Plasma Cell Proliferative Disorders: A Rational Approach to the Use of the Laboratory for Diagnosis and Prognostication

William G. Morice, M.D., Ph.D.
Associate Professor, Laboratory Medicine and Pathology
Chair, Division of Hematopathology
Mayo Clinic, Rochester, MN
DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Learning Objectives

• Describe the different types of plasma cell proliferative disorders (PCPDs)

• Describe the role of the laboratory in establishing a diagnosis

• Describe role of laboratory in guiding therapy, monitoring response, and detecting relapse
Plasma Cell Proliferative Disorders

- Heterogeneous group of disorders
- Common feature: Clonal Plasma Cell (PCs)
- Categorized by clinical and laboratory features
- Disorders include
  - Monoclonal Gammopathy of Uncertain Significance (MGUS)
  - Solitary plasmacytoma
  - Smoldering multiple myeloma
  - Multiple myeloma
  - Amyloidosis
Multiple Myeloma/Smoldering Myeloma

• Serum M-spike >3 g/dL (IgA >2.5 g/dL)
• BM Clonal PCs >10%
• No end-organ damage = smoldering myeloma
• End-organ damage = multiple myeloma
• End-organ damage = CRAB
  • HyperCalcemia
  • Renal insufficiency
  • Anemia
  • Bone disease
Monoclonal Gammopathy of Uncertain Significance (MGUS)

- Serum M-spike <3 g/dL (IgG, IgA <2.5 g/dL)
- BM monoclonal PCs <10%
- No end-organ manifestations (lytic lesions, renal failure, amyloid deposition, etc) or lymphoma
- Progression: 10-15% @ 10 yr, 25-30% @ 20 yr
  - Progress to: Multiple myeloma, amyloidosis, lymphoma (IgM)
Monoclonal Gammopathies

- Lymphoproliferative: 3% (1,298)
- Amyloidosis: 9.5% (3,781)
- Multiple myeloma: 17.5% (6,974)
- MGUS: 58% (23,179)
- Smoldering MM: 4% (1,494)
- Plasmacytoma: 2% (774)
- Macro: 2% (940)
- Other: 4% (1,489)

Mayo Clinic 1960-2008
n=39,929
MGUS and SM: Prevalence and Progression Risk

- **MGUS:** Up to 2% of persons ≥50 years old and about 3% of those >70 years

- For SMM, maximum risk in first 5 years

- **Risk factors:** Higher M-spike, higher plasma cell burden, type of M-protein, abnormal free light chain ratio, circulating plasma cells

- Cases with >60% clonal BM PCs all progress, most in first year.

MGUS and Myeloma: PLCO Cancer Screening Trial

- Serially collected serum samples in 77,469 healthy adults
- 71 subjects developed multiple myeloma
- All 71 had preceding MGUS
  - 2 years before MM: 100% had MGUS
  - 5 years before MM: 94.6% had MGUS
  - ≥8 years before MM: 82.4% had MGUS
- PC biologic factors prognostic in MM NOT biomarkers for MGUS disease progression

Landgren O: Blood 5412, 2009
Diagnosis of PCPDs: Role of the laboratory

- Establish plasma cell clonality
- Assess disease burden
- In cases of multiple myeloma:
  - Determine risk
  - Guide therapy
  - Assess response
  - Detect relapse
Plasma Cell Clonality by Immunophenotype
Different Approaches

Ig light chain analysis
- Cytoplasmic
- Test for skewed ratio ($\kappa:\lambda$ ratio >4:1 or <1:1)
- Can be performed by flow cytometry or IHC

Surface immunophenotype
- Abnormal PC: CD56 bright, CD19 & CD45 neg
- Normal PC: CD56 neg, CD19 & CD45 positive
- Often combined with Ig light chain analysis

Manzanera et al

Selected-CD38$^{\text{high}}$ plasma cells

CD56

CD45

CD19

n-PC

my-PC
Plasma Cell Flow Cytometry
New Methodology for Bone Marrow Analysis

• 8-color methods have recently introduced into clinical practice

• Combines immunophenotyping with DNA content analysis and high event collection (500,000 cells)

• Simultaneous assessment of
  • PC light chain restriction
  • PC DNA content (ploidy status)
  • PC proliferation
  • Proportion of normal (nonmalignant) PCs
Plasma Cell Phenotype by Flow

All plasma cells CD38 and CD138 positive

Normal PCs

Abnormal plasma cells
Comparison 8-Color PC Flow (New) to 6-Color PC Flow (Old)

<table>
<thead>
<tr>
<th>6-color plasma cell flow</th>
<th>8-color plasma cell flow</th>
<th>Positive (n=159)</th>
<th>Negative (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=142)</td>
<td>142 (70%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Negative (n=60)</td>
<td>17 (8%)</td>
<td>43 (22%)</td>
<td></td>
</tr>
</tbody>
</table>

Positive = clone detected  
Negative = no clone detected  
1 of discrepant cases LPL
Risk Assessment in PCPDs

What is needed?

- PC proliferation
  - Flow cytometry
  - The old: Slide based immunofluorescence
- PC DNA content/Ploidy
  - FISH
  - Flow Cytometry
  - The old: Metaphase analysis
- Reciprocal translocations
  - FISH
  - NGS
- Gene expression profiling
- Gene mutational analysis
Plasma Cell DNA Content by Flow

- Monotypic Kappa PCs hyperdiploid DNA content
- Normal PCs DNA diploid

All PCs
Plasma Cell Proliferation by Flow

- **G0/G1**
- **G2/M**
- **S-Phase proliferating cells**

Clonal PC

CD19

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Comparison Flow-based DNA Index & FISH/Metaphase For Clonal PC Ploid

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>DNA Content Clonal PCs by Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diploid</td>
</tr>
<tr>
<td>Diploid (n=59)</td>
<td>42</td>
</tr>
<tr>
<td>Aneuploid (n=49)</td>
<td>0</td>
</tr>
</tbody>
</table>
Comparison Flow & Genetics for PC Ploidy: Conclusions

• DNA ploidy by flow appears equally sensitive to cytogenetic studies for detecting aneuploidy

• Small percentage of cases with aneuploid PCs not detected by flow

• Flow appears more sensitive when the percentage of abnormal PCs is low

• Allows for detection of multiple aneuploid populations
mSMART 2.0: Classification of Active MM

**High risk**
- FISH
  - Del 17p
  - t(14;16)
  - t(14;20)
- GEP
  - High-risk signature

**Intermediate risk***
- FISH
  - t(4;14)‡
- Cytogenetic deletion 13 or hypodiploidy
- PCLI ≥3%

**Standard risk***†
- All others including
  - Hyperdiploid
  - t(11;14)**
  - t(6;14)

* Note that a subset of patients with these factors will be classified as high risk by GEP
† LDH > ULN and beta-2 M >5.5 may indicate worse prognosis
‡ Prognosis is worse when associated with high beta-2 M and anemia
** t(11;14) may be associated with plasma cell leukemia

ONLY NEEDED IF
CRAB present OR
Bone Marrow PCs>60%
mSMART – Off-Study

Transplant Eligible

High risk

4-6 cycles of bortezomib containing regimen (CBD, VRd, VTD, etc)

Collect stem cells

If not in CR, consider autologous stem cell transplant (ASCT)

All patients receive Rd† until progression

Standard risk

4 cycles of Rd*

Collect stem cells**

Autologous stem cell transplant (ASCT)

or

Continue Rd†

If not in CR/VGPR after 1st ASCT, consider consolidation (eg, 2nd ASCT or IMiD)

* Bortezomib-containing regimens preferred in renal failure or if rapid response needed
**If age >65 or >4 cycles of Rd, consider G-CSF plus cytoxan or plerixafor
† Continuing Rd is an option for patients responding well to induction with low toxicities; Dex is usually discontinued after 1st year

When to prognosticate: What is the role of screening studies?

- Cases received in the reference laboratory often have low #s of abnormal PCs.
- Can screen by FISH using Ig staining and IGH break apart probes.
- Can also use high sensitivity flow cytometry:
  - If less than 0.5% clonal PCs OR no clonal PCs
  - Cytogenetic studies are almost always non-informative
PFS and OS of Symptomatic MM Patients by Presence (N=80) or Absence (n=514) of >5% N-PCs/BMPCs at Diagnosis

- **>5% N-PC/BMPC (n=80)**
  - Median PFS: 51 mo
  - Median OS: NR

- **≤5% N-PC/BMPC (n=514)**
  - Median PFS: 39 mo
  - Median OS: 89 mo

Flow Cytometry for Circulating PCs

- A six-color multi-parameter flow cytometer examined 150,000 events (mononuclear cells) from each sample.
Circulating Clonal PCs: The Mayo Experience in New Myeloma

New MM patients:
ROC analysis determined best cut-off for cPCs that predicted worse 1 and 2-yr mortality was 400 cPCs

Newly diagnosed MM patients (N = 157)

Less than 400 cPCs (N = 120)

400 or more cPCs (N = 37)

Median follow up: 23 months
Newly diagnosed MM patients
Overall Survival (OS)

Median: Not Reached

Median: 32 months

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th># Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 400 cPCs present</td>
<td>120</td>
<td>12 (10%)</td>
</tr>
<tr>
<td>≥ 400 cPCs present</td>
<td>37</td>
<td>13 (35%)</td>
</tr>
</tbody>
</table>
Multiple Myeloma: Approach to Treatment

1. Establish diagnosis
2. Risk stratify
3. Control disease and treat complications
4. Consolidate initial response
5. Maintain response
6. Identify and treat disease relapse
7. Supportive care at all stages!
Relapsed Myeloma: Laboratory Considerations

• High sensitivity bone marrow flow cytometry evolving as the standard of care
  • Efforts for method standardization
  • Role of NGS Ig analysis unclear

• Repeat of risk stratification not required

• Laboratory studies that provide prognostic information
  • FISH for 1q duplication, 17p (TP53) deletion, MYC translocation
  • Bone Marrow Flow Cytometry
  • Peripheral blood flow cytometry
Progression-Free Survival and Overall Survival According to Presence or Absence of MM-PCs in Bone Marrow at Day 100 After ASCT

Results

Actively Relapsing patients:
ROC analysis determined best cut-off for cPCs that predicted worse 1 and 2-yr mortality was **100 cPCs**

actively relapsing patients  
(N = 145)  

- Less than 100 cPCs  
  (N = 92)  

- 100 or more cPCs  
  (N = 53)

Median follow up: 23 months
Actively Relapsing MM patients
Overall Survival (OS)

Survival from time of PB flow analysis

Median: 12 months

Median: 33 months

P < 0.001

Time since flow cytometry analysis (months)

Percent surviving

< 100 cPCs present
92 33 (36%)

≥ 100 cPCs present
53 37 (70%)

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Laboratory Studies in Suspected PCPDs
A Rationale Approach

- Bone Marrow
  - PC Flow Cytometry

- No Clonal PCs
  - STOP!

- Clonal PCs
  - No CRAB
    - <60% PCs
    - STOP!
  - 1st CRAB
    - >60% PC
    - PC Prolif
      - Ploidy
      - Translocations
      - ?GEP
  - Relapsed MM
    - FISH for
      - 1q duplication
      - TP53 deletion
      - MYC translctn
**Laboratory Studies In PCPDs and MM Closing Considerations**

- Ideally use BM flow method that quantifies normal PCs, DNA content, and proliferation
- FISH can also assess ploidy (less sensitive), no role for conventional metaphase analysis
- Semi-quantitative flow for circulating PCs can be a useful PROGNOSTIC test
- Potential role for NGS technologies for mutational analysis and Ig rearrangement needs to be studied
Questions & Discussion