninges), traumatic LP. No anticoag prior to LP!
Pemetrexed ~ folate antimetabolite
- **MoA:** Inhibits folate-dependent processes, disrupting the biosynthesis of purine and thymidine nucleotides. It has three-fold enzyme inhibition of: thymidylate synthetase (TS), DHFR, and GRAFT. Thus pemetrexed shuts down ALL of folate synthesis.
- **Dosing:** 500mg/m² IVPB over 10 mins every 3 weeks. It is used to treat non-small cell lung cancer and malignant pleural mesothelioma.
  - ContraX: Do not use in patients with CrCl < 45mL/min
  - DDI: Avoid use with NSAIDs. While no dose-adjustment is required in cases of normal renal function, NSAIDs may lead to accumulation. Take precaution in CrCl 45-79mL/min
  - Avoid short t½ NSAIDs (diclofenac, indomethacin) 2 days before and 2 days after pemetrexed
  - Avoid long t½ NSAIDs (naproxen, piroxicam) 5 days before and 2 days after pemetrexed
- **Toxicities:** Leucovorin is not required or effective for pemetrexed
  - Myelosuppression: Minimize risk by having ANC > 1500cells/mL prior to cycle. Use Vitamin B₁₂ 1000µg IM 1 week prior to therapy and then q9weeks. Take folic acid 1mg PO QDaily.
  - Rash: Adherence to dexamethasone regimen can curb rash risks: 4mg PO BID x3days before chemo.
  - Gastrointestinal: mucositis, diarrhea. (comparably lower emetogenic potential)

6-Mercaptopurine (6MP) ~purine antimetabolite
- **Indication:** 5FU is used in many different cancers and is delivered by many routes. It can be used for radiosensitization.
- **Dosing:** 50-75mg/m² PO QDaily on an empty stomach. Calculate weekly dose, then round to the nearest 50mg tablet. Divide your result by 7, and configure how to make the regimen easier for your patient. Do not consider cutting/crushing tabs.
  - ADRs: Myelosuppression: This is a dose-limiting toxicity – will require dose-adjustment
    - Hepatic: Intrahepatic cholestasis, liver failure
    - Gastrointestinal: mucositis (comparably lower emetogenic potential)
  - Drug Interactions: Avoid use with allopurinol, febuxostat, AZA, TAC, 5-ASAs
    - Allopurinol + febuxostat (XOI): Concurrent use will elevate [6MP] – toxicities
  - Dose-Adjustments: 6MP requires dose-adjustments for renal dysfunction, hepatic dysfunction, toxicities (myelosuppression), and TPMT deficiencies. Adjusting to TPMT deficiency is employed after initiation

5-Fluorouracil (5-FU) ~pyrimidine antimetabolite
- **Indication:** 5FU is used in many different cancers and is delivered by many routes. It can be used for radiosensitization.
- **Dosing:** 200-1000mg/m²
  - Concurrent leucovorin: In this case, leucovorin increases the binding affinity of 5FU, enhancing the tumor cytotoxic abilities only of the 5FU bolus (half-life related). It is given prior to 5FU bolus. Well-tolerated.
  - ADRs: Cardiac (angina, myocardial ischemia), Dermal (hand-foot syndrome, discoloration, nailbed changes)
    - Hand-Foot Syndrome (HFS): Cracking, burning, peeling, tenderness, perhaps a bleeding sensation in the palms of the hand and soles of the feet. This is d/t leakage of 5FU from capillary beds.
      - Management: Avoid hot water, keep hands and feet moisturized, analgesics
    - Dihydropyrimidine Dehydrogenase (DPD) Deficiency: DPD is the rate-limiting enzyme of 5FU catabolism. It has a 3-4% prevalence in the population, and having this genotype results in severe toxicity when exposed to 5FU. If a patient is having a greater response/toxicity than expected, suspect DPD def.
  - Toxicities are route-dependent
    - Bolus/Fast: Myelosuppression, mucositis, diarrhea
    - Continuous Infusion/Slow: Hand-Foot syndrome

Capecitabine ~pyrimidine antimetabolite
- **Capecitabine is a prodrug of 5FU.**
- **Dosing:** Start at 1000mg/m² per dose (q12º x14 days, every 21 days) and adjust to intended response. Although capecitabine is FDA-labeled for 1250mg/m², this dose is poorly tolerated. Dose-adjustments are required for renal and hepatic impairment.
- **ADRs** HFS: A dose-limiting toxicity, occurring more frequently than 5FU

<table>
<thead>
<tr>
<th>Capecitabine Dose Adjustments</th>
<th>Renal</th>
<th>Hepatic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CrCl</strong></td>
<td><strong>Dose</strong></td>
<td><strong>Liver Function</strong></td>
</tr>
<tr>
<td>30-50mL/min</td>
<td>75% normal dose</td>
<td>Mild-Mod</td>
</tr>
<tr>
<td>&lt;30mL/min</td>
<td>ContraX</td>
<td>Severe</td>
</tr>
</tbody>
</table>
Diarrhea is a DLT. Manage with loperamide + fluids. Low emetogenic
Liver: hyperbilirubinemia
Other: Myelosuppression, alopecia, mucositis

- DDIs: Phenytoin: Will increase [5FU] levels
  o [BBW]: Warfarin’s anticoag effect will be increased. ContraX. Switch to LMWH
  o Capecitabine is a CYP2C9 substrate.

5FU Overdose/Capecitabine: Uridine triacetate (10g packet PO q6º x 20 doses) starting within 96h from the end of tx.

Cytarabine ~pyrimidine antimetabolite
- Select Indications: ALL, AML, CLL, Meningeal leukemia, APL, various lymphomas
- Dosing: High-dose IV/SC cytarabine is >1500mg/m³. It is associated with profound toxicities so pre-medications and prophylactic measures must be involved. Cytarabine is also administered IT, but not at these high doses.
  o Pre-medications: Antiemetics prior to IV and SC dosing. Dexamethasone 0.1% eye drops, I-II gtt OU q6º during and for 2-7 days following completion of cytarabine course
  o Renal Dose-Adjustments:
    ▪ SCr 1.5-1.9mg/dL: 1g/m²/dose
    ▪ SCr ≥ 2mg/dL: 0.1g/m²/day
- ADRs: Myelosuppression: Neutropenia and thrombocytopenia are DLT
  o GI: mucositis, D, and moderate emetogenic potential (consider antinausea meds)
  o High-Dose Toxicities: CNS toxicity (hand-writing changes), conjunctivitis (ppx dexta), rash
    ▪ Cytarabine Syndrome: Fevers, myalgia, arthralgia, bone pain, rash

Gemcitabine ~pyrimidine antimetabolite
- Select Indications: STs, such as: Pancreatic cancer, non-small cell lung cancer, MBC, ovarian cancer
- Dosing: 1000-1250mg/m² IV over 30 minutes. No renal or hepatic dose adjustments currently required.
  o Sarcoma-specific: Capitalizing on the fact of gemcitabine’s saturable metabolism, protocols for treating sarcoma utilized a fixed-dose per minute regimen: 10mg/m²/min
  o Premedications: Antiemtics prior to dosing.
- ADRs: Albeit relatively well-tolerated, gemcitabine still has some of the usual ADRs.
  o Myelosuppression: Thrombocytopenia is a DLT
  o Other: Flu-like symptoms, low emetogenic potential

Trifluridine/Tipiracil (Lonsurf) ~pyrimidine antimetabolite
- Indicated for relapsed metastatic colorectal cancer
- Dosing: 35mg/m² (based on Trifluridine) PO BID on days 1-5 and 8-12 q28days. So like M-F, 2 weeks straight. Maximum Trifluridine dose should be 80mg.
  o Growth Factor Ppx: D/t myelosuppressive effects
  o Renal Adjustments: None, so long as CrCl ≥ 30mL/min
  o Hepatic Adjustments: None, so long as Tbili ≤ 1.5x ULN
- ADRs: Myelosuppression, N/V/D, fatigue and weakness
DNA supercoiling is an important physiologic process that serves to regulate access, expression, and metabolism of DNA. Winding (+supercoiling) and unwinding (-supercoiling) may introduce or relieve strain in the 3D helical DNA structure. While the most obvious form of supercoiling is seen in bacterial plasmids, eukaryotic cells also involve supercoiling activities due to the radical length of the linear dsDNA. DNA wrapping around nucleosomes incorporates DNA supercoiling. The enzymes responsible for this activity are topoisomerases. Topoisomerases catalyze three types of reactions with DNA: relaxation/supercoiling, knotting/unknotting, and catenation/decatenation.

**Eukaryotic Topoisomerases (human)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type I</strong> (5’ adduct):</td>
<td>Found in virtually all organisms. In humans, they are referred to as Topo IIIα and Topo IIIβ. There are no significant small molecule inhibitors, so we will not discuss them.</td>
</tr>
<tr>
<td><strong>Type II</strong> (5’ adduct):</td>
<td>There are α and β topo IIs. They catalyze the same reaction, but have distinct biological functions.</td>
</tr>
</tbody>
</table>

- **Topo I: Cut one strand**
  - **Type IA** (5’ adduct): After cutting 1 DNA strand free, the enzyme allows the free strand to unwind/rotate X number times, then resealing it.
  - **Type IB** (3’ adduct): Engages in a unique duplex pass-through type reaction, then resealing it. This is useful in replication fork conversion and for resolution of catenated duplexes.

- **Topo II: Cut two strands**
  - **Type II** (5’ adduct): There are α and β topo IIs. They catalyze the same reaction, but have distinct biological functions.
  - **α**: Replication and cell division
  - **β**: Transcription

**Phosphotyrosyl Linkage**: The purpose of formation is two-fold:
- (1) Energy Conservation!: Hydrolyzing the high-energy phosphodiester bond repeatedly during cellular processes would become an expensive endeavor. Converting most of this energy to a phosphotyrosyl bond saves on valuable cofactor stores (i.e., ATP) when reversal back to the phosphodiester is required.
- (2) Hide the Break!: Cellular machinery is highly sensitive towards detecting breaks in DNA polymers. Uptregulation of this machinery involves costly repair mechanisms. Hence, enzymatic shielding of the temporarily cleaved DNA via a phosphotyrosyl linkage is effective.

- **Vulnerable target**: Topoisomerases produce DNA damage when they cleave the DNA backbone. Once they make that cut, inability to proceed whether drug-induced or naturally occurring may lead to permanent damage.

**Poisoning Topoisomerase**

Directed therapy blocks the intermediate step when Topo is covalently bound to DNA and cleavage has already occurred. This reversibly inhibits activity.
- But how could reversible inhibition lead to the desired cell death?

- **Topo I**: When the replisome/replication fork encounters the trapped Topo I, collision occurs and the fork collapses, mediating a ds, irreversible break.
  - Treatment: Drugs here are **schedule-dependent**, as only replicating cells will be affected. Therefore, more frequent dosing is required.
  - Drugs: camptothecins (irinotecan, topotecan)
  - Camptothecins bind to the TopoI-DNA complex interface for intercalation, displacing active site residues to **prevent re-ligation**

- **Topo II**: Less schedule dependent
  - Any protein/enzyme tracking along the DNA collides with the trapped Topo II, leading to inappropriate recombination
  - Drugs: etoposide, doxorubicin, mAMSA

→ The DNA damage, **not the enzyme inhibition**, results in clinical activity

- **Resistance**: The aforementioned Topo-poisoning activity induces apoptosis. Cells that are defective in the process of apoptosis, however, will be resistant to the effects of topoisomerase inhibitors. There’s other processes as well
  - **Tyrosyl DNA phosphodiesterase 1 (Tdp1)**: Specific to Topo-Inhibitor activity, Tdp1 recognizes trapped Topos and initiates a special repair pathway to correct DNA damage associated with bound proteins. It is capable of fixing both 5’ and 3’ breaks, via hydrolysis at the phosphotyrosyl linkage.
Topoisomerase I Inhibitors: Recall, these agents are schedule-dependent cell killers. While animal models showed significant promise, the application of Topo I-inhibitors in practice and their associated results has been disappointing. Dr. Nitiss divulges that he does not believe the dosing schedule they are currently being used on is the issue.

- **Topotecan (Hycamptin):** A camptothecin analog, topotecan was the first on the market, used as a second-line therapy option for ovarian cancer and small cell lung cancer
- **Irinotecan (Camptosar):** Irinotecan is a prodrug used as the front-line therapy with 5-FU and leucovorin for metastatic carcinoma of the colon or rectum.
  - Activity: Irinotecan is metabolized to its active metabolite in serum by carboxylesterase to SN-38. SN-38 is inactivated via glucuronidation in the liver and secreted for excretion in the bile. When it hits bacteria of the colon, the bacteria remove the glucuronide moiety regenerating SN-38. As a consequence, there is a significant degree of late-onset diarrhea
  - Prevention of Severe Diarrhea: Pre-treat with antibiotics.

Topoisomerase II Inhibitors:

- **Etoposide:** Used in the treatment of germ-cell tumors. Shown to be curative. It also has application in small cell lung cancer and leukemias, though its use in leukemias has dwindled d/t the risks of secondary malignancy
  - **Secondary Malignancies:** First observed with etoposide, the Topo-II-targeting drugs can cause secondary malignancies, typically AML, via translocation of Mll1, an oncogene. Translocation and activation of Mll1 provokes rapid development of AML. The latency period for such a malignancy is frighteningly brief, only 1 year! Whereas the secondary malignancies associated with alkylating agents are like 7 years. Similar to etoposide, epipodphyllotoxins can generate carcinogenic damage.
- **Doxorubicin:** An anthracycline, doxorubicin is the most commonly used Topo-targeting drug, owing to its broad, potent activity across many cancer types. It is used both in leukemias and solid tumors. It is given IV.
  - **Cardiotoxicity:** Cardiotoxicity is a significant side effect associated with anthracyclines. Its development is highly age-related, older patients are more prone, as well as dose-related. Therefore, conventional regimens have a maximum lifetime exposure.
    - **Exposure:** The likelihood of cardiotoxicity with doses $\leq 300$mg/m$^2$ is low, but doses greater than 300 are strongly associated w/ myocardial damage. Alternative approaches have been developed to improve its therapeutic profile
    - **Pegylation:** Drug delivery via pegylated liposomes helps to limit the cardiotoxicity risks
    - **Concomitant Dexrazoxane:** Dexrazoxane is a catalytic inhibitor of Topo-II. When given concurrently with doxorubicin, it is able to block the cardiotoxic effects while preserving the anticancer property. With such a miraculous property, clinicians questioned whether dexrazoxane also prevents cell-killing, therefore lessening the efficacy of doxorubicin. Two trials showed there was no difference in efficacy, one suggested there may be a decrease. All three of those trials were underpowered, so this question remains unanswered. *(no ref available)*
  - **Extravasation:** When administered IV, there is leakage at the site of the needle. Local release of doxorubicin causes extreme, debilitating necrosis. Similar to as for cardiotoxicity, dexrazoxane is an effective antidote. If extravasation-type side effects are recognized, start dexrazoxane.
- **Daunorubicin:** An anthracycline with little use. But it is effective for some adult leukemias.
- **Mitoxantrone (Novantrone):** Mitoxantrone is an anthrancenedione effective for numerous cancers, including nonlymphocytic leukemias and hormone-resistant prostate cancers (pain relief).
  - Indication for Multiple Sclerosis: In MS, mitoxantrone inhibits immune cell function, thereby providing a clear clinical benefit to MS patients by reducing symptoms and disease progression
  - **Cardiotoxicity:** D/t its severe risks for cardiotoxicity and secondary leukemias, it is limited to 5 total lifetime courses and should be used with caution.

Future Prospects: We want to design new topoisomerase inhibitors that have lower risk of secondary malignancies.

- Role of Pharmacogenomics: Many tumors have defects in repairing DNA damage, as such, we have detected certain cancers to be more sensitive to topoisomerase inhibitors, whether that is good or leads to more secondary malignancies, who knows. More research is needed.

Types of Resistance Mechanism

- **Intrinsic Resistance:** Cancer cells maintain many of its inherent qualities specified by its genome
- **Acquired:** Treatment with cytotoxic agents exerts a strong selection for resistance variants

Resistance mechanism: Drug Transporters

- Doxorubicin is transported by MDR1. High MDR1 expression tends to provide resistance to doxorubicin
- Etoposide is transported by Mrp
- Camptothecins are substrates for ABCG2
Resistance mechanism: Topo Expression
- Cells expressing high levels of Topoisomerases will be hypersensitive to topoisomerase inhibitors. Cancer cells adapt to the inhibitors presence by expressing low levels of topoisomerases, therefore reducing the amount of cell damage that occurs.

Revisiting Bruzik
Cyclophosphamide (CPO) and ifosfamide (IFO) are phosphoramidate alkylating prodrugs. In this brief section, I review the metabolism of these compounds.

In lecture, we discussed the mechanisms of action for the alkylating mustard drugs. Pertinent to their efficacy, is having a nucleophilic Nitrogen. The nucleophilic nitrogen supports intramolecular formation of the electrophilic aziridinium cation for subsequent attack by DNA-based nucleophiles. For purposes of efficient drug delivery, R-substituents are added to lower nucleophilicity and reactivity.

Shown to the right is CPO. CPO (and IFO) require metabolic activation by the CYP3A4/5 or CYP2B6 enzymes in order to exert anticancer activity. As is, CPO’s nitrogen is not nucleophilic enough to have anticancer activity for two reasons: (1) The inductive effect whereby phosphorous pulls nitrogen’s electron density, as well as (2) partial π-bond conjugation conferred via mesomeric shifts.

CYP-mediated 4’-hydroxylation forms 4-OH-CPO, which is in spontaneous equilibrium with its ring-opened aldehyde compound, aldophosphamide. Aldophosphamide’s α-carbon’s protons are somewhat acid, to the extent that a weak-ish base can abstract a proton. In such a case, aldophosphamide undergoes β-elimination to form two molecules, the active anticancer drug phosphoramide and the potent α,β-unsaturated michael acceptor: acrolein.

Acrolein is a potent electrophile which interacts with protein thioles (-SH), amines, and other cellular components that can cause toxicity. Detoxification mechanisms of the body include GST’s sacrifice of glutathione to form inactive acrolein adducts for elimination. This reaction, however, can be readily saturated, especially with high dose CPO chemotherapy. Acrolein is known to produce urotoxicity, and is the causative agent of hemorrhagic cystitis. To prevent toxicity, mesna is given to react with acrolein and form inactive adducts.

Also notice the phosphoramidate mustard’s ionic charge. Negatively charged, the oxygen anion satisfies the phosphorous’ electronegative pull, thereby reducing the inductive and π-bond impact on nitrogen’s lone pair by phosphorous. Consequently, the nitrogen has become far more nucleophilic, thus satisfying our ideal alkylating mustard property.

CPO and the active phosphoramidate drug have multiple metabolic pathways for elimination. The one previously discussed was specifically mentioned because of this toxic byproduct, acrolein. The other pathways for elimination form otherwise inactive compounds for elimination.
Abbreviations

abx: antibiotics
ADL: Activity of Daily Living
BBB: Blood Brain Barrier
DDI: Drug-Drug Interaction
DLT: Dose-limiting toxicities
ECF: Extracellular Fluid
ECOG: Eastern Cooperative Oncology Group
EKG: Electrocardiogram
EWG: Electron Withdrawing Group
HSCT: Hematopoietic Stem Cell Transplant
ICF: Intracellular Fluid
LP: Lumbar Puncture
NCI: National Cancer Institute
PE: Physical Exam
PK: Pharmacokinetics
PMW: Post-Menopausal Women
Ppx: Prophylaxis
QoL: Quality of Life
RLS: Rate-Limiting Step
ULN: Upper limit of normal
Biochemical
ANC: Absolute Neutrophil Count
dNTP: deoxyriboNucleoside TriPhosphate
IMP: Inosine Monophosphate
PTH: Parathyroid Hormone
TS: Thymidylate Synthase

Drug
3-FU: 5-Fluorouracil
AG: Aminoglycosides
AI: Aromatase Inhibitor
AZA: Azathioprine
BCNU: carmustine
CCNU: lomustine
IBU: Ibuprofen
PCN: Penicillin
SSZ: Sulfasalazine
TKI: Tyrosine Kinase Inhibitor
ZLN: Zolendronic Acid

Parameters
CR: Complete Response
DFS: Disease-Free Survival
EF: Ejection Fraction
ORR: Overall Response Rate
PR: Partial Response

Condition
FN: Febrile Neutropenia
HA: Headache
HF: Heart Failure
HM: Hematologic Malignancy
MBC: Metastatic Breast Cancer
MM: Multiple Myeloma
N/V/D/C: Nausea, Vomiting, Diarrhea, Constipation, in any order or combination
SCD: Sickle Cell Disease
ST: Solid Tumor

References: