Test Utilization:
- Chronic Lymphocytic Leukemia

Initial Evaluation
- Diagnostic Criteria
- Selection of Tests for Prognosis
Response to Therapy
- Challenges
- Assessment for persistent disease

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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Chronic Lymphocytic Leukemia
Development of Test Utilization Algorithms

• Close collaboration with CLL disease oriented group
• Typical points for blood / bone marrow analysis in CLL patients
  • Diagnosis
  • Assessment of initial prognosis
  • Assessment of response to therapy
  • Assessment of progressive disease
    • Genetic progression
    • Transformation
  • Assessment of new cytopenias/systemic symptoms
Challenge:
--Correctly diagnose CLL
--Optimize prognostic testing
CLL Initial Evaluation on Blood

It is important to get the diagnosis correct right from the beginning

- Establish CLL Dx
  - Lymphocyte morphology
  - Flow cytometry results
  - Lymph node biopsy

- Consider Absolute B cell Count
  - Chronic lymphocytic leukemia
  - Monoclonal B cell lymphocytosis

Suspicion of Chronic Lymphocytic Leukemia
Flow Cytometry

B-CLL Phenotype

Hematopathology Consistent CLL diagnostic criteria

DDx:
B cell count ≥5000 = CLL
B cell count <5000 = MBL

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CLL Blood Morphology
Diagnostic Criteria for CLL
Flow Cytometry

- **CD5+ B cells**
  - Dim CD20

- **CD23+**
  - Dim monotypic light chain
CLL Lymph Node Morphology

Proliferation Center
CLL Lymph Node Immunohistochemistry

<table>
<thead>
<tr>
<th>CD20</th>
<th>CD3</th>
<th>CD5</th>
<th>CD43</th>
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</table>

CD23  | Cyclin D1 | kappa | lambda |
|------|-----------|-------|--------|
**CLL Initial Evaluation on Blood**

**Prognostic Studies**

- **B-cell count <1000**
  - AND
  - NO SLL presentation

- **B-cell count 1000-5000**
  - OR
  - **B-cell count <1000**
    - AND
    - SLL presentation
      - (i.e. lymphadenopathy)

- **B-cell count >5000**

  - Flow Cytometry
    - CD38
    - CD49d
    - Zap-70

  - **FISH:**
    - B-CLL Panel
    - or
    - Array CGH

  - **IGVH sequencing**
### Typical CLL FISH Panel*

- 6q-, MYB/Cen6
- 11q-, ATM/Cen11
- +12, D12Z3/MDM2
- 13q-, D13S319/LAMP1
- 17p-, TP53/Cen17
- t(11;14), CCND1/IGH
- *IGH BAP reflex to:*
  - t(14;18), IGH/BCL2
  - t(14;18), IGH/BCL3

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**Prognosis**

Excluding Mantle Cell Lymphoma

Unusual CLL Subgroups

*For Prognosis NOT Diagnosis*
Survival Significance of Standard CLL Risk Factors

**IGVH unmutated**
- CD38 hi
- Zap-70 hi
- p53 mutations
- High risk FISH (17p-, 11q-)

Median survival 8-10 years

**IGVH mutated**
- CD38 lo
- Zap-70 lo
- p53 normal
- Low risk FISH (13q-)

Median survival 25 years

CLL Bone Marrow Assessment for Response to Therapy

- Previously diagnosed CLL patients who have been treated
- No absolute lymphocyte count progression
- No progression of adenopathy or hepatosplenomegaly
- Minimal risk for MDS
- Assess bone marrow to determine:
  - Complete response (CR), minimal residual disease (MRD) negative or
  - CR, but MRD positive or
  - Partial response (nodular) or
  - Persistent disease
CLL Bone Marrow Assessment for Response to Therapy—Challenges
Is Minimal Residual Disease Testing Necessary?

- Level of MRD predicts progression free survival and overall survival:
  - After routine chemotherapy
  - After immunochemotherapy (FCR/alemtuzumab)
  - After stem cell transplantation

*Moreton C, et al JCO 2005;23:2971-9*
### CLL Bone Marrow Assessment for Response to Therapy—Challenges

What is the optimal test for MRD detection?

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Analytical Difficulty</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR--consensus primers</td>
<td>Low (10^-3)</td>
<td>Low</td>
</tr>
<tr>
<td>PCR--allele specific oligonucleotide</td>
<td>High (10^-6)</td>
<td>High</td>
</tr>
<tr>
<td>CD5/CD19 flow cytometry</td>
<td>Low (10^-2)</td>
<td>Low</td>
</tr>
<tr>
<td>MRD flow cytometry*</td>
<td>High (10^-4)</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

CLL Bone Marrow Assessment for Response to Therapy

MRD Flow Cytometry Assay Design

- 6 color, one tube
- CD45, CD5, CD19, CD20, kappa, lambda
- Collect 500,000 events
- Gating strategy
  - Exclude doublets/debris
  - Identify lymphocyte events (CD45/side scatter)
  - Gate on CD19 positive B cells
  - Identify dual CD20 (dim) CD5 positive cells
  - Evaluate for kappa and lambda
  - Calculate %MRD:

\[
%\text{MRD} = \left( \frac{\text{monoclonal CLL events}}{\text{non-aggregated WBC}} \right) \times 100
\]
CLL MRD By Flow Cytometry

MRD=0.35%

MRD=0.02%
CLL MRD By Flow Cytometry

MRD Negative

MRD=0.00%
CLL Minimal Residual Disease
Can Immunohistochemistry Substitute for Flow Cytometry?

- 82 patients
- Confirmed CLL
- Treatment with chemoimmunotherapy
- Bone marrow aspirates and biopsies:
  - Morphology assessment
  - MRD flow cytometry
  - Immunohistochemistry (IHC) for CD3, CD5, CD23 and PAX-5
- Compare MRD flow and IHC results

Amador-Ortiz C, et al; 2013
MRD Flow Cytometry vs. IHC Concordance

PAX-5+  CD23+  CD5+  CD3-

Amador-Ortiz C, et al; 2013
# CLL Minimal Residual Disease

**MRD flow cytometry vs. Immunohistochemistry**

<table>
<thead>
<tr>
<th></th>
<th>Flow MRD Positive (n=64)</th>
<th>Flow MRD Negative (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC MRD Positive (n=55)</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td>IHC MRD Negative (n=24) / Suspicious (n=3)</td>
<td>7/3</td>
<td>17</td>
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</tbody>
</table>

**Concordance = 87%**

**Conclusion:** With a slight loss in sensitivity, IHC can substitute for MRD flow cytometry.

- Discrete IHC positive lymphoid aggregate, hemodilute aspirate
- Very low flow MRD:
  - IHC negative: 0.005 - 0.200%
  - IHC suspicious: 0.040 - 0.890%

Amador-Ortiz C, et al; 2013
CLL Bone Marrow Assessment for Response to Therapy—Challenges

What is the significance of lymphoid aggregates?

Residual involvement by CLL (nodular partial remission) VS. Reactive lymphoid aggregates VS. Rituximab effect—T cell rich lymphoid aggregates
CLL Bone Marrow Assessment for Response to Therapy

MRD post therapy?

Intent to perform BM transplant within 3 mo of this bone marrow

No

Yes

Cytogenetics to evaluate occult MDS
CLL Bone Marrow Assessment for Response to Therapy

Morphologic involvement by CLL/SLL >30%

Flow: CLL MRD

Diagnosis: “Residual involvement by CLL/SLL (%), nodules present/absent” NO ancillary studies
CLL Bone Marrow Assessment for Response to Therapy

Flow: CLL MRD

Positive

BM lymphoid nodules present?

No

Diagnosis: “Involved by SLL/CLL (%), nodules present”

Yes

Concordant % involvement MRD flow and morphology

Yes

Perform IHC—see next slide

No

Negative

Lymphoid aggregates/intersitial infiltrates

Yes

Diagnosis: “Negative for CLL/SLL by morphologic and MRD assessments”

No
CLL Bone Marrow Assessment for Response to Therapy

Do the lymphoid aggregates represent CLL missed by the MRD flow assay or are they T cell aggregates in a Rituximab treated patient?

Flow: CLL MRD
- Negative
  - Lymphoid aggregates/intersitial infiltrates
    - Yes

Immunohistochemistry
- CD3, CD5, CD20, CD23, PAX-5
  - Yes
  - CLL Phenotype
    - Yes
      - Diagnosis: “Residual involvement by CLL/SLL (%) nodules present/absent”
    - No
      - Diagnosis: “Negative for CLL/SLL by morphologic and MRD assessments”
CLL Bone Marrow Assessment for Response to Therapy

Positive

BM lymphoid nodules present?

Yes

Concordant % involvement MRD flow and morphology

No

Flow: CLL MRD

? What is the degree of BM involvement by CLL:
--CLL involvement underestimated by the MRD flow
--MRD with abundant reactive T cells

Diagnosis: “Residual involvement by CLL/SLL(%)”

Immunohistochemistry CD3, CD5, CD20, CD23, PAX-5

Nodules rich in CLL B cells

No

Yes

Diagnosis: “Minimal residual involvement by CLL(%)”
CLL Test Utilization Summary

• Initial diagnosis
  • Get it right
    • Strict flow cytometry criteria (histograms!)
    • LN biopsy
    • Be patient
  • Judiciously order prognostic studies

• Evaluation of Response to Therapy
  • Importance of MRD testing
  • Use a highly sensitive flow cytometry assay possibly supplemented by IHC
  • Recognize the non-specificity of lymphoid aggregates post-therapy
  • Clinician/Hematopathologist joint algorithm development for evaluation of MRD