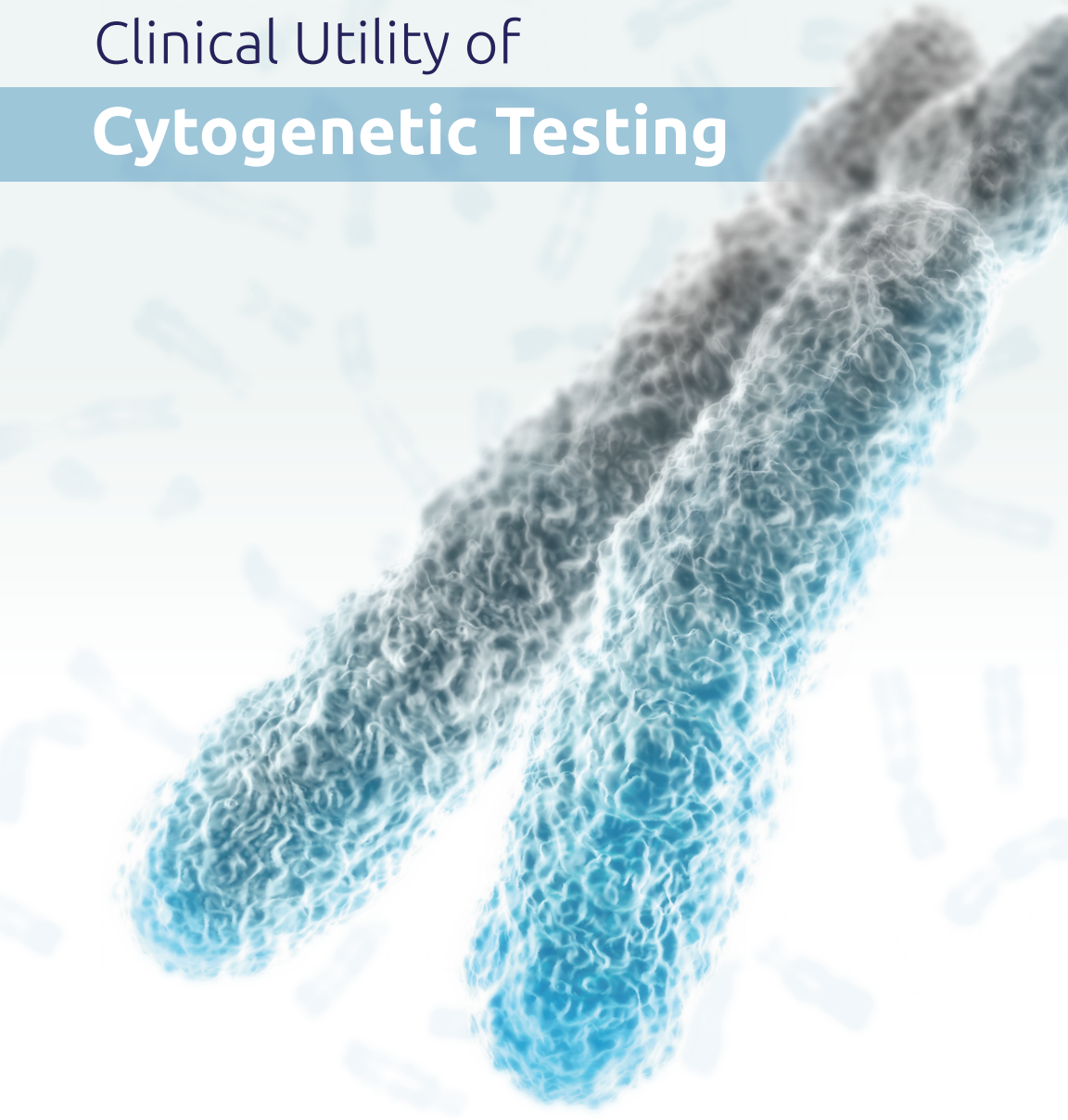
Multiple Myeloma:

Clinical Utility of Cytogenetic Testing



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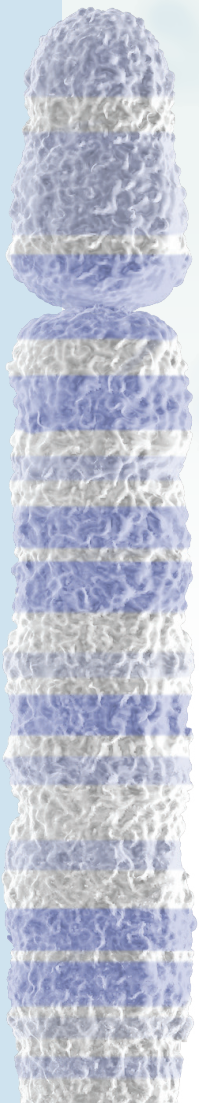
Multiple Myeloma

Multiple myeloma (MM) is a heterogeneous disease with many prognostic factors.[1] About 30% to 50% of patients exhibit abnormal karyotypes—with a median of 8 chromosomal abnormalities per MM patient, and a median of 10 abnormalities per karyotype at diagnosis in patients with hyperdiploid MM.[2-4]

Because cytogenetic abnormalities may correlate with clinical status and prognosis,[1] testing for cytogenetic abnormalities in newly diagnosed patients may help inform treatment decisions.[5,6]



Primary Cytogenetic Abnormalities

Primary cytogenetic abnormalities have been shown to initiate the development of some myeloma cells and are observed in all stages of gammopathy.[7] Nearly all MM patients have a primary abnormality—either a translocation and/or hyperdiploidy.[8]

TRANSLOCATIONS

Translocations, occurrences of one piece of a chromosome breaking off and attaching to a different chromosome, are common in MM, with IgH translocations occurring in 50% to 70% of cases.[2,9]

HYPERDIPLOIDY

Hyperdiploidy, the presence of extra copies of chromosomes, is an aberration typically affecting odd-numbered chromosomes and is typically mutually exclusive with IgH translocations; one analysis (n=120) found 17% of hyperdiploid MM contained 14q32 translocations, and 63% of cases with 14q32 translocations had nonhyperdiploid karyotypes.[10] Hyperdiploidy is involved in more than 50% of MM cases and may be associated with improved outcomes in the absence of adverse genetic lesions.[10-12]

Secondary Cytogenetic Events

In addition to primary cytogenetic events, secondary cytogenetic events in MM may warrant repeat cytogenetic testing.[6]

IgH Translocations

IgH translocations most likely occur in the germinal center during B cell activation and may be present during all stages of monoclonal gammopathy of undetermined significance (MGUS) and myeloma.[13,14] When these translocations occur, derivative chromosomes may contain enhancers juxtaposed with oncogene promoters.[13] IgH translocations and hyperdiploidy are considered the main transforming events in the transition from MGUS to MM, according to a standard model of molecular pathogenesis. (Limitations of this model include a lack consideration for the influence of the micro-environment and heterogeneity of MM clones over the course of the disease.) Nonetheless, this model predicts that primary 14q32 IgH chromosomal translocations are stable over time and correlate with clinical outcomes.[2,6,8]

IgH translocations are commonly detected in MGUS or smoldering MM, and intermedullary MM, with the incidence increasing as the disease progresses; IgH translocations are also extremely common in plasma cell leukemia.[15]

Translocations of IgH Locus[2]

Translocation	Gene(s)	Prevalence
t(11;14)(q13;q32)	*CCND1*	15% to 20%
t(4;14)(p16;q32)	*FGFR3 and MMSET*	10% to 15%
t(14;16)(q32;q23)	*MAF*	2% to 5%
t(6;14)(p21;q32)	*CCND3*	2%
t(14;20)(q32;q12)	*MAFB*	1%

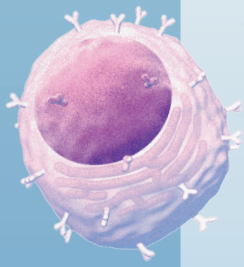
Abbreviations: *CCND1*, cyclin D1; *FGFR3*, fibroblast growth factor receptor 3; *MMSET*, multiple myeloma SET domain; *MAF*, v-maf musculoaponeurotic fibrosarcoma oncogene homolog; *CCND3*, cyclin D3; *MAFB*, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B.

Risk Stratification

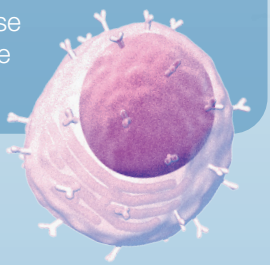
Mayo Clinic mSMART 3.0 risk stratification guidelines classify the following* as either standard or high risk events[5,16-18]:

Standard Risk	High Risk
t(11;14)	t(4:14)
t(6;14)	t(14;16)
Hyperdiploid	t(14;20)
	del(17p)
	Nonhyperdiploid

**This is not an exhaustive list.*



Standard risk is defined as having an indolent course of disease and lengthy survival, while high risk patients experience a more aggressive course and shorter survival.[17,18]



Cytogenetic analysis is recommended for all MM patients [6,19]

Testing for IgH Translocations

The National Comprehensive Cancer Network (NCCN) and the International Myeloma Working Group (IMWG) recommend cytogenetic analysis for all MM patients at the time of initial diagnosis.[6,19]

Conventional Karyotyping—is widely available, however, cells must be in metaphase to perform the assay.[20,21] Due to the low proliferation rate of MM in vitro, cytogenetic abnormalities are only detected in about 30% to 40% of patients using this method.[21-23]

Fluorescent In-Situ Hybridization (FISH)—has high sensitivity, with >90% detection rate when using enriched plasma.[23] Its advantage over conventional karyotyping is that FISH can be used on plasma cells in interphase.[23,24] Using FISH is especially important for identifying IgH translocations because some cannot be detected by standard chromosome analysis.[24,25] It should be noted, however, that because breakpoints with the IgH gene are variable, the selection of FISH probes is important.[13,26] **IgH break-apart probes** can help clearly identify the presence of an IgH translocation.[27,28] **Dual-color dual-fusion probes** can be used to definitively identify the specific type of IgH translocation.[27,28]

Depending on the facility, the testing strategy may be variable. Some laboratories may test for any IgH rearrangement using an IgH break-apart probe followed by dual-color dual-fusion probes, whereas other laboratories may only test for translocations associated with poor prognosis.[2] Variability in analytic technique may lead to missed translocations.[26]

TESTING APPROACHES

Because key decisions rely on accurate information, it is important that testing be performed appropriately, especially in samples containing <20% plasma cells.[23] According to the College of American Pathologists, plasma cell enrichment or cytoplasmic immunoglobulin-enhanced FISH can improve sensitivity, compared to interphase FISH performed on unenriched samples.[29,30]

Plasma cell enrichment, which improves detection of cytogenetic abnormalities by 3-fold when used with FISH, may still yield false negatives due to loss of CD138 over time, or if, in rare cases, the neoplasm does not express CD138.[23,30,31] Enrichment may be achieved through *flow cytometric analysis*, which is useful for comparison and quality control of plasma cell enrichment and helps to ensure adequate CD138 expression if implemented before plasma cell separation.[32] Enrichment can also be accomplished using *magnetic-activated cell sorting (MACS)*, which enables positive CD138 selection.[33] With MACS, timing is important because plasma cells may lose CD138 expression when outside the bone marrow environment.[34] One analysis found that MACS-enriched plasma cell concentration from a single specimen declined from about 58% on day 2 to about 13% on day 8.[32]

Fluorescence-activated cell sorting (FACS) offers a more pure separation of plasma cells than MACS and permits the analysis of specimens with decreased CD138+ expression.[32]

Plasma cell staining, or cytoplasmic immunoglobulin-enhanced FISH (clg FISH), permits identification by light chain-specific immunofluorescence. Specimens with loss of CD138 expression can be analyzed using clg FISH.[32]

Next Generation Sequencing (NGS)—has the potential to perform as well as FISH for structural changes.[35] Like FISH, NGS typically requires sample preprocessing with CD138 cell purification.[31,35,36] Given the extent of genomic profiling needed in the diagnosis and management of MM, NGS may provide a more comprehensive, cost-effective approach in the near future.[35,36]

Because aspirates typically do not contain a large number of plasma cells, timing is sensitive and multiple tests may be needed; close communication among laboratories, pathologists, and oncologists is crucial.[31]

AbbVie is actively conducting research and investigating potential therapeutics in multiple myeloma.

REFERENCES

1. Pawlyn C, Cairns D, Kaiser M, et al. The relative importance of factors predicting outcome for myeloma patients at different ages: results from 3894 patients in the Myeloma XI trial. *Leukemia.* 2020;34(2):604-612.
2. Saxe D, Seo, E, Bergeron MB, Han J. Recent advances in cytogenetic characterization of multiple myeloma. *Int J Lab Hematol.* 2019;41(1):5-14.
3. Nilsson T, Hoglund M, Lenhoff S, et al. A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal multiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences. *Br J Haematol.* 2003;120(6):960-969.
4. Chng WJ, Ketterling RP, Fonseca R. Analysis of genetic abnormalities provides insights into genetic evolution of hyperdiploid myeloma. *Genes Chromosomes Cancer.* 2006;45(12):1111-1120.
5. Dispenzieri A, Rajkumar V, Gertz MA, et al. Treatment of newly diagnosed multiple myeloma based on Mayo stratification of myeloma and risk-adapted therapy (mSMART): consensus statement. *Mayo Clin Proc.* 2007;82(3):323-341.
6. Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. *Leukemia.* 2009;23(12):2210-2221.
7. Fonseca R, Bailey RJ, Ahmann GJ, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. *Blood.* 2002;100(4):1417-1424.
8. Capp J-P, Bataille R. Multiple myeloma exemplifies a model of cancer based on tissue disruption as the initiator event. *Front Oncol.* 2018;8:355.
9. Dictionary of Cancer Terms. Translocation. National Cancer Institute. Accessed July 13, 2021. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/translocation
10. Mohamed AN, Bently G, Bonnett ML, Zonder J, Al-Katib A. Chromosome aberrations in a series of 120 multiple myeloma cases with abnormal karyotypes. *Am J Hematol.* 2007;82(12):1080-1087.
11. Walker BA, Wardell CP, Murison A, et al. APOBEC family mutational signatures are associated with poor prognosis translocations in multiple myeloma. *Nat Commun.* 2015;6:6997.
12. Shah V, Sherborne AL, Walker BA, et al. Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. *Leukemia.* 2018;32(1):102-110.
13. Walker BA, Wardell CP, Johnson DC, et al. Characterization of IgH locus breakpoints in multiple myeloma indicates a subset of translocations appear to occur in pregerminal center B cells. *Blood.* 2013;121(17):3413-3419.
14. Yellapantula V, Hultcrantz M, Rustad EH, et al. Comprehensive detection of recurring genomic abnormalities: a targeted sequencing approach for multiple myeloma. *Blood Cancer J.* 2019;9:101.
15. Chesi M, Bergsagel PL. Advances in the pathogenesis and diagnosis of multiple myeloma. *Int J Lab Hematol.* 2015;37(suppl 1):108-114.
16. mSMART Risk Stratification of Newly Diagnosed Myeloma. Accessed April 28, 2021. https://static1.squarespace.com/static/5b44f08ac258b493a25098a3/t/5b802d8270a6adbc6a79a678/1535126914646/Risk+Strat+3.0rev_svr.pdf
17. Mikhael JR, Dingli D, Roy V, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus guidelines 2013. *Mayo Clin Proc.* 2013;88(4):360-376.
18. Kumar SK, Mikhael JR, Buadi FK, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus guidelines. *Mayo Clin Proc.* 2009;84(12):1095-1110.
19. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology. Multiple myeloma. V7.2021. April 26, 2021.
20. Soekojo CY, Wang G, Chen Y, et al. Role of conventional karyotyping in multiple myeloma in the era of modern treatment and FISH analysis. *Clin Lymphoma Myeloma Leuk.* 2019;19(8):e470-e477.
21. Rajkumar SV, Fonseca R, Dewald GW. Cytogenetic abnormalities correlate with plasma cell labeling index and extent of bone marrow involvement in myeloma. *Cancer Genet Cytogenet.* 1999;113:73-77.
22. Aydin C, Ulas T, Hangul C, et al. Conventional cytogenetics and interphase fluorescence in situ hybridization results in multiple myeloma: a Turkey laboratory analysis of 381 cases. *Indian J Hematol Blood Transfus.* 2020;36(2):284-291.
23. Pozdnyakova O, Crowley-Larsen P, Zota V, Wang SA, Miron PM. Interphase FISH in plasma cell dyscrasia: increase abnormality detection with plasma cell enrichment. *Cancer Genet Cytogenet.* 2009;189(2):112-117.
24. Kapoor P, Fonseca R, Rajkumar V, et al. Evidence for cytogenetic and fluorescence in situ hybridization risk stratification of newly diagnosed multiple myeloma in the era of novel therapies. *Mayo Clin Proc.* 2010;85(6):532-537.
25. Chng WJ, Dispenzieri A, Chim C-S, et al. IMWG consensus on risk stratification in multiple myeloma. *Leukemia.* 2014;28(2):269-277.
26. Ross FM, Avet-Loiseau H, Ameye G, et al. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. *Haematologica.* 2012;97(8):1272-1277.
27. Smol T, Daudignon A. Comparison of IgH profile signals using t(4;14) and IgH break-apart probes by FISH in multiple myeloma. *Cytogenet Genome Res.* 2017;153(1):18-21.
28. Cook JR, Hartke M, Pettay J, Tubbs RR. Fluorescence in situ hybridization analysis of immunoglobulin heavy chain translocations in plasma cell myeloma using intact paraffin sections and simultaneous CD138 immunofluorescence. *J Mol Diagn.* 2006;8(4):459-465.
29. Khoury JD, Dogan A, Foucar K, et al. College of American Pathologists. Protocol for the examination of specimens from patients with plasma cell neoplasms. Based on AJCC/UICC TNM, 7th ed. Posted January 2015. Accessed May 27, 2021. https://documents.cap.org/protocols/cp-plasma-cell-neoplasms-2015-v1001.pdf
30. Lu G, Muddasani R, Orlowski RZ, et al. Plasma cell enrichment enhances detection of high-risk cytogenomic abnormalities by fluorescence in situ hybridization and improves risk stratification of patients with plasma cell neoplasms. *Arch Pathol Lab Med.* 2013;137(5):625-631.
31. Pugh TJ, Fink JM, Lu X, et al. Assessing genome-wide copy number aberrations and copy-neutral loss-of-heterozygosity as best practice: an evidence-based review from the Cancer Genomics Consortium working group for plasma cell disorders. *Cancer Genet.* 2018;228-229:184-196.
32. Hartmann L, Biggerstaff JS, Chapman DB, et al. Detection of genomic abnormalities in multiple myeloma. *Am J Clin Pathol.* 2011;136(5):712-720.
33. Put N, Lemmens H, Wlodarska I, et al. Interphase fluorescence in situ hybridization on selected plasma cells is superior in the detection of cytogenetic aberrations in plasma cell dyscrasia. *Genes Chromosomes Cancer.* 2010;49(11):991-997.
34. Zlei M, Egert S, Wider D, Ihorst G, Wäsch R, Engelhardt M. Characterization of in vitro growth of multiple myeloma cells. *Exp Hematol.* 2007;35(10):1550-1561.
35. Manzoni M, Marchica V, Storti P, et al. Application of next-generation sequencing for the genomic characterization of patients with smoldering myeloma. *Cancers.* 2020;12(5):1332.
36. Cuenca I, Medina A, Rosinol L, et al. Clinical validation of a NGS capture panel to identify mutations, copy number variations and translocations in patients with multiple myeloma. *Blood.* 2020;136(suppl 1):13-14.

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