CLL/SLL

Updates and other things you should know

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ARUP Laboratories
Outline

- Background
- Diagnosis
- Prognostic Testing
- Theranostic Testing
Learning Objectives

• Understand the process of diagnosing CLL/SLL
• Differentiate between diagnosis, prognosis, and theranosis.
• Recognize interference of assays in the setting of targeted therapies.
Introduction

• CLL vs SLL
  • Chronic Lymphocytic LEUKEMIA
  • Small Lymphocytic LYMPHOMA

• Same disease, different locations

• Very common
  • 21,040 new cases per year in the US
    *Does not include monoclonal B cell lymphocytosis (MBL)

• Quite Indolent
  1. 85% 5 year survival
  2. Lots of people who live with the disease (clinical or subclinical)
Diagnosis

• SLL
  • Nodal involvement

• CLL
  • Bone marrow and Peripheral Blood Involvement
Morphology (Lymph Node)

- Effaced Architecture
  - Lighter zones with ‘pseudo’-proliferation centers
- Small cell infiltrate (small = normal resting lymphocytes)
- Soccer Ball like nuclear chromatin
- Scant Cytoplasm
- Occasional larger forms
H&E Images
Morphology (Smear)

- Smudge Cells
- Albuminated slide
  - Soccer ball like nuclear chromatin
Wright Giemsa Images (ASH image bank)
Conventional EDTA Smears

Albumminated vs EDTA cells

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Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Cellavision Images

Immunophenotype

- Retained CD19
- Decreased CD20
- Low to intermediate CD5 (can be less than or equal to background T cells)
- Decreased CD22, CD79b
- Aberrant CD200, CD23, CD43, loss of FMC7, decreased CD81

Immunohistochemistry

- LEF1+
- CyclinD1-negative (rare weak cells in proliferation centers)
Flow Cytometry

• Fastest and cheapest way to make the diagnosis of CLL

• What is flow cytometry?
  • Fluorescently labeled antibodies bound to single cells

Fluorescent Signal is proportional to quantity of target
It’s a toomah
Are Light Chains useful?

- Yes and No
- Light chains can be low to negative
Biclonal CLL
Diagnostic Criteria

• >5k/uL neoplastic lymphocytes
  • Morphology
    • Can be difficult to separate normal lymphocytes from neoplastic lymphocytes
    • Trivial if WBC is high (e.g. 30k WBC with phenotypic evidence is diagnostic for CLL)
  • Flow Cytometry
    • Accurate measurement of the % neoplastic cells/WBCs
    • Cell concentration is trickier
      • Bead Standards
      • Multiplication with WBC count from analyzer
What about cases <5k/uL

- Monoclonal B cell lymphocytosis (MBL)
  - Precursor to CLL
  - High Count >2.5k – 2% rate of transformation to CLL
  - Low Count <2.5k – <1% rate of transformation to CLL

*Some use MBL as a generic term for any small monoclonal B cell population with immunophenotypic abnormality (CD5-/CD10- B-NHLs) but for this talk, MBL means a CLL/SLL like population
Monoclonal B cell lymphocytosis

• Very Rare in young (<40 y/o)
  • 1/365 at 10⁻⁵ (0.01%) sensitivity

• Common as you get older
  • 20% incidence at 80 y/o

• Genetic linkage (family member with CLL -> 17x increase in incidence MBL)
Exclusion of other entities...

• CD5+ B-NHL
  • CLL/SLL
  • Mantle Cell Lymphoma
  • Marginal Zone Lymphoma (very rare)

• Exclude MCL with cyclinD1, SOX11, or t(11;14) FISH
  • NCCN Guideline
  • Misdiagnosing MCL as CLL/SLL is a bad thing
Natural History of Disease

- 20k diagnoses a year
- 5k deaths a year

Take home point: most people die with CLL/SLL not of CLL/SLL

*not to say CLL/SLL is a nice disease, there is significant morbidity (e.g. fatigue due to cytopenia)
How do things go south?

1. Marked leukocytosis >100k/uL

2. Disseminated lymphadenopathy

3. Richter transformation
   - 2-10% of CLL cases end up here

How do we predict who will die of disease?
What can we do to change that?
Prognosis
• Not all CLLs are created alike
• IgHV mutation status\(^1\)
  • Unmutated = Bad
  • Mutated = Good
• TP53
  • Wild type = Good
  • Mutated = Bad
• Karyotype
  • >=3 abnormalities in 1 cell) = Bad

• Cytogenetics\(^2\)
  • Del(17p) – bad
  • Del(11q) – bad
  • Del(13q) – good
  • Trisomy 12 – so-so
  • Normal – so-so

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)

Chronic Lymphocytic Leukemia/
Small Lymphocytic Lymphoma

Version 1.2021 — September 28, 2020

NCCN.org

NCCN Guidelines for Patients® available at www.nccn.org/patients
Stage vs Grade

• Stage
  • Degree of anatomic involvement correlated with worse outcome

• Grade
  • Morphologic Findings correlated with worse outcome
### Staging Systems for CLL

#### Rai System<sup>a</sup>

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Modified Risk Status</th>
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<tbody>
<tr>
<td>0</td>
<td>Lymphocytosis, lymphocytes in blood $&gt;5 \times 10^9$/L clonal B cells and $&gt;40%$ lymphocytes in the bone marrow</td>
<td>Low</td>
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<tr>
<td>I</td>
<td>Stage 0 with enlarged node(s)</td>
<td>Intermediate</td>
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<tr>
<td>II</td>
<td>Stage 0–I with splenomegaly, hepatomegaly, or both</td>
<td>Intermediate</td>
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<tr>
<td>III&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Stage 0–II with hemoglobin $&lt;11.0$ g/dL or hematocrit $&lt;33%$</td>
<td>High</td>
</tr>
<tr>
<td>IV&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Stage 0–III with platelets $&lt;100,000$ mm$^3$</td>
<td>High</td>
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#### Binet System<sup>b</sup>

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<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Hemoglobin $\geq 10$ g/dL and Platelets $\geq 100,000$ mm$^3$ and $&lt;3$ enlarged areas</td>
</tr>
<tr>
<td>B</td>
<td>Hemoglobin $\geq 10$ g/dL and Platelets $\geq 100,000$ mm$^3$ and $\geq 3$ enlarged areas</td>
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<tr>
<td>C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Hemoglobin $&lt;10$ g/dL and/or Platelets $&lt;100,000$ mm$^3$ and any number of enlarged areas</td>
</tr>
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<sup>c</sup> Immune-mediated cytopenias are not the basis for these stage definitions.
IgHV mutation status

Fig 1. Kaplan-Meier survival curve comparing CLL patients with mutated and unmutated V<sub>H</sub> genes. Median survival for unmutated CLL: 117 months; median survival for mutated CLL: 293 months. The difference is significant at the \( P = .001 \) level (log-rank test).
What is the Immunoglobulin Heavy Chain Variable region?

CAP today, May 2019
Somatic Hypermutation of the IgH locus

• Mutated
  • >2-3% difference in base pair sequence compared with germ line (of which there are many!)

• Methods
  • Sanger Sequencing
  • NGS
  • Bioinformatics
Cytogenetic Prognosis

Dohner et al.
Prognosis

• **Flow Cytometry**
  - ZAP-70 – associated with IgHV mutation status
    - Rarely done – difficult to do well
  - CD38 – associated with IgHV mutation status
    - Some studies suggest it may be an independent prognostic indicator
  - CD49d – independent prognostic indicator
    - Not in current guidelines

Prognosis cont

• Molecular (PCR) Findings
  • TP53 mutations – Bad
  • ATM (11q)
  • SF3B1
  • NOTCH1 – Bad
  • Formerly done as single gene assays, now done as part of NGS panel (40+ genes)

• Biochemical
  • Beta-2 microglobulin
Minimal Residual Disease

• Prognostic vs Predictive
  • I know you’ll do poorly but there is nothing I can do about it.
  • I know you’ll do poorly and I can treat you differently with good results.

• CLL MRD certainly prognostic, studies on going regarding predictive value
MRD Modalities

• Flow Cytometry
  • 0.01% to 0.001% Sensitivity (4 to 5 log)
  • Looks for an immunophenotypically aberrant population
  • Widely applicable
  • <24h turnaround time

• NGS
  • 0.0001% sensitivity (6 log)
  • Looks for same Ig sequence as original tumor
  • Only applicable if original tumor is also sequenced
  • 1-2 week turnaround (optimistically)
CLL MRD by Flow Cytometry

- ERIC (European Research Initiative on CLL) consensus panel
- CD45, CD19, CD20, CD43, CD81, CD5, CD79b
- Platform/reagent independent panel
- Shown to be reliable down to 5 log (0.001%)
- Collecting 5 million cells for analysis
- Peripheral blood based assay
CLL MRD by NGS

- ClonoSEQ assay by Adaptive Biotechnologies
- Send out to centralized facility, 1-4 wk turn around.
- Requires a priori knowledge of sequence
- Costs $1900
- Question of whether $10^{-5}$ vs $10^{-6}$ matters
Surrogate Endpoint

• Survivals for CLL are measured in years
• Can MRD be used to predict poor outcomes of therapies? Very important for drug trials!

• FDA approval of CLL MRD as a surrogate endpoint
Targeted Therapies

• CD20 – Rituximab
  • FCR – Fludarabine, Cyclophosphamide, Rituximab
  • CR – Chlorambucil, Rituximab

• Burton Tyrosine Kinase (BTK) Inhibitors
  • ibrutinib, Acalabrutinib
  • Useful even in high risk patients
  • Resistance mechanisms evolve in long treated patients

• Phosphoinositide 3-kinase (PI3K) inhibitors
  • Idelalisib

• BCL2 inhibitors
  • Ventoclax
What are the implication of targeted therapies?

1. Loss of target for detection of residual disease
   Do what is necessary to treat the patient!

2. Request to test for target for therapeutic purposes
   Needs to be done at the time of diagnosis or relapse!
Conclusion

• CLL is a common and usually indolent disease
• Flow cytometry is the fastest and cheapest way to make the diagnosis
• It is clinically important to separate indolent from aggressive disease (prognostication)
• Prognostication can be based on:
  • Grade, Stage, Molecular Mutations, Cytogenetic Abnormalities