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
  - *IGH*/*CCND1* t(11;14)
  - *CKS1B* (1q21)
  - *IGH* (14q32)
  - *p53* (17p13.1)
  - 11q13/15q22/9q34 for ploidy analysis
- If *IGH* rearrangement detected does not involve *CCND1*, additional probes are added
  - *IGH*/*FGFR3* t(4;14)
  - *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
  - Bone disease
  - Impaired renal function
  - Anemia
  - Hypercalcemia
  - Recurrent or persistent bacterial infection
  - Hyperviscosity

Initial diagnostic workup
- CBC
- BUN/creatinine, electrolytes
- LDH, calcium
- M-protein workup
  - Serum free light chain test
  - Serum quantitative immunoglobulins
  - Serum and 24-hr urine protein electrophoresis (SPEP plus UPEP)
  - 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
  - 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
  - M-protein in serum and/or urine
  - BM (clonal) plasma cells or biopsy-proven plasmacytoma
  - 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 MMs
  - FISH increases this to >90% of MMs
- 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		
Markers	**Survival**	**Recurrent Testing**
Established and validated		
14q32 (IGH) 5 major oncogenes involved in translocation with 14q32 (11q13 [*CCND1]*, 16q23 [*C-MAF]*, 4p16 [*FGFR3/MMSET]*, 6p21 [*CCND3]*, 20q11 [*MAFB]*) Presence – 55-70% of MM	• Depends on translocation partner o 6p21, 11q13 are associated with good prognosis o 4p16, 16q23, 20q11 are associated with poor prognosis	Test only once
t(4;14) Detectable only by FISH Presence – 15-20% of MM	• Associated with poor survival with conventional therapy • Short remission duration after high-dose chemotherapy with stem-cell support • Bortezomib partially abrogates adverse effects	Test only once
deletion 17p (p53) Presence at diagnosis – <10% Presence with advanced disease – much higher	• More aggressive disease • Associated with poorest prognosis • Predictive of short duration of response after high-dose chemotherapy • Predictive of CNS involvement	Repeated testing justified
t(14;16) Presence – 5-7%	• Confers an adverse outcome	Test only once
Gains/amplification of 1q21 Presence at diagnosis – ~30-40% Presence at relapse – >70%	• Associated with increased risk of MM progression • May be associated with other high risk markers such as t(4;14) which worsen prognosis	Repeated testing justified
With modest effects		
Hyperdiploidy Characterized by odd numbered chromosomes (3, 5, 7, 9, 11, 15, 19, 21) Presence – ~45%	• Weak tendency towards more favorable outcome	Ploidy characteristics are stable over time (test only once)
t(11;14) Presence – 15% of MM Light chain amyloidosis – 35-50% IgM MM – >90%	• Weakly associated with improved survival	Test only once