

Multiple Myeloma Testing



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Disclosures

- None

Utilization Message

- As you view this presentation, consider the following important points regarding testing:
 - How is the testing going to be used in your practice?
 - When should the tests be used?
 - How will results impact patient management?

Diagnostic Criteria for Multiple Myeloma (WHO 2008/2016; IMWG 2014)

- Symptomatic
 - Monoclonal BM plasma cells (>10%) or plasmacytoma
 - Myeloma-defining event
 - End organ damage* (Ca²⁺, renal, anemia, bone lesions = CRAB)
 - >60% BM plasma cells, serum free light chain ratio >100, or 2 or more focal lesions on MRI
- Smoldering (asymptomatic)
 - BM plasmacytosis (10%-60%) or high M-protein (≥3 g/dL in blood, ≥0.5 g/24 hour in urine)
 - No myeloma-defining events

mSMART 2.0: Classification of Active MM

High-Risk	Intermediate-Risk ^a	Standard-Risk ^{a,b}
<ul style="list-style-type: none"> ▪ FISH^c <ul style="list-style-type: none"> ▪ Del 17p ▪ t(14;16) ▪ t(14;20) ▪ GEP <ul style="list-style-type: none"> ▪ High risk signature 	<ul style="list-style-type: none"> ▪ FISH <ul style="list-style-type: none"> ▪ t(4;14)^d ▪ 1q gain 	<p>All others including:</p> <ul style="list-style-type: none"> ▪ Trisomies ▪ t(11;14)^e ▪ t(6;14)

<https://www.msmart.org/>

^a Note that a subset of patients with these factors will be classified as high-risk by GEP
^b LDH >ULN and beta-2 M > 5.5 may indicate worse prognosis; ^cTrisomies may ameliorate
^d Prognosis is worse when associated with high beta-2 M and anemia
^e t(11;14) may be associated with plasma cell leukemia; ^f Cut-offs vary

Testing: Immunophenotypic Assessment

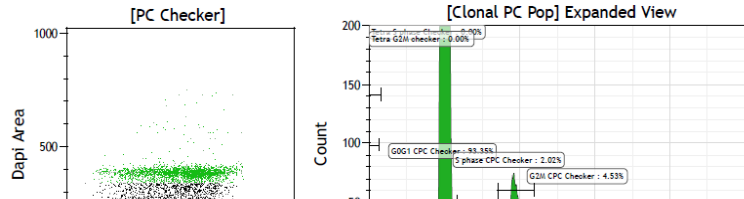
- Immunophenotypic assessment:
 - PC proliferation rate –
 - PCPRO/Plasma Cell DNA Content and Proliferation, Bone Marrow (flow)
 - MSMRT/Mayo Algorithmic Approach for Stratification of Myeloma and Risk-Adapted Therapy Report
 - % bone marrow polyclonal PCs – PCPRO and MSMRT
 - % Circulating clonal PCs – PBLI/Plasma Cell Assessment, Blood (flow)
 - Minimal residual disease (MRD) – Multiple Myeloma MRD by Flow

Testing: Cytogenetic Abnormalities

- Cytogenetic abnormalities
 - -13/13q-, RB1/LAMP1
 - t(11;14), CCND1/IGH
 - 14q32 rearrangement, IGH*
 - 17p-, TP53/D17Z1
 - +3/+7, D3Z1/D7Z1
 - +9/+15, D9Z1/D15Z4
 - 1q gain, TP73/1q22
 - 8q24.1 rearrangement, MYC
- PCPDF (FISH) or MSMRT

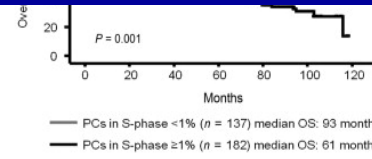
*Reflex testing for t(4;14)(p16.3;q32) *FGFR3/IGH*, t(6;14)(p21;q32) *CCND3/IGH*, t(14;16)(q32;q23) *IGH/MAF*, and t(14;20)(q32;q12) *IGH/MAFB*

PCPRO: Staining with DAPI Measures DNA Content in Cell



Best S-phase cutoff: 2%
 Sensitivity for detection of small clones:
 - variable: 10^{-4} – 2×10^{-5}

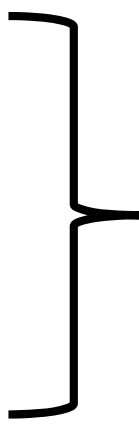
Tetra Index (only use if tetra)	2.00
Poly PCs per Non Aggs	0.00%
Poly PCs per all PCs	0.00%
Clonal Dapi Mean	199.79
Poly Dapi Mean	191.47
Clonal HPCV	3.47
Poly HPCV	N/A



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More Antigens + More Collected Events = Higher Sensitivity

- CD45-negative
- CD19-negative
- CD38-dim
- CD56-positive
- CD117-positive
- CD81-dim
- CD27-dim
- Light chain-restricted

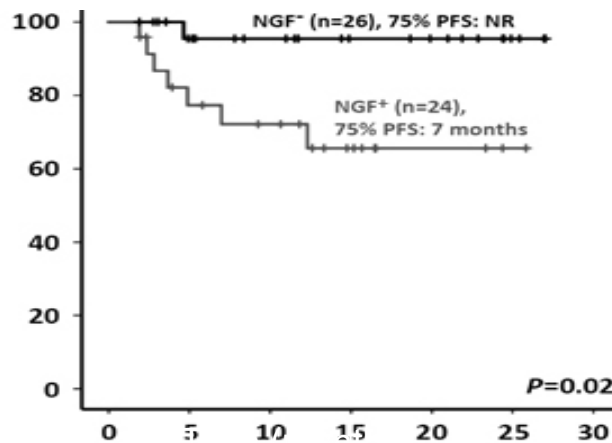


Abnormal Plasma Cells



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Importance of High-Sensitivity MRD²



Myeloma MRD Test at Mayo

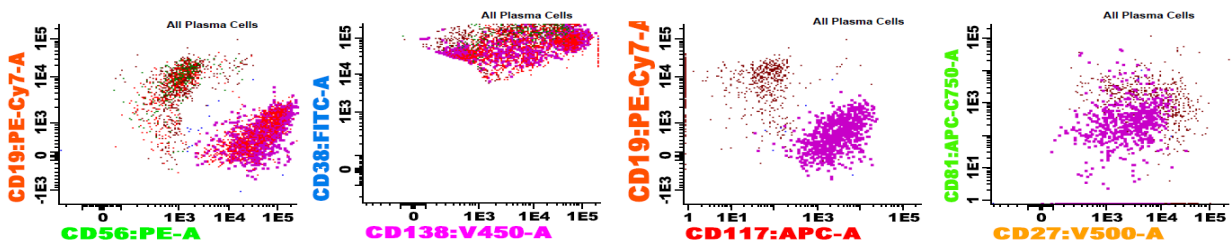
- Used IMWG-recommended next-generation flow cytometry technology
- 5x10⁶ events collected per tube (total 107)
- Sensitivity 10⁻⁵

Tube 1: CD138/CD27/CD38/CD56/CD45/CD19/CD117/CD81

Tube 2: CD138/CD27/CD38/CD56/CD45/CD19/cyKappa/CyLambda

Myeloma MRD Test at Mayo

- Analyzed with the assistance of the Infinicyte software



Myeloma MRD Test at Mayo

- When to order
 - Indicated only for patients post-therapy with:
 - Complete response (Negative serum and urine immunofixation, <5% BM plasma cells, no plasmacytoma)
 - OR
 - Stringent complete response (CR + normal free light chain ratio, polyclonal BM PC by IHC)
- For diagnosis or relapse – order PCPRO or MSMRT test (PCPRO + FISH)

Myeloma MRD Test at Mayo

- What requirements for minimal residual disease testing
 - Need minimum 2 mL of bone marrow aspirate
 - Indicate whether the patient is on a therapeutic antibody regiment (anti-CD28 Daratumumab)

NGS for Multiple Myeloma

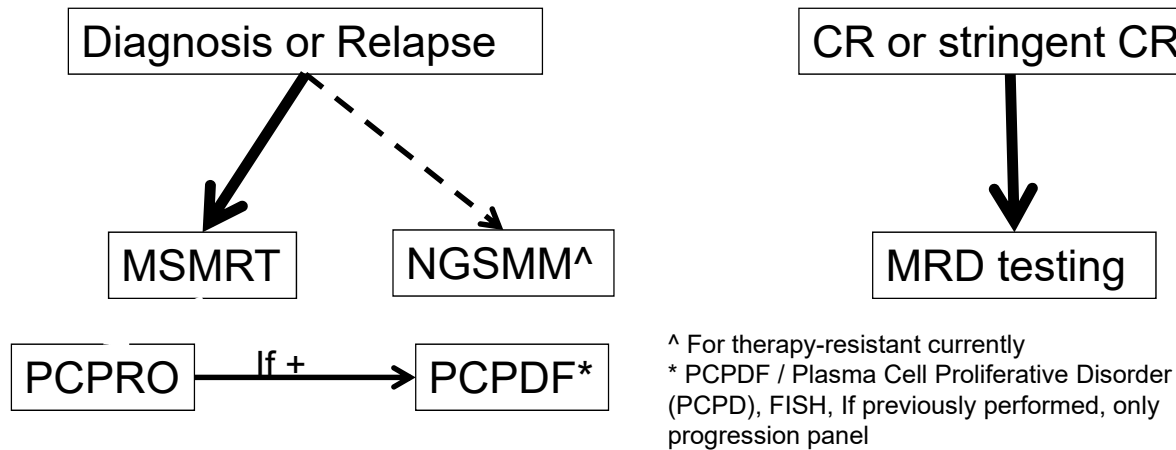
- NGSMM / NGSMM Next-Generation Sequencing (NGS), Multiple Myeloma
- Identification of mutations important for plasma cell signaling, proliferation, survival and sensitivity to chemotherapy
- 61 genes plus 7 intron regions
- 250x minimum per base coverage; Illumina sequencing
- Plasma cells sorted by flow cytometry laboratory (minimum 5% plasma cells in the specimen)

<i>AKT1</i>	<i>CD38</i>	<i>DIS3</i>	<i>IFNGR2</i>	<i>KRAS</i>	<i>PIK3R2</i>	<i>RB1</i>
<i>AKT2</i>	<i>CDK4</i>	<i>DIS3-G</i>	<i>IGF1R</i>	<i>MYC</i>	<i>PIM1</i>	<i>STAT3</i>
<i>AKT3</i>	<i>CDK7</i>	<i>EGFR</i>	<i>IKZF1</i>	<i>MYD88</i>	<i>PIM2</i>	<i>TGFBR2</i>
<i>AKT3-G</i>	<i>CDKN1B</i>	<i>FAM46C</i>	<i>IKZF3</i>	<i>NFKB2</i>	<i>PIM3</i>	<i>TLR4</i>
<i>ATM</i>	<i>CDKN2A</i>	<i>FGFR3</i>	<i>IL6</i>	<i>NR3C1</i>	<i>PSMA1</i>	<i>TP53</i>
<i>B2M</i>	<i>CDKN2A-G</i>	<i>FGFR3-G</i>	<i>IL6R</i>	<i>NRAS</i>	<i>PSMB5</i>	<i>TRAF3</i>
<i>BIRC2</i>	<i>CRBN</i>	<i>GRB2</i>	<i>IRF4</i>	<i>PIK3CA</i>	<i>PSMB5-G</i>	<i>WHSC1</i>
<i>BIRC3</i>	<i>CUL4A</i>	<i>IDH1</i>	<i>JAK2</i>	<i>PIK3CG</i>	<i>PSMD1</i>	<i>XBP1</i>
<i>BRAF</i>	<i>CUL4B</i>	<i>IDH2</i>	<i>KDM6A</i>	<i>PIK3R1</i>	<i>PSMG2</i>	
<i>CCND1</i>	<i>CXCR4</i>	<i>IDH3A</i>	<i>KDM6A-G</i>	<i>PIK3R1-G</i>	<i>PTPN11</i>	

Indications for NGS Test

- Therapy-resistant relapsed myeloma
- Possibly also at initial diagnosis (for prognostic information)
- The report identifies mutations and describes their potential clinical importance and therapeutic implications

Mayo Algorithm for MM Testing



References

- Paiva B, Vidriales M-B, Montalban M-A, et al: Multiparameter Flow Cytometry Evaluation of Plasma Cell DNA Content and Proliferation in 595 Transplant-Eligible Patients with Myeloma Included in the Spanish GEM2000 and GEM2005 <65 y Trials. Am J Pathol 2012 Nov;181(5):1870-1878
- Flores-Montero J, Sanoja-Flores L, Paiva B, et al: Next-generation Flow for Highly Sensitive and Standardized Detection of Minimal Residual Disease in Multiple Myeloma. Leukemia e-pub 10 March 2017, doi: 10.1038/leu.2017.29