Overview: Today’s Take-away

• Understanding of:
  - Cell phenotype and clinical presentation of CLL
    - Diagnosis
    - Disease course
  - Population of CLL patients
    - Risk factors for CLL
  - Current treatments for CLL
  - Typical gene changes and cytogenetic rearrangements seen in CLL and their prognostic and treatment indications

Chronic Lymphoid Leukemia

• AKA B-cell Chronic Lymphocytic Leukemia
  - Accumulation of long-lived mature monocolonal B lymphocytes
  - Accumulate in marrow and blood and overtake healthy blood cells
  - CLL is a stage of small lymphocytic lymphoma (SLL) which presents in the lymph nodes
Patient Population
• Most common form of leukemia found in adults in Western countries
• 30% of all leukemias in USA
• 16,000 new cases per year in the United States
  - 4,580 deaths per year*
• Generally adult onset†
  - Median diagnosis 72 years
  - Rarely seen in younger patients, >2% younger than 45 at diagnosis
  - Twice as frequent in men as women
  - Extremely rare in children
• Survival‡
  - Median survival of Rai stage 0 is ~12 years
  - Stages II-IV rates of 5-8 years
  *Siegel et al., CA: Cancer Clinicians, (2012), 62: p10-29
  †Shanshal and Haddad, Dis Mon 2012, 58:153-167
  ‡Rai et al., Blood. 1975; 46:219-234

Contributing Factors and Causes
• Increased age
• Exposure:
  - no known association with radiation, alkylating agents or leukemogenic chemicals
  - Herbicides and pesticides linked to higher risk of CLL
• Familial Risk
  - Family members of patients with CLL have 2-7X higher risk of developing CLL
  - 5% of patients report family history of leukemia*


Clinical Presentation of CLL
• ~25% of patients present with no symptoms, with a routine CBC revealing lymphocytosis
• Many patients consult physician due to pain-free swelling of lymph nodes
• Other presenting symptoms
  - Unintentional weight loss
  - Extended fevers for greater than 2 weeks
  - Night sweats
  - Extreme fatigue
  - Immunodeficiency disorder-like symptoms
Diagnosis of CLL

- Often discovered initially after CBC performed for another reason*
- Complete blood count (CBC)
  - 30-50x10⁹/L lymphocytosis
- Peripheral blood smear to confirm
  - Presence of smudge cells
- Flow cytometry detecting circulating clonal B-lymphocytes expressing CD5, CD19, and CD20
- Low mitotic rate
  - Traditional karyotyping tends to fail
  - FISH relatively successful

*Shanshal and Haddad, DM, April 2012

Rai system and the Binet system for Staging of CLL

- Rai classification defines disease based upon lymphocytes with leukemia cells in the blood and/or marrow.
- Binet Staging is based on the number of involved areas as defined by the presence of enlarged lymph nodes.

Disease Course

- Lymphadenopathy
  - Vary in size, non-tender
- Splenomegaly, Hepatomegaly
- Infiltration of skin with CLL cells
- Anemia
- Invasion of CLL to other tissues
  - Disruption of function of organs
Drug Treatment for CLL

- Chemotherapy not needed until patients become symptomatic or display progression
- Chemotherapy – several regimens used
  - Nucleoside analogues, alkylating agents, and biologics, often in combination
  - Combinations show higher response rates and longer survival than single agents
    - Initial often fludarabine, cyclophosphamide, rituximab (FCR)
- Lenalidomide*
  - Immunomodulatory drug shows response in studies


Refractory CLL

- CLL that no longer responds favorably to standard treatment
- Stem cell transplant
- Inhibitor and monoclonal antibody therapies
  - Alemtuzumab – p53 patients†
  - Bruton’s tyrosine kinase (BTK)-inhibitor (ibrutinib) showing some success in CLL*
  - Ofatumumab
  - Anti-CD20 monoclonal approved by FDA for treatment of patients with CLL refractory to fludarabine and alemtuzumab‡
  - Lumiliximab
  - Anti-CD23 antibody investigated in Phase I and II trials for CLL¥

*Bird et al., NEJM, June 19, 2013

Gene and Chromosomal abnormalities associated with CLL

- Poor Prognosis
  - CD38 expression
  - Lack of IgVH mutations
  - ZAP-70 expression
  - NOTCH1 mutations
  - 17p deletions
  - Mutations in genes on 17p
  - 11q deletions
  - 6q deletions
- Intermediate Prognosis
  - Trisomy 12
- Favorable Prognosis
  - del(13)(q14)
Additional CLL anomalies of note

- t(11;14)
- BCL2
- c-Myc

CD38 expression

- A trans-membrane protein initially developed as an identification marker for T-cells
- Regulator of intracellular calcium levels
- Major role in B cell growth and survival
- >30% CD38 expressing cells by flow cytometry are "CD38+"
  - indicates poor prognosis and aggressive disease

IgVH Gene

- Immunoglobulin variable region heavy chain
  - Variation in immunoglobulin proteins
- <98% homology to germline is considered mutated
  - Conversely, >98% homology to germline is considered unmutated
    - Lack of mutation informs clonality and lack of appropriate mutation variation
- LACK of IgVH gene mutations associated with advanced and progressive disease*
  - Worsened survival

*Hamblin TJ et al., Blood 1999;94:1848-54.
ZAP-70 Expression

• Gene involved in T-cell receptor intracellular signaling
• Expression associated with lack of IgVH mutations
  Patients with >20% ZAP-70-positive cells had a worse prognosis compared with those having <20% ZAP-70-positive cells*
  - Increased risk for adverse outcome
• Flow cytometry, RT-PCR, staining techniques


NOTCH1 mutations

• Signaling protein on the surface of CLL cells
• Activating mutations of the NOTCH gene in ~10% of CLL patients at diagnosis*
• Reduced overall survival, increased disease aggressiveness

*Del Guidice et al, 2012, Haematologica

del(17p), del TP53, or mutated TP53

• TP53 key tumor suppressor gene
  - Cell cycle checkpoint
  - Halt for repair
  - Signal apoptosis for non-repaired cells
• Poor prognosis
• Resistance to therapy with alkylating agents and purine analogues*
• Responsive to alemtuzumab†
• Not responsive to rituximab

*Bird et al., NEJM, June 19, 2013
11q deletion/ATM

- Deletion of the ATM gene
  - DNA repair gene involved in Bloom, FA, and RAD51 repair pathways
- Loss of 11q predicts reduced survival times*
- Often associated with bulky nodes without high blood counts


6q deletions*

- 6q21 deletion is associated with an intermediate to poor prognosis†
  - with cMyc amplification, this deletion indicates a poor prognosis‡
- 6q23 (MYB) deletions present in significant percentage of CLL patients¥
  - Associated with transition to PLL (Prolymphocytic Leukemia), a more aggressive form of the disease

*Lawce and Olson, J Assoc Genet Technol, 2009
†Cuneo et al., Leukemia, 2004.
‡El Gendi et. al., J of App Hemat., 2012
¥Qui et al., Leuk & Lymph, 2008.

Trisomy 12

- Common cytogenetic finding
  - Intermediate prognosis
- Significant fraction have NOTCH mutation*
  - Prognosis for trisomy 12 may eventually rely on NOTCH status

*Del Guidice et al, 2012, Haematologica
13q14

- del(13)(q14.3) is associated with a favorable survival
  - Median prognosis 133 months*
- Most frequent chromosomal anomaly in CLL
- Monoallelic deletions are present in ~70% of patients
- Biallelic and mosaic monoallelic-biallelic deletions affect ~19% of patients


Risk-Stratification of CLL

- Poor Prognosis
  - CD38 expression
  - Lack of IgVH mutations
  - ZAP-70 expression
  - NOTCH1 mutations
  - del(17p)
  - 11q deletions
  - 6q deletions
- Intermediate Prognosis
  - Trisomy 12
- Favorable Prognosis
  - del(13)(q14)

t(11;14)

- Translocation
  t(11;14)(q13;q32)
  - IGH-CCND1 fusion
  - Seen in mantle cell lymphoma
- Atypical subset of CLL
- Frequent cytologic and cytogenetic evolution
- Shares features with mantle cell lymphoma*

*Cuneo et al., Br J Haem, 1995
**BCL2**

- Anti-apoptotic protein
- High levels of BCL2 expression is a hallmark of patients with CLL
  - Can be translocation or mutation-based
  - Has lead to work looking at BCL-targeted therapies
- Translocation often t(14;18)(q32;q21)(IgH/BCL2)
  - Also found often in follicular lymphoma
- 938C>A mutation in BCL2 promoter associated with high non-translocation expression and adverse prognosis*
- Higher levels of BCL2 expression found in patients with progressive disease†

*Majid et al., Blood, 2008
†Marschitz et al., Am J Clin Pathol, 2000

**C-MYC**

- Gene maps to 8q24 and encodes a nuclear transcription factor which plays a critical role in regulation of several cellular functions
  - Dysregulation of c-Myc is present in transformation of many lymphoid proliferative disorders
- c-Myc amplification associated with aggressive disease and poor prognosis*


**Prognosis for CLL**
Therapy Selection for CLL

Accurate assessment of biomarkers and chromosomal abnormalities is essential in establishing prognosis, and determining course of treatment in patients with CLL.

CLL cell deleted for TP53