NEOPLASIA

- Abnormal new growth of cells or tissue that
  - Expands more rapidly than normal tissue
  - Continues to grow in the absence of external stimulatory factors
  - May show a lack of structural organization
  - May form a discrete mass

- Multi-step model of carcinogenesis
  - Vogelgram: normal → dysplasia → in situ cancer → invasive cancer → metastasis
    - Dysplasia – premalignant condition of almost any tissue characterized by an abnormal histopathological appearance
    - In situ cancer (Carcinoma in situ – CIS) – malignant-appearing cells that do not yet show invasion of basement membrane (appearance is malignant but behavior is dysplastic) → form of very high grade dysplasia and almost always mandates therapy
    - Eg: activating mutations in K-RAS oncogene → inactivation of p16INK4a tumor suppressor gene → loss of p53 tumor suppressor gene

→ CONTINUUM
  - Oncogene –
    - Gene that facilitates transformation (malignant progression) when inappropriately activated
    - Activation by:
      - Amplification - cyclin D1
      - Point mutation – K-RAS
      - Translocation – c-MYC
      - Loss of an inhibitor – akt activation by PTEN loss
      - Or combo of mechanisms
  - Tumor suppressor genes
    - Inhibit malignant transformation (represses tumor formation)
    - Loss of genes is assoc with familial cancer syndromes
    - Can lead to defects of anti-cancer mechanisms like senescence (p16INK4a) or impaired DNA repair (BRCA1)
    - Accepted mechanisms of action include modulator of DNA repair, inducer of permanent growth arrest in response to oncogenic stimuli, inducer of apoptosis in response to hypoxia
    - p16INK4a, Rb, p53, PTEN, BRCA1
  - Epigenetic
    - Heritable modification of genome that does not change DNA sequence order of the genome
    - Most often refers to permanent silencing of a gene or locus thru modification of proteins that bind to DNA (histones) and/or hypermethylation of cytosines (CpGs) within gene’s promoter or regulatory regions
    - Contribute to malignant transformation by inactivating tumor suppressor genes in assoc with promoter DNA methylation or inducing genomic instability by inhibiting DNA repair (MLH1)
  - Errors in DNA repair crucial for serial mutations within a single cells and progeny → genomic instability and aneuploidy is critical in multi-step carcinogenesis

- All cancer cells show:
  - Inappropriate proliferation: dysregulation of cell cycle
    - Abnormal and inappropriate growth is sensed by would-be cancer cells → potent anti-proliferative signals (p16INK4a and/or p53 activation) → induce senescence (permanent growth arrest)
    - Pathways are epistatic: only one lesion per pathway required in general
    - Traversal of senescence barrier → requirement for pre-malignant cells to undergo transformation to cancer cells
      - Occurs thru perturbation of p16INK4a-cyclin D-cdk4 – Rb pathway → p16INK4a/Rb loss, cdk4 pt mutation, cyclin D overexpression
      - Not required for malignancy
    - Sensing abnormal growth
      - One mechanism: telomeres
        - Ends of chromosome that shorten with each cell division
        - Length provides a way of counting cell divisions
        - When telomeres get really short, p53 and p16INK4a are activated and growth arrests
        - Cancer cells require a mechanism to elongate telomeres, generally by re-activating telomerase
      - Another mechanism
Certain types of oncogene activation (ie K-RAS pt mutation) induce senescence in normal cells by inducing p16INK4a.

RAS activation causes senescence in normal cells with p16INK4a, but transformation in cells with inactivated p16INK4a.

Resistance to differentiation and apoptosis: dysregulation
- Vast majority of progeny cells in rapidly dividing tissues are scheduled to differentiate.
- Differentiated cells either die (apoptosis), are shed, or are destroyed in performance of physiologic function.
- Cancer cells require a means to evade/ignore signals to differentiate → remain in less committed, progenitor state that is compatible with self-renewal and resistance to apoptosis.
  - WNT-APC-β-catenin pathway: Solid tumors
    - APC mutation is a common, early event in colon cancer.
    - Allows mutant cells to remain in crypts in a less-differentiated state.
    - Cells do not migrate to tips of colonic villi to be sloughed but instead accumulate → lead to hyperplastic polyp (precursor of colon cancer).
  - Acute Promyelocytic leukemia (APML)
    - Abnormal retinoid receptor prevents cellular differentiation.
    - Treatment = high dose retinoids → inhibits abnormal retinoid receptor → leukemia cells differentiate in vivo.
    - Pathways seem to be involved in stem-cell maintenance in corresponding tissues.
- Malignant growth also induces an apoptotic signal.
  - Hypoxia, DNA damage, and certain forms of oncogene activation can all induce apoptosis.
  - Signal frequently requires p53.
  - Evasion can be accomplished by p53 loss, overexpression of survival molecules (BCL-2) or activation of pro-survival pathways (PTEN loss, AKT activation).

Genomic instability
- Malignant transformation requires multiple genetic events (amplifications, deletions, pt mutations, whole chromosome loss/gain).
- Normally, genome is too stable to permit accumulation of different lesions in a single cell by chance alone → impairments of DNA repair and metabolism are a requirement for transformation.
- Examples
  - Defects in repair of DNA photo-adducts → predilection for skin cancer.
  - Loss of mismatch repair proteins (MLH1 and MSH1) → colon cancer.
  - Mutations of BRCA1 → breast/ovarian cancer.
  - Many forms of DNA damage signal to p53 → activates senescence and apoptosis.
- Ability to grow where it ought not (malignant growth – invasion, angiogenesis, metastasis).
  - Not all cancers metastasize, but all grow malignantly.
  - Malignant is not synonymous with deadly and benign is not synonymous with curable.
  - In many endocrine tumors, metastasis is the sine qua non of malignancy (not just invasion).
  - In most cases, to cause harm, a tumor cell must:
    - Locally invade.
    - Grow into lymphatics or blood vessel.
    - Spread to distant site.
    - Attach to endothelium and leave blood vessel or lymphatic.
    - Grow in new home, often destroy normal stroma in the process.
    - Induce new blood vessels.

THE ABILITY TO GROW MALIGNANTLY (metastasize or invade) IS THE MOST CLINICALLY IMPORTANT CHARACTERISTIC OF CANCER.

Most therapeutic efforts to find a metastasis inhibitor have appeared to fail → but some of known anti-cancer therapies do inhibit metastasis (bisphophonates, anti-VEGF Ab, Taxol, etc).

Molecularly targeted therapy
- Almost all drugs used were developed empirically.
Specific inhibitors of oncogenic pathways that are required for tumor maintenance are now being rapidly developed for several important human malignancies

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Target</th>
<th>Tumor type treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib (Gleevec)</td>
<td>inhibits ABL and KIT kinases</td>
<td>CML and GI stromal tumors</td>
</tr>
<tr>
<td>Erlotinib (Tarceva)</td>
<td>inhibits epidermal growth factor receptor kinase</td>
<td>minority of nonsmall cell lung cancer, possibly other solid tumors</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>monoclonal Ab against vascular endothelial growth factor (VEGF)</td>
<td>colon cancer, renal cell CA, breast CA</td>
</tr>
<tr>
<td>Rituximab (Rituxan)</td>
<td>monoclonal Ab against CD20</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
</tbody>
</table>

- Cancer Mortality
  - Ability to cure varies dramatically with tumor type
  - Cellular biology of tumor and genes/proteins it expresses constitute in part the underlying basis for drug sensitivity or resistance
  - Tumor heterogeneity (from both tumor microenvironment and differentiation state of cells that comprise tumor) contribute to chemoresponsiveness ➔ possible examples
    - Tumor stem cells express high level of efflux pumps and spend a greater % of time in G0 ➔ more resistant
    - Normal tissue contains a high % of cells in G0 or a quiescent state while early tumors contain more cells that are in cell cycle ➔ tumors more tractable to chemotherapies
    - Late stages of tumors with bulky disease contain many non-cycling cells as well as necrotic and hypoxic regions of the tumor ➔ more resistant
  - Cure requires killing or removing last cancer cell

- Clinical importance of metastasis
  - Tumor that has spread to different sites
  - Helps determine patient prognosis
  - Metastases
    - Most commonly involve lymph nodes, lungs, liver, bones, brain, adrenals, subcutaneous sites
    - Usually multiple but solitary ones are not uncommon and should be biopsied
    - Responsible for majority of cancer deaths ➔ interrupt function of a normal organ ➔ organ failure
    - Formation is an inefficient process largely due to highly complex and interdependent steps involved in terminal aspect of metastasis formation
  - Micrometastases
    - Metastases that cannot be detected clinically at time of tumor diagnosis, generally b/c they are too small to be seen by CT scan
    - Existence can be inferred b/c many patients are put into remission by surgical resection of their primary tumor, only to relapse with distant disease at some later time
    - Target of neoadjuvant or adjuvant therapy
    - If risk of micrometastatic disease is high enough, a rational strategy is to recommend systemic treatment as adjuvant therapy, hoping to eradicate microscopic disease

- Cancer staging
  - Defines extent of cancer and predicts ultimate outcome for the patient
  - Objectives include definition of prognosis, treatment plan, outcome eval, and facilitating cancer treatment eval and reporting end results
  - TNM staging system
    - T stage: denotes extent of primary tumor
      - T1 – T4, T0 if primary not identified
      - Describes increasing size or local spread of primary tumor
    - N stage: denotes extent of regional lymph node involvement
      - N, 0 – 3
      - Describes increasing size, number or location of regional lymph node involvement
    - M stage: denotes presence or extent of metastatic disease
      - M, 0 or 1
      - Describes distant metastatic spread and site
  - Stage is not absolute ➔ clarified by clinical and pathological
Sometimes can change after further testing or surgery

- Tumor in large intestine by radiologic eval (clinical stage II) but at time of surgery, a small liver metastasis is noted (surgical stage IV)
- More accurate staging always makes a given therapy appear more active

**Performance status**
- Measured predominantly by either Karnofsky score or ECOG performance status
- Most universal predictor of patient outcome across a wide variety of tumor types
  - PS0: no symptoms, no limits from disease
  - PS1: restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature
  - PS2: ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hrs
  - PS3: capable of only limited self-care, confined to bed or chair more than 50% of waking hours
  - PS4: completely disabled; cannot carry on any self-care; totally confined to bed or chair
  - PS5: dead

**Chemotherapy**
- Tumors are generally clinically and radiologically undetectable when cell numbers are less than $10^9$, the time period in which they are most responsive to treatment
- A lethal tumor burden is $>10^{12}$ cells
  - Tumor burden established by extrapolating from size of lesion using radiologic assessment and/or measuring a marker protein produced by tumor (eg immunoglobulin in multiple myeloma)
- Chemo results in a several log kill and may lead to a clinical remission despite a large number of tumor cells still being present
  - One of most important determinants of response is sensitivity to chemotherapeutic agents
  - Drug resistance limits curability and resistant clone will expand in response to selective pressure of single drug administration
  - Treatment must be dose intensive and at frequent intervals to suppress emergence of malignant clone

**Principles**
- Single drugs are rarely curative due to development of resistance by cancer cells
- Combination regimens should be used based upon
  - Component drugs must be active as single agents
  - Toxicities should be non-overlapping
  - Drugs should be used in optimal dose/schedule
  - Combinations should be given at consistent intervals

**TREATMENT IS MULTIDISCIPLINARY**

**Mechanisms of drug resistance**
- Overexpression of drug metabolizing enzymes
- Overexpression of drug targets
- Expression of drug efflux pumps (MDR gene)
- Increase in DNA repair mechanisms
- Transport defects

**Major settings for use**
- Adjuvant – adjunct (usually chemotherapy) to local treatment methods, primarily surgical resection (solid tumors) → given to people who do not have detectable cancer, but are likely to relapse (NED during treatment)
- Neoadjuvant – chemo b/f surgery or radiotherapy → theoretically makes surgery simpler by shrinking tumor and allows oncologist to tell if tumor is sensitive to chemo being given
- Induction – intense, high-dose chemotherapy with remission as goal
- Consolidation/intensification – chemo repeated after remission is obtained to solidify remission and increase cure or prolong remission → may be similar to previous regimens (consolidation) or intensified (intensification); usually refers to hematopoetic malignancies
- Maintenance – prolonged chemo (can be >18 mts) attempting to maintain a remission (some leukemias and peds tumors, not solid tumors)
- Concurrent – use in conjunction with radiotherapy (localized but unresectable cancers)
- Treatment of adv disease – usually palliative in nature with a few exceptions where it can be curative (testicular, Hodgkin’s, NHL, etc)
- Site-specific instillation – intrathecal, intraperitoneal, hepatic, or carotid artery infusions
Role of surgical oncology
  o In general
    ▪ Initial diagnosis
    ▪ Staging
    ▪ Excision for cure or palliation
    ▪ Prevention (genetic predisposition to breast or colon cancer)
  o Applications
    ▪ Excisional biopsy – breast tumors, isolated lung nodule
    ▪ Definitive surgical treatment – appropriate local therapy and integration with other modalities
    ▪ Surgery to reduce or resect bulk residual disease – Burkitts, ovarian, testicular
    ▪ Resection of metastatic disease – lung mets in sarcoma, liver mets in colon cancer
    ▪ Oncologic emergencies – laminectomy for spinal cord compression, resection and lavage for bowel perforation
    ▪ Palliation – relief of bowel/biliary obstruction
    ▪ Reconstruction
  o Response rate (RR)
    ▪ Measure of therapeutic efficacy
      ▪ PR (rate of partial response) + CR (rate of complete response)
      ▪ PR – >50% decrease in tumor mass that lasts for more than 3 mts
      ▪ CR – complete resolution of disease for more than 3 mts
  o Evaluation of new drugs
    ▪ Phase I: find safe dose; usually with more than one type of tumor → toxicity ascertainment, use of surrogate markers
    ▪ Phase II: single tumor type → efficacy ascertainment
    ▪ Phase III: compare standard of care, large/multicenter trials

HEMATOPATHOLOGY OF NEOPLASIA
  o Properties of malignant tumors
    ▪ Abnormal growth and proliferation
      ▪ How do abnormal growth appear in patients?
        ▪ Lymphadenopathy
        ▪ Splenomegaly
        ▪ Mass lesion
        ▪ Abnormal CBC (cytopenias, leukocytosis)
      ▪ Lymphoma may cause abnormal growth pattern in lymph node (loss of architecture… absence of germinal centers and sinuses)
    ▪ Resistance to differentiation and apoptosis
      ▪ In acute leukemia, normal bone marrow cells have been replaced by leukemic blasts; virtually no mature nucleated cells are seen
    ▪ Clonal
      ▪ Detected by
        ▪ Look at genes → tumor cells will share the same abnormal genes
        ▪ Look at chromosomes
        ▪ Look at Ig proteins on cell surface (B-cells only) – Kappa or Lambda
        ▪ Look at Ig proteins secreted in serum or urine (B-cells only)
      ▪ Monoclonality of B-cells
        ▪ Rearranged Ig gene → detect using molecular biology techniques
        ▪ Light chain restriction on cell surface → tumor cells will have either kappa or lambda light chains on cell surface
          ▪ Normal mature B-cells have surface Ig with either kappa or lambda light chains on surface; normal B cells in peripheral blood and lymphoid tissues consist of a mixture of kappa and lambda positive cells
          ▪ B/C LYMPHOID NEOPLASMS ARE CLONAL, THEY WILL CONSIST OF EITHER KAPPA OR LAMBDA POSITIVE CELLS
        ▪ Diagnostic procedures
          ▪ Flow cytometry
            ▪ Identifies Ags on cells using Abs labeled with fluorescent tag
            ▪ Useful for
              ▪ Detecting light chain restriction (monoclonality)
              ▪ Detecting abnormal phenotypes
              ▪ Differentiating myeloid from lymphoid proliferations
Flow cytometric analysis of benign lymphoid tissue

This analysis was performed using fluorescently labeled antibodies to the B-cell antigen CD19 and to the kappa and lambda light chains. Note the presence of kappa and lambda light chains.

Flow cytometric analysis of a B-cell neoplasm

This analysis was performed using fluorescently labeled antibodies to the B-cell antigen CD19 and to the kappa and lambda light chains. Note the predominance of kappa positive cells indicative of a monoclonal population.

- Immunohistochemistry
- In situ hybridization
  - Secretion of monoclonal Ab
    - Serum and urine protein electrophoresis
    - Serum and urine immunofixation
  - T-cell Clonality
    - Precursor T-cells undergo gene rearrangement of T-cell receptor genes
    - Can detect using molecular studies (southern blot, PCR)
  - Assoc with genetic abnormalities and instability
    - Cytogenetic studies
      - Conventional karyotyping
      - Fluorescence in situ hybridization (FISH)
    - Philadelphia chromosome is assoc with CML
      - 9 and 22 translocation
    - Abnormalities of c-myc are assoc with Burkitt lymphoma
      - C-myc is on chromosome 8
      - Immunoglobin genes are on chromosomes 8, 14, 22

- Categories
  - Myeloid
    - Myeloproliferative disorders
    - Myelodysplasia (preleukemia)
    - Acute leukemia
  - Lymphoid
    - Acute leukemia
    - Chronic leukemia
    - Lymphoma
    - Plasma cell dyscrasia

CLINICAL RADIATION THERAPY

- Radiation overview
  - Function preserving treatment modality
  - Destroys dividing cells by producing damage to DNA
  - Affects both normal and tumor tissue
    - Increasing tumor control and increasing normal tissue complications with increasing radiation dose
    - Improved therapeutic ratio will result if tumor curve can be moved to left or the normal tissue complication curve can be moved to the right
  - Of clinical value when relative injury to tumor can be made greater than that to the normal tissue \( \rightarrow \) maximization of therapeutic ratio

- Uses
  - Primary therapy for a tumor mass in order to obtain local tumor control while preserving organ function
  - In combo with surgery where the surgeon removes the major tumor mass and radiation therapy is used for control of microscopic local residual disease
  - In combo with chemo where radiation is used for control of primary tumor mass and chemo for control of metastatic disease
  - As primary therapy for locally adv disease where the tumor cannot be removed surgically
  - For palliation for tumor infiltration causing pain, to prevent local bleeding, or tumor obstruction of a hollow viscus
Generally given as a fractionated course of therapy
  o This decreases relative damage to tumor compared to normal tissues → improves therapeutic ratio
    ▪ Typically, one fraction of radiation is given per day, 5 days per wk, with total # of treatments extending over 5-7 wks (for curative therapy)
    ▪ Radiation kills cells by 1st order kinetics → same percentage of cells is killed in each fraction, although absolute number of cells killed decreases as tumor mass decreases
  o Dose is related to details of clinical situation
    ▪ Dose delivered in a fractionated course is biologically very different and represents a biologically less effective dose than the same physical dose delivered at higher dose per fraction or in a single dose
  ▪ Cell survival curve
    • Plotted with dose on x-axis and log of survival fraction on y-axis
    • Initial relatively flat portion of curve (shoulder) followed by exponential portion of curve
      o The survival curve “shoulder” reflects accumulation of damage which can be repaired by the cell
      o Less killing per rad in the shoulder portion
    • If there is a time interval between two doses of radiation
      o Some of the radiation damage is repaired
      o The shoulder of the cell survival curve is repeated with the next dose of radiation
    ▪ Different cell types have differing shapes of survival curves
    ▪ Biological effect of multiple fractions is much less than the same physical dose given in one fraction since the “shoulder” of the curve is present for each dose of radiation
  o Killing from multiple single doses of RT is the product of the individual killing times the number of fractions
  o Normal tissues which produce late complications are more sensitive to high single doses of radiation than tumor or epithelium
    ▪ Spared preferentially by multiple small doses
  o Biological factors
    ▪ Repair of radiation damage
      • After a radiation dose, cells will have accumulated non-lethal damage
      • If cell is allowed to rest after a radiation dose, this sub-lethal injury can be repaired
      • Differential repair capability can result in relatively more injury to tumor than normal tissue
    ▪ Redistribution of cells in various phases of cell cycle
      • Cells which are in late G2 and M are most sensitive to radiation; most resistant in late S
      • By dividing the radiation dose into multiple fractions, one can allow cells to redistribute themselves into more sensitive phases of cell cycle → tumor cell killing is more effective
    ▪ Reoxygenation of hypoxic cells b/t radiation fractions
      • Hypoxic cells are more resistant to radiation than are well oxygenated cells
      • Hypoxic cells are usually present in tumors b/c of angiogenesis
      • With each dose of radiation the oxygenated cells are preferentially killed, leaving a population of primarily hypoxic and radiation resistant cells
        o Hypoxic cells will reoxygenate if left alone for 24 hours → more sensitive to subsequent radiation doses
    ▪ Repopulation of tumor cells thru radiation course
      • Cells continue to divide while a radiation course is being given
      • Giving the radiation dose in the shortest time possible is necessary to minimize growth of residual tumor cells
      ⇒ single dose is not effective b/c
        Reoxygenation and redistribution, radiation is more effective if given in multiple fractions
        Repair and repopulation radiation is less effective if given in multiple fractions
        Normal tissues are relatively spared from radiation injury by fractionation, because their repair characteristics differ from tumors
  o Physical factors
    ▪ Type of radiation modality
      • X-rays, electron beams, and radioactive implants
      • Energy of beam defines penetration of beam into tissue
      • Minimum dose to normal tissue, maximum dose to tumor
- Field arrangement
  - Beam can be adjusted to enter from various angles → resulting in an optimized dose distribution
- Field size and shaping
  - Side effects and complications from radiation are directly related to amount of normal tissues irradiated
- Beam and field modifications
  - Use wedges and normal tissue compensators
- Intensity modulation
  - Modify beam orientation and intensity during treatment
  - Dose distributions that sculpt around normal tissues

**Acute Leukemias**
- Malignant disorder which results from the progressive expansion of a population of cells that are derived from a single, malignantly transformed progenitor cell (blast)
  - Separated from other hematopoietic clonal disorders by the findings of at least 25-30% blast cells in bone marrow
- If not treated, proliferation will result in rapid replacement of bone marrow → death from bleeding and/or infection
- Disorder growth properties
  - Increased proliferation (usually growth factor dependent)
  - Decreased apoptosis (esp in lymphoid… BCL2 overexpression)
  - Blocked differentiation (esp in myeloid… t(15:17) translocation)
- Lab diagnosis
  - Present with bone marrow failure characterized by one or more of:
    - Neutropenia (leading to fever, infection)
    - Anemia (leading to fatigue, pallor, malaise, weakness)
    - Thrombocytopenia (leading to bruising and hemorrhage)
  - Can also present with symptoms attributable to organ infiltration
  - Confirm cell lineage via
    - Peripheral blood morphology
      - Neutropenia, anemia, thrombocytopenia
      - Few blast in circulation
        - Blasts with Auer rods = AML
        - Small blasts with very high N:C ratios and inconspicuous nucleoli = ALL (childhood)
    - Bone marrow morphology
      - Increased number of immature cells (blasts)
      - 20-30% or more of nucleated bone marrow cells are blasts
      - Marked decrease in normal hematopoietic cells
    - Cytochemistry (AML)
      - Classify AML
      - Detect enzymes found in cytoplasm of myeloid cells (but not lymphoid cells)
      - Stains such as MPO, esterase
    - Flow cytometry (ALL)
      - Detect antigens on surface and in nucleus of leukemic cells
    - Cytogenetic analysis
      - Karyotypic analysis is very useful in predicting prognosis for both ALL and AML
    - Molecular assays
      - Detect translocations using PCR and/or FISH
      - PCR useful for classifying leukemias and for detection of minimal residual disease
- **Acute myeloid leukemias (AML)**
  - All types are derived from the multipotent stem cell giving rise to pure myeloid, mixed myeloid, and monocytic, erythroid and megakaryocytic subtypes (M3 and other)
  - Classification based on
    - Blast morphology
      - Surface Ags specific for each cell type (identified by flow cytometry of blasts with monoclonal Abs)
    - Histochemical stains for cell-specific enzymes
  - Incidence increases with age
    - In adults, 50% of leukemia is AML
    - In kids, only 20% is AML
  - Etiologic assoc (usually not definable)
    - Genetic risk factors: Down syndrome (MDS → M7 AML)
    - Drugs: cyclophosphamide (alkylating agents)
    - Radiation
Secondary leukemias: arise as a consequence of DNA damage resulting from prior chemo, radiation, or pre-existing marrow disorder; esp important in treatment of pediatric malignancies

- Presenting clinical features
  - Low peripheral blood counts → anemia, infection, bleeding
  - Fatigue, weakness, fevers, bruising
  - Pallor, petechiae, occasionally splenomegaly
  - Coagulopathy → abnormal procoagulant activities released from myeloblasts that are rapidly turning over OR assoc with co-existing infections → DIC

- Lab:
  - Sometimes blasts in peripheral blood, usually decreased RBCs and platelet counts, WBC can be low, normal, or elevated
    - High WBC can lead to leukostasis → intravascular clumping of blasts leading to acute infarction of CNS and/or lungs
    - High blasts can raise risk of tumor lysis syndrome → hyperuricemia with subsequent renal failure; hyperphosphatemia, hyperkalemia, hypercalcemia
  - If myeloblasts not seen in peripheral blood, diagnosis awaits bone marrow aspirate and/or biopsy

Morphology
- Neutropenia, anemia, thrombocytopenia, circulating blasts
- BM has increased blasts (>20% of nucleated BM cells) and decreased hematopoiesis
- Blasts may contain Auer rods

Cytochemistry
- Most common
  - Myeloperoxidase (MPO) – myeloblasts + neutrophilic series, not lymphoblasts
  - Chloroacetate esterase (CAE) – myeloblasts + neutrophilic series
  - Alpha naphthyl acetate esterase (ANAE) – monoblasts + monocytes
- STUDIES ALL NEGATIVE IN LYMPHOBLASTS

Cytogenetics
- Good prognosis
  - t(15:17) – acute promyelocytic leukemia → M3
  - t(8:21)
  - t(16:16) or inversion 16
- Poor prognosis
  - -5
  - -7

Treatment options
- Supportive care
  - Preventing/treating tumor lysis – allopurinol, iv fluids, urine alkalinization, antibiotics, red cell and platelet transfusions as necessary, patient/family education/support
  - Coagulopathies – FFP, platelets

Chemotherapy
- Goal: eradicate abnormal leukemic clone, allowing normal progenitors to repopulate bone marrow
- Prolonged periods of pancytopenia after treatment
- Drugs: Anthracycline/ara-C combinations
- Other than M3: intense and mostly inpatient; lasts 6-12 months
- M3: ATRA (all-trans-retinoic acid) and less-intensive chemo → highly curative

Remission
- Defined at <5% blasts in marrow + no evidence of leukemia on physical exam, in peripheral blood, or in CSF
- Not cure, but it can be prognostic
  - Patients who achieve remission quickly are more likely to be cured
  - Allogeneic BMT with HLA-matched sibling donor gives greater than 50% DFS (disease free survival) at 5 years with stable survival curve
  - Younger patients do better
  - Problems: lack of suitable donors, transplant morbidity and mortality, cost, age-related issues, relapse rate still high (due to drug resistance)

Acute Lymphoblastic Leukemias (ALL)
- Classification based on
  - Blast morphology (L1-L3)
  - Expression of lymphoblast-specific enzymes (TdT) and lymphoid surface markers
- Marker of pre-B cells: CALLA (common acute lymphoblastic leukemia antigen) \( \rightarrow \) 80% of ALL cases are CALLA+
- T cell markers \( \rightarrow \) 15% of ALL
- Mature B cell markers \( \rightarrow \) 1% of ALL
  - Disease of childhood \( \rightarrow \) incidence peak at 2-10 yrs old
  - Does not commonly occur as a 2nd leukemia
  - Etiologic assoc (usually not definable)
    - Genetic risk factors: Down syndrome
    - Drugs: immunosuppressants
    - Viral/post-viral: EBV for L3 ALL
- ALL IS A RARE CLONAL EVENT FOLLOWING A RATHER COMMON EXPOSURE
- Clinical features
  - Higher incidence of CNS, lymph node, testicular disease
  - T-cell ALL can present with mediastinal mass on chest x-ray
  - B-cell ALL (L3) can occur in conjunction with Burkitt’s lymphoma
  - Fever, infection, mucocutaneous bleeding
  - DIC is UNCOMMON
- Lab:
  - Morphology
    - Neutropenia, anemia, thrombocytopenia, circulating blasts
    - Marrow shows increased blasts (>30% of nucleated BM cells) and decreased hematopoiesis
  - Flow cytometry
    - Used to classify ALL
      - Precursor B-cell
        - Most common phenotype of ALL in children
        - Type with best prognosis in children
        - Surface Ig negative
      - Mature B-cell
        - Typically assoc with L3
        - Poor prognosis in adults and children
        - Surface Ig +
        - Light chain restricted \( \rightarrow \) either kappa or lambda
        - Blue cytoplasm, prominent cytoplasmic vacuoles
        - Abnormalities of c-myc oncogene on chrom8 \( \rightarrow \) t(8:14)
        - Closely assoc with Burkitt’s lymphoma
      - Precursor T-cell
        - Best prognosis in adults
        - Characteristically positive for T-cell antigens, CD3,4,8 in addition to TdT and CD34
        - Predilection for teenage, young adult males
        - Often present with mediastinal mass
  - Cytogenetic analysis
    - Favorable
      - Hyperdiploidy (50+ chromosomes) \( \rightarrow \) tumors more sensitive to chemo
    - Unfavorable
      - t(9:22) – Philadelphia chromosome
      - t(8:14)
      - t(4:11)
      - Hypodiploidy (fewer than 46 chromosomes)
- Differential diagnosis
  - ITP – low plt count, easy bruisability, otherwise normal peripheral blood cts; BM aspirate shows normal RBC and WBC precursors with increased megakaryocytes
  - Aplastic anemia – anemia or pancytopenia; BM shows reduction in all 3 cell lines
  - Reactive lymphocytoses – assoc with viral infections; atypical lymphocytes that have more cytoplasm than lymphoblasts
- Treatment options
  - Supportive care as above
  - Chemotherapy
    - Usually less intensive than AML
    - Given for longer period of time (2-3 yrs, usually outpatient)
    - Vincristine + prednisone \( \rightarrow \) lymphocytotoxic but not myelosuppressive
    - Methotrexate and 6-MP
- CNS disease – treated with intrathecal chemo
- Remission same as AML
- Prognosis
  - Overall cure rate (5 yr DFS) for ALL in childhood is over 75%
  - Most important prognostic factors are
    - Age (2-10 yr optimal)
    - Initial white count (< 10,000 optimal)
    - Quick remission
    - Non-B, non-T cell phenotype
    - Hyperdiploidy
    - Absence of important translocations
- BMT sometimes used for patients with relapsed ALL or up-front for high risk patients

### Chronic Leukemias
- Usually result from proliferation of more differentiated cells
- Are generally compatible with some normal blood cell formation and longer survival of affected patients
- Laboratory diagnosis
  - Leukocytosis is an elevated WBC count and can be assoc with benign or malignant conditions
  - Peripheral blood morphology
    - Neutrophilia or lymphocytosis?
      - Need absolute count number not percentages
  - Immunophenotype
    - Flow cytometry → leukocytosis due to increase in number of B-cells, T-cells, or myeloid cells?
    - Immunophenotyping → leukocyte proliferation clonal?
      - Clonal B-cell population will have cells that make only one light chain
    - Benign proliferations of B-cells will have a mixture
  - CD numbers
    - Low numbers: T-cell phenotype
      - CD 1, 2, 3, 4, 5, 7, 8
      - CD 4 + AIDS → low T cells
    - Midteens: Myelomonocytic
      - CD 13, 14, 15 are myeloid markers
    - 19 and early 20s: B-cell phenotype
      - Be all you can B in the army at age 19-23
      - CD 19, 20, 21, 22, 23 are B-cell markers
    - Other markers
      - CD34 – stem cells
      - CD11c, CD25, CD103 – Hairy Cell Leukemia
  - Cytogenetics
    - Conventional
      - FISH → 9;22 translocation
    - Molecular pathology
      - RT-PCR → 9;22 translocation
      - Gene rearrangement studies → each B/T cell will have a slightly different rearrangement unless this group is clonal as in a malignancy

#### Chronic Myeloid Leukemia (CML)
- Epidemiology
  - 1 in 100,000
  - Median age at diagnosis – 50 to 60
  - Risk factor – radiation
- Clinical features
  - Symptoms: fatigue/weakness, fevers, sweats, anorexia, weight loss, abd fullness, early satiety, bleeding
  - PE: hepatomegaly, splenomegaly
  - Lab: leukocytosis of granulocytes (blasts to neutrophils), basophilia, eosinophilia, anemia, thrombocytosis, low leukocyte alkaline phophatase (LAP) score
- Pathophysiology
  - Transforming event occurs in a hematopoietic pluripotent stem cell (HSC) → reciprocal translocation of long arms of chromosomes 9 and 22 (Philadelphia chromosome)
• CML HSCs properties that lead to expression of disease
  • Capacity for self-renewal
  • Ability to differentiate preferentially along granulocytic pathway
  • Increased proliferative rate and increased apoptotic threshold conferred by Bcr-Abl expression provides CML HSCs with competitive edge over normal HSCs

• Ph chromosome
  • t(9:22)
  • Leads to creation of fusion gene product (Bcr-Abl)
    o Varying lengths of Bcr are fused to 2nd exon of Abl
      • P210 Bcr-Abl – majority of CML cases
      • P190 Bcr-Abl – rare in CML; 20-30% of adult ALL
    o Constitutively active intracellular tyrosine kinase that activates a number of downstream signaling events that lead to increased proliferation and decreased apoptosis
  • WHO criteria for diagnosing CML REQUIRE the presence of a (9,22) translocation or PCR evidence of Bcr-Abl expression
  • 5% of CML harbor (9;22) translocations that are not detected by conventional cytogenetics → use FISH

  o Differential Diagnosis
    • Leukemoid rxn
      • Reactive leukocytosis seen in infection, malignancy, drug rxns, and inflammatory disorders
      • Normal or increased LAP score
      • Never see basophilia
    • Other myeloproliferative disorders
      • Possibilities
        o ET
        o Idiopathic myelofibrosis
        o P. vera
        o Others
      • LAP score normal or increased
      • Test for Ph chromosome

  o Diagnostic workup
    • Peripheral smear and BM aspirate/biopsy → phase of disease
      • WBC elevated – 50,000-200,000
      • Lots of myeloid cells – varying levels of maturation → predominance of mature granulocytes
      • Platelets often elevated
      • Basophils
      • Very few erythroid precursors
      • BM – hypercellular → elevated M:E ratio (granulocytic hyperplasia)

    • Cytochemistry
      • LAP (leukocyte alkaline phosphatase) score is low

    • Cytogenetic studies
      • Conventional cytogenetic eval
        o Detects Ph chromosome in most CML cases, detects other cytogenetic abnormalities that may impact prognosis
        o Not as sensitive as FISH or PCR for monitoring disease response (requires cells in metaphase)
        o Misses cryptic (9;22) translocations
      • FISH
        o Detects Ph chromosome in metaphase and interphase
        o Detects cryptic translocations
        o Does not look for other translocations

    • Molecular studies
      • RT-PCR
        o Shows bcr/abl transcript
        o Highly sensitive technique → suitable for monitoring disease response and detecting minimal residual disease
        o Does not look for other cytogenetic abnormalities

  o Clinical course pre-Gleevec
    • Chronic phase
      • Median 3-5 yrs
      • <10% blasts
- **Accelerated phase**
  - 6-9 mts
  - Increasing WBC count and worsening splenomegaly despite therapy
  - Blasts in 10-29% of cells
  - Increasing basophilia
  - New cytogenetic abnormalities

- **Blast crisis**
  - 3-6 mts
  - Essentially transformation to acute leukemia → AML (66-75%) or ALL (25-33%) 
    - **Treatment**
      - **Conventional chemo**
        - Busulfan, hydroxyurea
        - Good at controlling leukocytosis, thrombocytosis, splenomegaly in chronic phase
        - Almost no cytogenetic responses
        - Does not change disease course
      - **Interferon alpha**
        - Major cytogenetic responses
        - 3 year median survival – 79%
        - Achievement of MCR assoc with better survival
        - IFN-α assoc with substantial toxicities
        - Cytarabine (Ara-C) added to IFN-α improves hematologic responses, cytogenetic responses and survival at cost of increased toxicity
      - **Allogeneic stem cell transplantation (SCT)**
        - High-dose chemo +/- whole body radiation followed by donor HSC infusion
        - Only know curative approach
        - Substantial morbidity and mortality
        - Graft vs leukemia effect
          - GVHD assoc with lower risk of relapse
          - T cell depletion of donor stem cell product leads to increased risk of relapse
          - Patients who relapse after SCT can be treated with an infusion of donor-derived lymphocytes and go back into remission
        - Disease free survival after a sibling-matched SCT for chronic phase CML is 45-70%
      - **Gleevec**
        - Inhibits Bcr-Abl kinase function
        - Achieves unprecedented cytogenetic response rate outside of transplant
        - Well tolerated with few side effects
        - Resistance to Gleevec can occur
          - Amplification/over-expression of Bcr-Abl
          - Pt mutations in kinase domain of Bcr-Abl that block binding of Gleevec
          - Constitutive activation of downstream effectors
          - Strategies to circumvent Gleevec resistance include
            - Increased dose
            - 2nd generation Bcr-Abl inhibitors that are more potent and can bind Bcr-Abl despite the presence of pt mutations
            - Gleevec + novel drugs that target downstream effectors of Bcr-Abl

- **Chronic Lymphocytic Leukemia (CLL)**
  - **Epidemiology**
    - 3.5-5.5/100,000
    - Most common leukemia
    - Median age at diagnosis – 55 yrs
    - Family members at 2-7 times increased risk of developing disease
  - **Clinical features**
    - Symptoms: fatigue/weakness, fever, night sweats, malaise, abd fullness, early satiety
    - Incidental finding in at least 20% of cases
    - PE: splenomegaly, lymphadenopathy, hepatomegaly rare
    - Labs: lymphocytosis (all mature lymphocytes), anemia, thrombocytopenia, hypogammaglobulinemia
    - Autoimmune phenomena
      - AIHA – 10-25% of cases
      - Diffuse large B-cell non-Hodgkin lymphoma (Richter’s syndrome)
      - Hodgkin lymphoma
- Clonally related to original CLL
- All disease transformations assoc with bad prognosis and short survival
  - Pathophysiology
    - Transformation of mature lymphocyte
    - Increased expression of Bcl-2 (anti-apoptotic protein) → survival advantage to malignant clone
  - Prognostic systems
    - Clinical stage (Rai staging system)
      - Stage 0: lymphocytosis only (median survival > 15 years)
      - Stage 1: lymphocytosis + lymphadenopathy (8 yrs)
      - Stage 2: lymphocytosis + splenomegaly (6 yrs)
      - Stage 3: lymphocytosis + anemia (3 yrs)
      - Stage 4: lymphocytosis + thrombocytopenia (2 yrs)
    - Cytogenetic abnormalities
      - Best detected by FISH
      - Abnormalities
        - 13q deletion – 133 mts
        - Normal – 111 mts
        - Trisomy 12 – 114 mts
        - 11p deletion – 79 mts
        - 17p deletion – 32 mts
      - P53 dysfunction assoc with worse prognosis
        - Deletion 17p (where p53 is located)
        - P53 mutation
        - Mutation of ATM (kinase that functions to activate p53)
        - Deletion of 11p (where ATM is located)
      - Somatic hypermutation of variable region of IgVH
        - Lack of somatic hypermutation is assoc with malignant transformation at a pre-germinal site → bad prognosis
        - Clinical stage loses its prognostic utility when IgVH mutational status and cytogenetics are considered
    - ZAP70
      - Intracellular kinase that facilitates signaling downstream of T cell receptor
      - Expression in CLL cells is strongly assoc with lack of somatic hypermutation of IgVH
      - Expression is assoc with worse prognosis
      - May be a better prognostic marker than IgVH mutational status
  - Laboratory diagnosis
    - Peripheral blood and BM
      - Lymphocytoses of >5,000 is seen in peripheral blood → mature-appearing with numerous smudge cells (cells that have been destroyed in preparation of the smear)
      - Increased lymphocytes in BM
    - Flow cytometry
      - Immunophenotyping shows a mature B-cell phenotype with CD5 (normal T-cell antigen) and CD23 expression
  - Cytogenetic studies
    - May show clonal abnormalities including trisomy 12
  - Treatment
    - Treatment of asymptomatic CLL does not confer a survival adv
    - Indications
      - Progressive symptoms related to disease
      - Fevers, night sweats, weight loss
      - Progressive adenopathy or organomegaly (bulky lymphadenopathy, painful splenomegaly, rapidly increasing lymphocytosis)
      - Autoimmune cytopenias (AIHA, ITP)
      - Increased freq of bacterial infections
    - Alkylating agents (Chlorambucil)
      - Well tolerated
      - Long-term use can increase risk of AML/MDS
    - Purine analogs (Fludarabine)
      - Better response rates compared with chlorambucil but no survival adv
      - Sensitizes cells to alkylating agents by inferring with DNA repair
      - Leads to profound cell-mediated immune compromise
• Monoclonal Ab
  o Rituxan
    ▪ Monoclonal anti-CD20 Ab
    ▪ CD20 is a pan-B-cell surface marker present of CLL cells
  o Campath
    ▪ Monoclonal anti-CD52 Ab
    ▪ CD52 is a pan-B and T cell marker (present on monocytes too)
  o Anti-tumor activity mediated by ADCC
  o Enhanced response rates when added to traditional chemotherapy agents

  ▪ Compare with
    o Benign neutrophilia
      ▪ Bacterial infections, non-hematopoietic malignancies, drug rxns
      ▪ Peripheral blood and BM morphology
      ▪ WBC elevated but usually less than 60,000
      ▪ Neutrophils may show signs of activation (toxic granules and/or vacuoles)
      ▪ Predominance of mature granulocytes with some immature forms
      ▪ BM is usually either normal or may show mild granulocytic hyperplasia (M:E of > 7:1 but < 10:1)
    ▪ Cytochemistry
      ▪ LAP score is elevated
    ▪ Cytogenetic and molecular studies
      ▪ Normal
    o Benign lymphocytosis
      ▪ Viral infections (mononucleosis)
      ▪ Peripheral blood and BM morphology
      ▪ Atypical lymphocytosis
        ▪ T lymphocytes with lots of pale blue cytoplasm (low N:C)
        ▪ Lymphocytes “kissing” RBCs
      ▪ Flow cytometry
        ▪ Immunophenotyping shows a mixture of T-cells and polyclonal B-cells (B-cells expressing both kappa and lambda light chains)
    ▪ Cytogenetic and molecular studies
      ▪ Normal
    o Hairy Cell Leukemia
      ▪ Middle age or elderly male with massive splenomegalgy and pancytopenia
      ▪ Peripheral blood smear and BM aspirate
      ▪ Hairy cells in peripheral blood
      ▪ BM – increased fibrosis and infiltrate of lymphocytes
    ▪ Cytochemical studies
      ▪ Positive for TRAP stain (Tartrate resistant acid phophatase)
    ▪ Immunophenotyping shows a mature B-cell expressing CD11c, CD25 and CD103

LYMPHOMAS

• Lymphadenopathy – disease of lymph nodes (lymph node enlargement)
  o Reactive
  o Metastases
  o Malignant lymphoma
• B cell lymphomas occur when mistakes are made during Ig processing
  o Chromosome 14 (Ig heavy chain), 2 (kappa light chain), 22 (lambda light chain)
• Most lymph nodes with lymphoma grow over wks to mts and without pain
• Risk of lymphoma
  o Increasing events
    ▪ Age – Small lymphocytic, Follicular, Diffuse, HD (bimodal)
    ▪ Chronic Ag stimulation (HP)
  o Immune dysregulation
    ▪ HIV – Follicular, Diffuse, Burkitt’s, HD
    ▪ Solid organ transplant
    ▪ Autoimmune disease (Sjogren’s, RA)
  o Viruses
    ▪ EBV: >95% of endemic Burkitt’s; 40% of HD
    ▪ HCV
• When to consider lymphoma
- Isolated LN > 1 cm
  - LN that has grown over wks to mts
  - LN that has growth w/o pain
  - LN that is not assoc with a site of infection
- “B” symptoms
  - Weight loss > 10%
  - Drenching night sweats
  - Fever

- What to do
  - Excisional > incisional > core needle > fine needle
  - CT scan

- Staging
  - I: single nodal region or single extranodal site
  - II: two or more nodal regions or an extranodal site and regional nodal involvement on same side of diaphragm
  - III: lymphatic involvement on both sides of diaphragm
  - IV: liver or bone marrow involvement or extensive involvement of another extralymphatic organ

- Benign, reactive lymphadenopathies → rxn pattern due to immune stimulus; pathological pattern relates to type of stimulated cell; normal nodal architecture is preserved; most common cause of lymph node enlargement
  - Follicular hyperplasia
    - Proliferation of B lymphocytes
    - Increased need for Ab production
    - Enlargement of germinal centers with tingible-body macrophages and increased mitotic activity
    - Eg: strep throat → enlarged nodes
  - Paracortical hyperplasia
    - Proliferation of T lymphocytes
    - Increased need for cell-mediated immunity
    - Expansion of paracortical regions with increased mitotic activity and activated lymphocytes
    - Eg: enlarged neck nodes in adolescents with mono
  - Sinus histiocytosis
    - Proliferation of macrophages
    - Stimulation of antigen-presenting cells
    - Expansion of subcapsular and medullary sinuses
    - Eg: lymph nodes draining carcinomas

- Non-Hodgkin Lymphomas
  - Malignant clonal diseases of lymphocytes
  - NOT stem cell disorders
  - WHO classification – based on maturity of cell – for pathologic classification
    - Precursor B-cell neoplasm
      - Precursor B-cell lymphoblastic leukemia/lymphoma
    - Mature B-cell neoplasms
      - Small lymphocytic lymphoma
      - Follicular lymphoma
      - Diffuse Large B-cell lymphoma
      - Burkitt’s Lymphoma/leukemia
    - Precursor T-cell neoplasm
      - Precursor T-cell lymphoblastic leukemia/lymphoma
    - Mature T-cell neoplasms
  - Working formulation – for clinical classification
    - Low grade (medial survival 5-10 yrs)
      - Asymptomatic at presentation
      - Most present at high stage
      - Incurable
      - Older patients
      - Lymphomas
        - Small lymphocytic (SLL)
        - Follicular small cleaved cell (FSCL)
    - Intermediate grade (2-5 yrs)
      - Rapidly fatal if untreated
      - Present with symptoms
      - More likely to present at low stage
      - Potentially curable

<table>
<thead>
<tr>
<th>Lymphomas</th>
<th>H&amp;O Grouping</th>
<th>Working Formulation</th>
<th>Tumor Histology</th>
<th>Translocation</th>
<th>Prog- Oncogene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Lymphocytic (CLL)</td>
<td>CD5+</td>
<td>follicular</td>
<td>low grade</td>
<td>t(14:18)</td>
<td>BCL2</td>
</tr>
<tr>
<td>Follicular (FL)</td>
<td></td>
<td>diffuse large B-cell</td>
<td>intermediate grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burkitt’s Lymphoma</td>
<td></td>
<td></td>
<td>high grade</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• Age varies
• Lymphomas
  o Diffuse large cell (DLCL)
    • High grade (1/2-2 yrs)
      • Rapidly fatal if untreated
      • Present with symptoms
      • More likely to present at low stage
      • Potentially curable
      • Age varies
    • Lymphomas
      o Lymphoblastic (LL)
      o Burkitt (SNCCCL)
  o Small Lymphocytic Lymphoma (= CLL)
    • Tumors comprised of small, round lymphocytes
    • Occur in older adults
    • Mature B cell
      • CD19+, CD20+, CD22+, CD23+
      • Co-expressing CD5+
      • Kappa or lambda light chain
    • Behave like low grade lymphomas → incurable
    • Many present without symptoms and are treated palliatively
  o Follicular Small Cleaved Cell Lymphoma — You got BCL2 (18) on my heavy chain (14).
    • From the follicle
    • Low grade
    • Many years (5-10 yrs)
    • Middle-aged to elderly adults
    • Most common type of NHL
    • Morphologically similar to normal germinal center cells
    • Mature B-cell immunophenotype
      • CD19+, CD20+, CD22+, CD10+
      • Kappa or lambda light chains
    • Characteristically assoc with the t(14;18) → translocates bcl-2 proto-oncogene downstream of immunoglobulin regulatory sequences (over expression of bcl-2, which inhibits apoptosis)
    • Incurable
    • Often asymptomatic
    • Patients typically present with high stage disease (including bone marrow involvement) → III/IV stage
    • May progress to diffuse large cell lymphoma
    • Treatment: palliative
  o Diffuse Large Cell Lymphoma — What happened to my BCL6?
    • From the follicle
    • Intermediate grade
    • A few years (2-5 yrs)
    • 2nd most common type of NHL
    • 30% of DLCL assoc with abnormalities in BCL6; many have BCL2 as well
      • BCL6 often on non Ig genes
      • BCL6 indicates a better prognosis
      • BCL2 does not
    • Adults and children
    • Can be assoc with abnormalities of immune function → HIV, SCID, BM and organ transplants
    • Approx 1/3 of cases present in extranodal site (GI, brain)
    • Curable (20-80%) – low stage disease
      • Determining prognosis: IPI score
        • > normal LDH, Age > 60, stage III, IV; > 1 extranodal site; performance status ≥ 2 → add one pt per item
Low risk = 0, high risk = 3 to 4 → 5 yr survival decreases from 75% to 25%

- Sometimes asymptomatic
- Treatment: R-CHOP +/- XRT
  - Stage I, II: RADIATION + chemo
  - Stage III, IV: CHEMO + radiation
  - Rituximab
    - Chimeric monoclonal anti-CD20 Ab
    - Active as a single agent against low grade lymphomas
    - Particularly effective against BCL2 expressing lymphomas

**Burkitt’s Lymphoma** (ALL FAB L3) – You got MYC (8) on my Ig (2, 14, 22).

- Aggressive
- High grade
- Months (0.5-2 yrs)
- Includes tumors endemic in Africa (BL) and similar sporadic tumors occurring world-wide (SNCCCL)
  - Endemic: >95% EBV genome positive
  - Non-endemic: 15-20% EBV positive
- Predominantly children
- t(8;14) translocates c-myc proto-oncogene downstream of Ig heavy chain regulatory sequences
  - myc is a transcription factor → drives proliferation and directly transactivates LDH (elevates LDH)
  - can also have t(2;8) and t(8;22)
- Mature B cell phenotype
- Morphology
  - Macrophages with debris form “starry sky”
  - Characteristic cytoplasmic vacuoles
- Curable
- Usually symptomatic
- Treatment: like ALL

**Lymphoblastic Lymphoma**

- Lymph node correlate of ALL
- Diseases of children
- Composed of lymphoblasts with similar phenotypes to ALL
- Immunophenotype most often that of an immature T-cell
  - CD3+
  - Co-expressing CD4 and CD8
  - TdT
- Treated similarly to ALL
- BUT LL is more likely than ALL to occur in teenagers and to present with mediastinal mass

**Hodgkin’s Lymphoma**

- Malignant neoplastic disease of lymph nodes characterized by a distinctive cell (Reed-Sternberg cell)
  - RS cell is an activated B cell
  - RS cell has big nucleoli (2 eyes); big size
- Pathologic subtyping of HD
  - Morphologic diagnosis based on finding RS cells in appropriate cellular bkgd
    - Lymphocytes
    - Eosinophils
    - Histiocytes
    - Fibroblasts
  - Morphologic subtypes correlate with prognosis
    - Classical HL
      - Nodular sclerosis – good prognosis
      - Lymphocyte rich – very good prognosis
      - Mixed cellularity – intermediate prognosis
  - Hodgkin’s Disease
    - Nodular sclerosis – good prognosis
    - Lymphocyte rich – very good prognosis
    - Mixed cellularity – intermediate prognosis
  - Diffuse large cell/Hodgkin’s disease: Curative (R-CHOP)
    - Burkitt’s: Curative (ALL treatment)
PLASMA CELL DYSCRASIAS

- Disorders of immunoglobulin producing plasma cells
- Incidence of patients with monoclonal protein detected on serum protein electrophoresis and immunofixation rises with age
- May be related to H. pylori \( \rightarrow \) resolves with proton pump blockade and antibiotic therapy
- Disease of adults
- Differential Diagnosis
  - Monoclonal gammopathy of undetermined significance (MGUS)
  - Amyloidosis
  - Lymphoma, esp indolent NHL (follicular)
  - Chronic lymphocytic leukemia
  - Solitary plasmacytoma
  - Waldenstrom’s macroglobulinemia
  - Multiple Myeloma
  - Bony lesions + hypercalcemia
    - Metastatic solid tumor, esp lung, breast, head/neck, renal cell, prostate
    - Other hematologic malignancy, esp NHL and HTLV-1-assoc adult T-cell leukemia/lymphoma

**Multiple Myeloma**

- Malignancy that arises due to clonal, neoplastic proliferation of plasma cells, most commonly in BM
- 2\textsuperscript{nd} most common hematologic malignancy and 9\textsuperscript{th} leading cancer-related cause of death in women
- Median age = 65
- Female: male = 1.3:1.0
- Incidence of myeloma in African American pop is about twice that in the Caucasian population
- Etiology
  - Vast majority of cases remain largely unknown
  - Factors that may play causative roles include
    - Radiation exposure
    - Occupational exposures (agricultural, chemical, and pulp workers, leather tanners)
    - Exposure to certain chemicals (benzene, formaldehyde, hair dyes, paints, asbestos)
- Clinical and pathological findings consequence of either:
  - Tumor mass effect of the malignant plasma cells proliferating in the bone marrow
  - Abnormal secretory products from the malignant plasma cells including monoclonal immunoglobulins and cytokines
- Pathogenesis
  - Studies hampered b/c plasma cells often have a low proliferative activity, making routine karyotyping/cytogenetics difficult
  - FISH – up to 80% of patients or more have some chromosomal abnormalities
    - Chromosome 13 – poor prognosis
    - t(11;14) or t(4;14) – cyclin-D cell-cycle control protein \( \rightarrow \) over-expression of D1
    - can over-express c-Myc protein \( \rightarrow \) drives cells thru cell cycle
    - can over-express Bcl-2 protein \( \rightarrow \) protects them from apoptosis and chemo-mediated cell death
  - IL-6 – essential for growth of myeloma cells \( \rightarrow \) promotes survival
- Clinical presentation
  - Many symptoms are non-specific \( \rightarrow \) fatigue, dyspnea
- Up to 20% of patients are asymptomatic at presentation → diagnosed only on basis of routine studies (protein/albumin discordance)

### Symptoms
- Bony disease → lytic lesions, rib/bony fractures, bony pain
  - Cytokines induce increased OSTEOCLAST activity
- Anemia with assoc fatigue, dyspnea, pallor → generally normocytic and normochromic (suggesting anemia of chronic disease)
  - Formation of RBC rouleaux on peripheral blood smear suggests paraproteinemia
  - Marrow infiltration with plasma cells
- Renal insufficiency → often caused by presence of Bence Jones protein casts (distal tubules)
  - BJPs = monoclonal free light chains in urine → can accumulate in distal tubules and cause nephropathy
  - Can be exacerbated by dehydration, hypercalcemia, and hyperuricemia
  - Amyloidosis
- Hypercalcemia → constipation, confusion, polyuria
  - Generally due to bony resorption and decreased mobility → progressive bony destruction
  - Exacerbated by renal failure
- Recurring infections → Strep pneumo, H. flu
  - Likely the result of hypogammaglobulinemia, granulocytopenia, impaired cell-mediated immunity
- Neurologic involvement
  - Nerve root compression
  - Peripheral neuropathy (esp with amyloidosis)

### Diagnostic approach
- Tests to do
  - CBC with differential + Platelet count + smear
  - Calcium and creatinine levels
  - LDH, C-reactive protein, β2-microglobulin (useful prognostically)
  - Serum protein electrophoresis (SPEP) with immunofixation and quantitative immunoglobulins → to check for IgG, IgA, IgM
  - 24 hour urine collection (UPEP) with immunofixation → to check for kappa and lambda serum free light chains + creatinine clearance
  - Myeloma bony survey → risk for pathologic fracture?
  - Bone marrow aspiration and biopsy with cytogentic and FISH

### Findings
- Major criteria
  - Presence of biopsy-verified plasmacytoma (local infiltration of plasma cells in a tissue compartment outside BM)
  - BM plasmacytosis >30%
  - Presence of serum monoclonal protein (one of the options)
    - > 3.5 g/dL = IgG
    - ≥ 2.0 g/dL = IgA
    - ≥ 1.0 g of kappa or lambda light chains in 24 hour urine collection (normal = mixed)
- Minor criteria
  - BM plasmacytosis of 10-30%
  - Monoclonal serum protein in lower amts than indicated as major criterion
  - Lytic bony lesions
  - Depressed normal Ig with
    - IgM < 50 mg/dL
    - IgA < 100 mg/dL
    - IgG < 600 mg/dL
  → diagnosis of multiple myeloma is made if
  - 2 major criteria
    - One major + one minor criterion are present (major + BM of 10-30% is not diagnostic)
    - 3 minor criteria (as long as 2 of these include marrow plasmacytosis and serum monoclonal protein
- International working group criteria → relies solely on disease related symptoms
  - Serum and/or urine monoclonal protein
  - Clonal marrow plasmacytosis of at least 10%
  - Evidence of end-organ damage → one or more of following
Anemia
Bony lesions
Hypercalcinia
Renal insufficiency due to paraprotein (Bence Jones Protein)
Other: recurrent bacterial infections, amyloidosis, hyperviscosity

→ symptomatic multiple myeloma diagnosis requires all criteria
→ patients who meet 1st two criteria with monoclonal protein of at least 3.0 g/dL, but have no evidence of organ damage would have “asymptomatic multiple myeloma”

○ Staging → determine extent of disease
  ▪ MD Anderson Cancer Center
    • Stage I (low myeloma cell mass, with < 0.6 x 10^12 cells/m2 of BSA)
      o Hb > 10.5 g/dL, corrected serum Ca ≤ 11.0 mg/dL, and serum monoclonal protein < 4.5 g/dL
    • Stage II (intermediate myeloma cell mass, with 0.6-1.2 x 10^12 cells/m2)
      o Hb and Ca that don’t fit I or III
    • Stage III (high myeloma cell mass, with > 1.2 x 10^12 cells/m2)
      o Hb < 8.5 g/dL, corrected serum Ca > 11.5 mg/dL
    • Subclass → based on serum creatinine
      o < 2.0 mg/dL → A
      o ≥ 2.0 mg/dL → B
  ▪ International Working Group → serum albumin and serum β2-microglobulin → more accurate for prognosis
    • Stage I (β2-microglobulin < 3.5, albumin ≥ 3.5) → median survival = 62 mts
    • Stage II (other than I and III) → median survival = 44 mts
    • Stage III (β2-microglobulin ≥ 5.5) → median survival = 29 mts

○ Management and Therapy
  ▪ Stage I + asymptomatic + no poor prognostic features → watchful waiting
  ▪ Induction therapy
    • Oral alkylating agents with steroids (MP/melphalan + prednisone)
      o Generally well tolerated
      o Modest overall response rate of 55% + Risk of myelodysplasia and 2nd leukemia → generally not used for patients who have a stem cell transplant as an option
      o Reserved for older patients who have poor organ function and/or poor performance status and cannot tolerate more aggressive therapy
      o VELCADE –
        ▪ Higher overall and complete response rates; under study
        ▪ Ubiquitin-proteasome pathway, responsible for protein degradation; inhibited by bortezomib (VELCADE®)
        ▪ Inhibits p44/42 MAPK, NF-kB
        ▪ Activity against multiple myeloma in Phase I & II trials ⇒ FDA approval for relapsed/refractory disease
    • Combination of thalidomide and dexamethasone
      o Oral admin
      o Excellent response rate with lack of damage to stem cells → comparable to MP and VAD
      o Side effects of thalidomide (somnolence, constipation, neuropathy, increased risk of DVT and PE)
    • IV chemotherapy (VAD)
      o Continuous infusion over 4 days
      o Onset of action more rapid than with MP → used in patients with more adv disease who might be developing renal insufficiency
  ▪ Cannot be cured with std chemo → Peripheral blood stem cell transplantation (PBSCT)
    • Undergo therapy after completed induction and hopefully achieve a state of minimal residual disease
  ▪ Autologous transplantation
    o Most common type used
    o Allows patients to receive higher doses of chemo and then rescues BM by re-infusion of their own stem cells that were collected prior to high-dose therapy
    o Not curative → generally some contamination of collected stem cells with residual plasma cells and even high-dose chemo does not destroy all of patient’s abnormal plasma cells
- Provides a longer relapse-free and overall survival compared with continued std chemo alone
- Allogeneic transplantation
  - HLA-matched sibling as donor
  - Advantage of graft-vs-tumor immunologic rxn but also have higher risk of complications
- Min-transplants
  - Donor is not fully “ablated” but receives milder doses of chemo that only suppresses marrow, after which stem cells from a related donor are infused
  - Goal – create chimera with some recipient and some donor cells present → decrease toxicity compared with allogeneic transplant but improve immune rxn against tumor compared with autologous transplant
  - Still investigational
- After transplantation, some patients placed on a maintenance therapy
- Relapse is inevitable
- Supportive care plays an important role
  - Bisphosphonates –
    - Treat hypercalcemia → decrease incidence of bony fractures
    - May directly inhibit myeloma cell growth
    - Risks = renal insufficiency + osteonecrosis of jaw
  - Kyphoplasty and/or vertebroplasty may help alleviate pain in patients with vertebral compression fractures
  - Erythropoietin to simulate RBC production
  - G-CSF to raise neutrophils and prevent infections (?)
  - Immunizations with pneumonia and influenza vaccinations
  - Prophylactic antibiotics (?)
- MGUS – Monoclonal Gammapathy of Uncertain Significance
  - Almost 2/3s of patients with a monoclonal band on serum protein electrophoresis and immunofixation
  - Incidence increases with age
  - Broadly defined as serum monoclonal protein of < 3.0 g/dL, and the absence of a urinary monoclonal protein, lytic bony lesions, anemia, hypercalcemia, or renal insufficiency
  - Protein/albumin discordance
    - Normal total protein is approx 2 times albumin
    - Discordance – total > 2 times albumin
  - Only 25% will progress to multiple myeloma
  - No therapy indicated
  - H. pylori infection → treatment → resolution of monoclonal protein
- Waldenstrom’s Macroglobulinemia
  - Malignancy of mature, plasmacytoid lymphocytes (lymphoplasmacytic morphology)
  - Involves clonal proliferation of plasmacytoid lymphocytes (mature B cell phenotype) which secrete IgM
  - Low grade lymphoma with infiltrates in marrow, lymph nodes, spleen
  - Disease of adults, more common in men
  - Hyperviscosity → IgM stays in intravascular b/c pentameric
    - Manifestations
      - Neurologic symptoms – dizziness, headaches, visual changes, decreased consciousness, retinal vein engorgement, papilledema
      - Cardiopulmonary symptoms – CHF
      - Bleeding diathesis, esp with epistaxis
  - Diagnosis - serum viscosity determination; SPEP (monoclonal IgM spike)
  - Treatment – plasmapheresis with exchange + chemo
- Amyloidosis
  - Complication of up to 10% of patients with multiple myeloma
  - Can also be found in the absence of myeloma → primary and familial types
  - Characterized by organ deposition of amyloid fibrils, often with displacement and effacement of normal tissue architecture
  - Clinical features
    - Nephritic syndrome
    - Cardiomyopathy
    - Periorbital purpura
    - Hepatomegaly
    - Neuropathy
    - Macroglossia
Carpal tunnel syndrome

Diagnosis
- Rectal biopsy or subcutaneous fat pad aspiration
- Congo Red stains –
  - Stain – if positive, peach color
  - Polarized light \( \rightarrow \) amyloid appears greenish-yellow

Treatment
- MP chemo
- Stem cell transplantation
- But most patients do not benefit and prognosis is poor

**Pediatric Tumors (Neoplasia)**

- 1/1000 adults at age 21 are survivors of childhood cancer \( \rightarrow \) 20 yr cumulative probability of developing 2\(^{nd}\) malignancy is 5-15% for survivors, depending on initial diagnosis and treatment
- Primary tumor sites in children are most often derived from cells in hematopoietic, neural, and lymphatic Tissues, while common adult tumors tend to arise from epithelial cells of discrete Organs (carcinomas are rare in children)
- Tumors of children are more likely to be assoc with inherited genetic abnormalities than are adult tumors (many of the genes are suppressor genes)
- Factors contributing to development of childhood malignancies
  - Viral infections (EBV)
  - Inherited and acquired immunodeficiencies (NHL)
  - Germ-line and somatic mutations in tumor suppressor genes (retinoblastoma)
  - Amplification of proto-oncogenes (N-myc in neuroblastoma)
  - Ionizing radiation (brain tumors, osteogenic sarcoma as 2\(^{nd}\) malignancies)
  - Prior chemotherapy (2ndary leukemia)

Common presentations of childhood cancer
- Fever
- Bleeding, bruising
- Headaches, neurologic changes, seizures
  - Ocular and orbital signs – leukocoria (white reflex, instead of red), strabismus, orbital and periorbital soft tissue masses
- Lymphadenopathy
- Abdominal and pelvic masses – with or without pain
- Bone and soft tissue masses/pain

Common themes in peds malignancies
- Loss of tumor suppressor genes
- Rapidly fatal if untreated

**Small Round Blue Cell Tumor** morphology
- Primitive cell of origin
- Uniform round cell population
- High N:C ratios
- Hyperchromatic nuclei

Distribution of childhood cancers
- Leukemia (30%) – mostly ALL
- Brain tumors (20%)
  - Majority of brain tumors in infants and children are infratentorial
  - Subtypes
    - Supratentorial astrocytoma
    - Medulloblastoma
    - Cerebellar astrocytoma
    - Brainstem glioma
    - Ependymoma
    - Other
  - Treatment
    - Low cure rates
    - Multidisciplinary approach
      - Surgical management improving
      - CNS radiation – extremely toxic < age 3 years
      - Chemotherapy – must cross blood-brain barrier
      - Supportive care – corticosteroids to decrease edema; treat seizures
- Lymphoma (14%)
- **HD**
  - Rare in children < 10; fairly common in teens, incidence rises with age to peak in mid 20’s
  - Frequently presents are cervical/supraclavicular/mediastinal lymphadenopathy
  - Nodular sclerosing (teenagers) and mixed cellularity (children) most common subtypes
  - Less likely to use extended field radiation therapy in children

- **Non-HD**
  - High grade NHL’s are much more common in childhood than indolent, low grade lesions
  - Lymphoblastic, small non-cleaved cell, large cell
  - Similar considerations to diagnosis and treatment of ALL
    - Spectrum of disease (can involve BM and CSF)
    - Flow cytometric analysis of surface antigens
    - Cytogenetic and molecular analyses
    - Primary treatment is chemo, similar drugs

  - **Solid tumors**
    - High stage tumor = bad
    - Usually treated with goal of minimizing late effects

  - **Subtypes**
    - **Neuroblastoma**
      - Infants, children (85% of cases in children < 5 yrs old)
      - Derived from neural crest cells that normally form the sympathetic nervous system
      - Originate in sympathetic ganglia or adrenal medulla (40%)
      - > 90% secrete catecholamines
      - Diagnostic test: urine catecholamines often elevated
      - Smear: primitive small round undifferentiated cells, Homer-Wright rosettes
      - Widespread necrosis and dystrophic calcification common; metastases common; **infiltrative** (tend to cross midline, are firm/fixed)
      - Most important prognostic factors are
        - Age (children < 1 yr do best)
        - **Amplification of N-myc oncogene in tumor** (negative correlation with survival)
          - Stage II, no N-myc amplification
            - Surgical resection (even partial)
            - No chemo, no radiation
            - 2 yr DFS – 90%
          - Stage II, N-myc amplification
            - Surgical resection
            - Intensive chemo
            - Local irradiation
            - 2 yr DFS < 50%
      - Site (extra-adrenal tumors tend to be better differentiated and have better prognosis)
      - Stage (inversely correlates with survival, except IVS – can have spontaneous remission)
      - Grade (better differentiated tumors have better prognosis)

    - **Rhabdomyosarcoma**
      - Children → most frequent soft tissue sarcoma
      - Biphasic incidence (1st peak b/f age 5 (embryonal type predominant) and 2nd peak involving adolescents (embryonal and alveolar predominant)
      - Malignant tumor arising from primitive mesenchymal cells with striated/skeletal muscle differentiation (form Z-bands)
      - Most patients present with palpable mass and/or symptoms with mass
      - Most common sites are head and neck, genitourinary tract, extremities and trunk
      - Eval and treatment complex, site and stage specific → involves surgery, radiation, and chemo
      - Prognosis generally favorable
        - Extent of disease is most important
        - Alveolar has worst prognosis

    - **Wilm’s tumor**
      - WT1 or WT2 on chromosome 11 (loss of tumor suppressor genes)
      - Infants, children
      - Malignant neoplasm of embryonal nephrogenic (renal) elements
      - Tumor tends to be large, soft, encapsulated
TRIPHASIC TUMOR composed of
- BLASTEMAL – primitive small ovoid blue cells
- STROMAL – fibroblast-like cells, sometimes skeletal muscle, smooth muscle, cartilage, bone, fat, etc
- EPITHELIAL TISSUE - tubules

Most common pediatric renal tumor

Clinical presentation
- Patients usually asymptomatic → present with large, unilateral abd mass
- Hypertension
- Fever
- Abd pain
- Hematuria

Metastasis is uncommon (contrast to neuroblastoma)

Assoc with loss of tumor suppressor genes
- Sporadic (non-familial)
  - Most common form
  - Usually unilateral
  - Somatic cells have 2 copies
  - Acquired loss of both copies of suppressor gene in tumor
- Congenital
  - Patient born with one copy
  - No copy of gene in tumor
  - Tumor more likely to be bilateral/multifocal → probably set of tumor suppressor genes on 11p accounts for heritable fraction of patients

Tumor staging
- Stage I: tumor limited to kidney, capsule intact, complete resection, vessels of renal sinus not involved
- Stage II: tumor extends beyond kidney; capsule penetrated, but still a complete resection
- Stage III: residual non-hematogenous tumor in abd
- Stage IV: hematogenous metastases (lung, liver, bone, brain) or lymph node metastases outside abd/pelvis
- Stage V: bilateral renal involvement

Treatment – successful use of adjuvant chemo and multidisciplinary approach
- Surgical resection, chemo, radiation
- Current cure rate is approx 90% for low stage disease

Bone sarcomas
- Adolescents
- Subtypes
  - Osteosarcoma
    - RB1, p53 (loss of tumor suppressor genes)
    - Highly malignant bone tumor characterized by formation of malignant bone tissue → malignant osteoblastic cells producing woven bone or osteoid
    - Most common sites are around knee (distal femur of proximal tibia) or around humerus → 75% arise near knee or shoulder
    - Radiologic features: permeative, destructive, lytic/blastic, Codman’s triangle, freq soft tissue extension
    - Spreads thru bloodstream, freq to lungs → almost all patients die with lung mets
  - Ewing’s sarcoma
    - Peak incidence is 10-20 yrs old (similar to osteosarcoma)
    - Long bones are commonly involved but also pelvis
    - Small round blue cell tumor arranged in sheets, nests, and organoid patterns
    - Patients with metastatic disease do poorly
    - Treatment by surgery, chemo, radiation

Retinoblastoma
- Ocular symptoms in infants, children
- RB1 (loss of tumor suppressor genes)
- Most common malignant eye tumor of childhood
- Neoplasm arising from immature neurons of retina
- May be familial (diagnosed at early age + multiple tumors more likely) or sporadic (>90%)
- Unilateral (80%) or bilateral
  - With bilateral → mutations in tumor suppressor gene on 13q
- Cream colored tumor with scattered chalky white calcified flecks within necrotic zones
- Small round blue cell morphology + Flexner-Wintersteiner rosettes
- Very high risk of 2nd malignancies → esp those children with inherited retinoblastoma (malignancies like osteogenic sarcoma)
- Treatment: enucleation, cryotherapy, radiation