Multiple Myeloma by FISH

Indications for Ordering
Aids in stratifying individuals with newly identified multiple myeloma (MM) into risk groups for
• Prognostic counseling
• Selection and sequencing of therapy

Test Description
Fluorescent in situ hybridization (FISH)
• Plasma cells are isolated from a bone marrow (BM) aspirate using CD138+ microbeads
• CD138+ cells (plasma cells) are analyzed by FISH using specific probes for
  o IGH/CCND1 t(11;14)
  o CKS1B (1q21)
  o IGH (14q32)
  o p53 (17p13.1)
  o 11q13/15q22/9q34 for ploidy analysis
• If IGH rearrangement detected does not involve CCND1, additional probes are added
  o IGH/FGFR3 t(4;14)
  o IGH/MAF t(14;16)

Tests to Consider
Primary test
Multiple Myeloma Panel by FISH 2002294
• Detect molecular genetic abnormalities predictive of outcome in individuals with MM

Related tests
Chromosome Analysis, Bone Marrow 2002292
• Diagnosis, prognosis, and monitoring of MM
Chromosome FISH, Multiple Myeloma Panel Process and Hold 2006270
• Use when the clinical necessity of FISH is uncertain at the time of collection, so that CD138+ sorting on fresh specimen may be attempted and a pellet held for future testing, if desired

Disease Overview
Incidence – 3-9/100,000
Age of onset – median age at presentation ~70 years
• Only 15% of patients are <60 years

Symptoms
• Presenting clinical features include symptoms of
  o Bone disease
  o Impaired renal function
  o Anemia
  o Hypercalcemia
  o Recurrent or persistent bacterial infection
  o Hyperviscosity

Initial diagnostic workup
• CBC
• BUN/creatinine, electrolytes
• LDH, calcium
• M-protein workup
  o Serum free light chain test
  o Serum quantitative immunoglobulins
  o Serum and 24-hr urine protein electrophoresis (SPEP plus UPEP)
  o Serum and urine immunofixation electrophoresis (SIFE plus UIFE)
• Skeletal survey

To establish diagnosis
• BM aspirate and trephine biopsy
• Plasma cell immunophenotyping

To establish tumor burden/prognosis
• Albumin and beta-2 microglobulin
• FISH analysis

Diagnostic criteria
• Smoldering (asymptomatic) myeloma
  o Requires ≥1 of the following
    ▪ M-protein in serum ≥30 g/L
    ▪ BM clonal plasma cells ≥10%
    ▪ No related organ or tissue impairment (no end-organ damage, including bone lesions) or symptoms
• Active (symptomatic) myeloma
  o M-protein in serum and/or urine
  o BM (clonal) plasma cells or biopsy-proven plasmacytoma
  o Myeloma-related organ or tissue impairment
    ▪ Requires ≥1 of the following
      ▪ Calcium elevation
      ▪ Renal insufficiency
      ▪ Anemia
      ▪ Bone disease (lytic or osteopenic)
      ▪ Other examples of active disease include repeated infections, amyloidosis, or hyperviscosity
Prognostic issues using FISH

- Abnormalities are detected by conventional cytogenetics in ~30% of MM
  - FISH increases this to >90% of MM
- Patients with cytogenetic abnormalities have a different survival than those without
- Major genetic subtypes do not change over time
  - Need to only test once at diagnosis for most markers
  - Repeat testing is probably justified for
    - 17p13
    - Gains/amplification of 1q21
- Refer to table

Test Interpretation

**Analytical sensitivity/specificity** – >95%

**Results**

- Abnormal – gain/loss/rearrangement detected
  - Percentage of cells affected (out of 200) reported
- Normal – no evidence of gains, deletions, or rearrangements of loci tested

**Limitations**

Only detects aberrations specific to probes used

### Prognostic Issues Related to Genetic Markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Survival</th>
<th>Recurrent Testing</th>
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</thead>
<tbody>
<tr>
<td><strong>Established and validated</strong></td>
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<tr>
<td>14q32 (IGH)</td>
<td>Depends on translocation partner</td>
<td>Test only once</td>
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<tr>
<td>5 major oncogenes involved in translocation with 14q32 (11q13 [CCND1], 16q23 [C-MAF], 4p16 [FGFR3/MMSET], 6p21 [CCND3], 20q11 [MAFB])</td>
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<tr>
<td>Presence – 55-70% of MM</td>
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<tr>
<td>t(4;14)</td>
<td>Associated with poor survival with conventional therapy</td>
<td>Test only once</td>
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<tr>
<td>Detectable only by FISH</td>
<td>Short remission duration after high-dose chemotherapy with stem-cell support</td>
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<tr>
<td>Presence – 15-20% of MM</td>
<td>Bortezomib partially abrogates adverse effects</td>
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<tr>
<td>deletion 17p (p53)</td>
<td>More aggressive disease</td>
<td>Repeated testing justified</td>
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<tr>
<td>Presence at diagnosis – &lt;10%</td>
<td>Associated with poorest prognosis</td>
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<tr>
<td>Presence with advanced disease – much higher</td>
<td>Predictive of short duration of response after high-dose chemotherapy</td>
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<tr>
<td>t(14;16)</td>
<td>Predictive of CNS involvement</td>
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<td>Presence – 5-7%</td>
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<tr>
<td>Gains/amplification of 1q21</td>
<td>Associated with increased risk of MM progression</td>
<td>Repeated testing justified</td>
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<tr>
<td>Presence at diagnosis – ~30-40%</td>
<td>May be associated with other high risk markers such as t(4;14) which worsen prognosis</td>
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<td>Presence at relapse – &gt;70%</td>
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<td><strong>With modest effects</strong></td>
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<tr>
<td>Hyperdiploidy</td>
<td>Weak tendency towards more favorable outcome</td>
<td>Ploidy characteristics are stable over time (test only once)</td>
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<td>Characterized by odd numbered chromosomes (3, 5, 7, 9, 11, 15, 19, 21)</td>
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<tr>
<td>Presence – ~45%</td>
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<tr>
<td>t(11;14)</td>
<td>Weakly associated with improved survival</td>
<td>Test only once</td>
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<td>Presence – 15% of MM</td>
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<td>Light chain amyloidosis – 35-50%</td>
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<td>IgM MM – &gt;90%</td>
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