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REVIEW

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Recent advances in cytogenetic characterization of multiple myeloma

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Abstract

The detection of cytogenetic abnormalities in multiple myeloma (MM) has received more importance over last years for risk stratification and the new risk-adapted treatment strategies. Conventional G-banding analysis should be included in a routine procedure for the initial diagnostic workup for patients suspected of MM. However, the detection of chromosomal abnormalities in MM by conventional cytogenetics is limited owing to the low proliferative activity of malignant plasma cells as well as the low number of plasma cells in bone marrow specimens. Fluorescence in situ hybridization (FISH) or microarray-based technologies can overcome some of those drawbacks and detect specific target arrangements as well as chromosomal copy number changes. In this review, we will discuss different cytogenetic approaches and compare their strength and weakness to provide genetic information for risk stratification and prediction of outcome in MM patients.

KEYWORDS

chromosome abnormalities, cytogenetics, fluorescence in situ hybridization, microarray, multiple myeloma

1 | INTRODUCTION

Multiple myeloma (MM) is a clinically and biologically heterogeneous disorder where monoclonal gammopathy of unknown significance (MGUS) and smoldering multiple myeloma (SMM) are premalignant states susceptible to progress to active MM.[1,2] The International Myeloma Working Group (IMWG) and International Myeloma Workshop have developed the diagnostic criteria and guidelines for standard investigation of patients with suspected MM. In addition, the introduction of a range of new promising agents has extended the therapeutic possibilities. High-risk MM patients based on several cytogenetic abnormalities may show varying prognosis with the choice of therapy. Careful investigation of cytogenetic subgroups provide insight into the effect of new regimens in these patients.[3]

Multiple myeloma is a plasma cell cancer, but MM cells differ from healthy normal plasma cells because they retain a low potential of proliferation, generally 1%-3% of cycling cells. The progeny of proliferative plasmablastic cells from the germinal center migrate to bone marrow and then mature toward terminally differentiated plasma cells.[4-6] Two major primary genetic events have been implicated in the transformation of normal plasma cells into myeloma cells. One oncogenic pathway is hyperdiploidy with multiple trisomies of odd-numbered chromosomes. The other one is characterized by the occurrence of translocations involving the *IGH* gene. Recent studies have suggested that different genomic alterations coexist in mixed subclones. The dominant clonal abnormalities are observed throughout the course of the disease from MGUS to SMM to end-stage MM. Some abnormalities transform normal plasma cells to MGUS, while some minor clones occur later to be a reservoir for relapse.[1,2,4-6]

Conventional cytogenetic studies in MM can provide the advantage of whole genome analysis with one experiment. Abnormal karyotypes are identified in about 30%-50% of MM cases, more often in an advanced stage or a more proliferative form of diseases. However, the low mitotic index, especially in

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TABLE 1 Major cytogenetic abnormalities in multiple myeloma and their clinical impacts

Cytogenetic abnormalities	Frequency in newly diagnosed MM (%)	Prognostic impact
Primary events		
Translocations		
t(4;14)(p16;q32)/*IGH-MMSET/FGFR3*	10-15	Adverse
t(6;14)(p21;q32)/*IGH-CCND3*	2	Neutral
t(11;14)(q13;q32)/*IGH-CCND1*	15-20	Neutral
t(14;16)(q32;q23)/*IGH-MAF*	2-5	Adverse
t(14;20)(q32;q12)/*IGH-MAFB*	1	Adverse
Copy number aberrations		
Hyperdiploidy	50	Favorable
13q deletion	45-50	Adverse
Secondary events		
Translocations		
MYC translocation	15-20	Neutral or adverse
Copy number aberrations		
1q21 gain	35-40	Adverse
1p deletion	30	Adverse
17p deletion	10	Adverse

the early stage of diseases, and a difficult interpretation of some cryptic aberrations can be main limiting factors.[4-7] Fluorescence in situ hybridization (FISH) or microarray-based technologies can overcome some of those drawbacks and detect specific target arrangements as well as chromosomal copy number changes. In this review, we will discuss different cytogenetic approaches and compare their strength and weakness to provide genetic information for risk stratification and prediction of outcome in MM patients.[7-9]

2 | CONVENTIONAL CYTOGENETICS (TRADITIONAL G-BAND KARYOTYPING)

The detection of chromosomal abnormalities in MM by conventional cytogenetics is limited owing to the low proliferative activity of malignant plasma cells as well as the low number of plasma cells in bone marrow specimens. Clonal chromosomal abnormalities are observed in only 30% of the patients with MM.[10] The addition of interleukin-4 to cultures of bone marrow cells increases the proportion of abnormal metaphases and improves the detection rate of cytogenetic abnormalities to 50%.[11] Despite the low detection rate, abnormal metaphase cells predict adverse prognosis[12] and also provide both numerical and structural information regarding the myeloma clone. Patients with MM typically show complex karyotypes, with recurrent numerical and structural aberrations.[10,13,14] MM can be divided into hyperdiploid and nonhyperdiploid subtypes based on the number of chromosomes.[15] The structural abnormalities include translocations and copy number aberrations such as gains and deletions (Table 1).

2.1 | Hyperdiploidy vs Non-hyperdiploidy

2.1.1 | Hyperdiploidy

Hyperdiploidy is detected in about 50% cases of newly diagnosed MM and preferentially involves gain of the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21.[16] It is considered a primary cytogenetic event in MM. Furthermore, hyperdiploidy and primary translocations affecting the *IGH* locus are generally mutually exclusive. Only about 5% of the patients with myeloma have coexistence of hyperdiploidy and primary *IGH* translocations.[17] Single-cell analysis has shown that hyperdiploidy can precede *IGH* translocation in the clonal history of patients with both abnormalities. Hyperdiploidy is associated with a favorable outcome. However, patients with both hyperdiploidy and coexisting adverse cytogenetic abnormalities (such as del(17p), t(4;14) and 1q gain) have poor prognosis.[17] In terms of specific trisomies affecting prognosis, trisomies 3 and/or 5 significantly improve overall survival, whereas trisomy 21 is associated with an adverse outcome.[18] Hyperdiploid karyotype group usually has heterogeneous additional–structural–chromosomal aberrations. The median overall survival of patients with hyperdiploid myeloma is negatively correlated with the number of the additional–structural–chromosomal aberrations.[19]

2.1.2 | Nonhyperdiploidy

Hypodiploid, pseudodiploid, and near-tetraploid karyotypes are referred to as nonhyperdiploid subtypes. Myelomas with either hypodiploidy or pseudodiploidy are characterized by various structural chromosomal abnormalities and monosomies. Common monosomies

are of chromosome 13, 14, 16, 22, and sex chromosomes.[14] Near-tetraploidy usually refers to the duplication of pseudodiploid or hypodiploid karyotypes with a 4N DNA content. Among nonhyperdiploid myelomas, hypodiploid myelomas have the most aggressive clinical phenotype and a higher prevalence of molecular markers associated with poor outcome and disease progression.[20]

2.2 | *IGH* translocations

Chromosomal translocations are observed in 50%-70% of the patients with myeloma and over 90% of these translocations involve chromosome 14, which includes the *IGH* locus at 14q32.[13,16,21] *IGH* translocation leads to the upregulation of partner gene expression under the control of the *IGH* enhancer. Most recurrent translocations such as t(4;14)/*IGH-MMSET/FGFR3*, t(6;14)/*IGH-CCND3*, t(11;14)/*IGH-CCND1*, t(14;16)/*IGH-MAF*, and t(14;20)/*IGH-MAFB* are regarded as primary cytogenetic events that initiate tumor development. Recurrent 14q32 translocations display specific outcomes with respect to the oncogenesis and prognosis of MM. Primary *IGH* translocations are strongly associated with the nonhyperdiploid subtype.[22]

2.2.1 | t(11;14)(q13;q32)

t(11;14)(q13;q32) is the most frequently observed translocation in MM, accounting for 15%-20%.[13,16,23] Conventional cytogenetic method has shown that t(11;14) is balanced in most cases and is accompanied by monosomy 13 in only 25% of cases. The juxtaposition of *IGH* results in the upregulation of *CCND1* and patients with t(11;14) show cyclin D1 expression by immunohistochemistry. This translocation is associated with lymphoplasmacytic morphology, IgM secretion, nonsecretory myeloma, and the expression of CD20.[24] t(11;14) has a neutral impact on prognosis.[23]

2.2.2 | t(4;14)(p16;q32)

The t(4;14)(p16;q32) translocation is detected in 10%-15% of the patients with myeloma.[13,16,23] The breakpoints are located on distal parts of both chromosomes, and the translocation is undetectable by conventional chromosomal analyses. Interestingly, hypodiploidy and monosomy 13 are observed in the majority of t(4;14) cases. t(4;14) leads to the deregulation of *FGFR3* and *MMSET*,[25] and has been identified as an adverse prognostic marker.[23] In the mSMART risk stratification, t(4;14) indicates an intermediate risk, as survival is improved by treatment with the proteasome inhibitor, bortezomib.[26] However, the IMWG and revised International Staging System (ISS) defined t(4;14) as a high-risk cytogenetic abnormality.[3,27,28]

2.2.3 | t(14;16)(q32;q23)

The t(14;16)(q32;q23) translocation is present in 2%-5% of the patients with MM,[13,16] and it is difficult to detect by conventional cytogenetics techniques. This translocation results in the overexpression of the *c-MAF* proto-oncogene and is associated with a poor prognosis.[13] A study of t(14;16)-positive MM suggests that this translocation is associated with a lack of CD56 expression and high proliferative activity and has an unfavorable outcome, even in the novel drug era.[29]

2.2.4 | t(14;20)(q32;q12)

The t(14;20)(q32;q12) translocation is very rare, and it is found in only 1% of the patients with myeloma.[16] This translocation results in the upregulation of the *MAFB* oncogene and is a high MM risk marker.[3]

2.3 | *MYC* translocation

Translocations involving *MYC* are a secondary cytogenetic events and occur in 15%-20% of the patients with myeloma.[16,23,30] The partner loci in *MYC* translocations include *IGH*, *IGL*, *IGK*, *FAM46C*, *FOXO3*, and *BMP6*.[16] *MYC* translocation is frequently found in ISS stage II/III, the hyperdiploid subgroup, and in cases with 1q21 gain. *MYC* translocation is associated with a poor outcome and has a particularly negative impact in hyperdiploid subgroup.[16,30]

2.4 | Copy number aberrations

2.4.1 | 1q21 gain

1q21 gain is the most frequent structural abnormality, observed in 35%-40% of the patients with MM.[16] It is represented with duplication of 1q chromosome, unbalanced whole arm translocation of 1q, isochromosomes, or jumping translocation detected by G-banding. Rearrangements of 1q result from instability of the 1q12 region which is highly decondensed pericentromeric heterochromatin.[31] 1q gain is considered to be a secondary event that influences tumor progression through dosage effect of driver oncogenes such as *CKS1B* on 1q21. 1q gain is an independent poor prognostic factor. The number of 1q21 copies is correlated with both disease progression and prognosis.[32]

2.4.2 | 1p deletion

The deletion of 1p is detected in approximately 30% of the patients with myeloma,[16,33] and commonly deleted regions have been identified, including 1p32 (*CDKN2C*), 1p22, and 1p12. The 1p deletion appears to have an adverse impact on clinical outcome.[33]

2.4.3 | 13q deletion

Around 45%-50% of the patients with myeloma harbor a deletion of the long arm of chromosome 13 of which 85% involve monosomy 13 and the remaining 15% involve interstitial deletions encompassing the minimally deleted region at 13q14.11-13q14.3.[23] The 13q deletion is rarely observed as a sole anomaly. It is detected in both

the hyperdiploid and hypodiploid subtypes but is more frequent in hypodiploidy. Although the 13q deletion is detected in only 10%-20% of the patients by conventional cytogenetics, its detection by metaphase analyses is a critical prognostic factor in myeloma.[12] The results of a study published in 2017 suggest that abnormalities of chromosome 13, for example, monosomy 13 (adverse) and partial deletion of chromosome 13q (protective) show differential effects on overall survival.[34]

2.4.4 | 17p deletion

Chromosome 17p deletion is considered as a secondary event. It is observed in around 10% of patients with newly diagnosed MM,[16,23] and patients who relapse after treatment have an increased risk of developing the 17p deletion.[35] *TP53* is the most relevant tumor suppressor gene deleted on chromosome 17p, and mutations on the remaining allele are found in 25%-40% of patients.[36] *TP53* deletion in myeloma is a high-risk marker associated with adverse prognosis. A study published in 2017 reported that patients with a concomitant complex karyotype and *TP53* deletion show a significantly poorer overall survival compared with patients with a normal karyotype and *TP53* deletion. This study suggests that a comprehensive genetic assessment adds to *TP53* status in the risk stratification of MM.[37]

3 | FISH ANALYSIS

In bone marrow samples of MM patients, the plasma cells are still relatively fewer in number than the other cell types. As such, it is imperative to ensure that testing is performed on the appropriate cell type, particularly in samples with less than 20% plasma cells.[38] Common methods for enrichment include plasma cells selection by flow cytometry and positive CD138$^+$ selection by magnetic assisted cell sorting (MACS). Ideally, CD138$^+$ selection should occur within 2 days of sample collection as plasma cells have been reported to lose expression of CD138 when outside of the bone marrow environment, thus significantly reducing the yield of plasma cells selection on older samples.[39] The cell pellet obtained after plasma cells selection can then be fixed and dropped on slides for FISH testing or can be used to extract DNA to perform microarray or multiplex ligation-dependent probe amplification (MLPA). If only performing FISH testing, it is possible to specifically analyze plasma cells by applying on the FISH slides an antibody for the detection of the cytoplasmic immunoglobulin light chain and to only score the FISH signals of cells showing positive antibody staining, without the need for prior cell separation. This method, referred to cIg-FISH, has been gradually replaced by whole-sample enrichment for plasma cells, which is also compatible with other testing approaches.[40]

Given the diagnostic and prognostic significance of translocations involving the *IGH* locus in MM, FISH is an important component of testing on these samples as some of these translocations are cryptic and cannot be detected by standard chromosome analysis.[40] However, the testing strategy for the detection of these translocations is variable. Indeed, some laboratories may offer testing solely for the translocations that have been associated with a more dismal prognosis such as t(4;14) and t(14;16), while others may proceed with identification of the specific translocation in any sample with an *IGH* rearrangement. In the latter, first-line testing with an *IGH* break apart probe followed by reflex testing using dual color dual fusion probes is often performed. Testing for deletion 17p, gain 1q, and deletion 1p, which can be performed by FISH, microarray, or MLPA, is also performed in most instances, as recommended by experts and used in published guidelines for cytogenetic risk stratification of MM patients.[38,41,42]

As all but one of the *IGH* translocations lead to gene overexpression by juxtaposition of a given gene near the *IGH* promoter or enhancers but do not create a fusion transcript, breakpoints can be quite variable within the *IGH* gene.[43] As such, design of the FISH probe plays an important role in the ability to detect not only common but also rare or atypical rearrangements as demonstrated by Kim et al[44] in their comparison of various *IGH* break apart probes. It is thus important to remain alert to the presence of any atypical signal pattern when analyzing MM samples with an *IGH* break apart, particularly when using this type of probe as first line testing for the detection of *IGH* rearrangements. However, the cutoffs for these various signal patterns are typically less than 5%.

As for dual color dual fusion probes, the cutoffs for the 1O/1G/2F signal pattern corresponding to a balanced translocation are generally less than 5%. However, these probes are much less sensitive for the detection of unbalanced translocations. For example, as many as 30% of interphase nuclei in normal controls can show a 1O/1G/1F signal pattern due to random overlap of red and green signals, particularly in cells with a small nucleus. FISH studies by Fonseca et al[13] using dual color dual fusion probes for the detection of t(4;14), t(11;14), and t(14;16) showed the presence of a 1O/1G/1F signal pattern in approximately half of the patients tested. Although typically seen in a large proportion of cells and thus well above any cutoff, 5 of the 14 patients with an unbalanced t(4;14) had a fusion signal in less than 75% of the clonal cells, compared to 0/7 patients with an unbalanced t(14;16) and 6/35 patients with an unbalanced t(11;14). This limitation of the dual color dual fusion probes is thus particularly important for detection of unbalanced t(4;14) as it may be present in a smaller proportion of the clonal cells than the other translocations. Contrary to other *IGH* rearrangements, t(4;14) also leads to a fusion transcript on the der(4), and loss of *FGFR3* expression on the der(14) as well as complete loss of the der(14) has been shown in some cases by reverse transcriptase-polymerase chain reaction (RT-PCR), FISH, and microarray.[45,46] Studies on chromosomes territories have suggested that *FGFR3*, *CCND1*, and *IGH* loci are in closer proximity than *IGH* and *MAF* in nonmalignant B cells of MM patients and healthy donors,[47] possibly explaining not only the higher frequency of t(11;14) and t(4;14) but also the more frequent signals overlap for these probes than for t(14;16). In samples where an unbalanced t(4;14) is suspected in less than 50% of nuclei, reflex testing with an *IGH* break apart probe may be warranted to confirm the results.

Another aspect of FISH testing for which there is variability between laboratories is how the cutoffs for the various FISH signal patterns are established. While some laboratories monitor cutoffs on a regular basis and adjust them to report all results >2 or 3 standard deviations, other groups use their own predetermined cutoffs with no ongoing monitoring.[38,48] The first strategy could potentially yield false-positive results in instances where there are marked fluctuations in the cutoffs over time or between samples, for instance due to the difficulty to accurately differentiate between random signal overlaps and an unbalanced t(4;14). The second strategy could potentially lead to false-negative results in samples with low level abnormal clones, particularly for secondary abnormalities involving gain of 1q and loss of 1p or 17p. However, it is not clear if these abnormalities have the same prognostic significance when present at low levels at diagnosis.[48] In both instances, it is important to understand the limitations of the FISH assay when interpreting and reporting the results and to perform reflex testing when appropriate.

4 | COPY NUMBER CHANGE BY SNP MICROARRAY

For many years, the standard techniques for identifying genetic abnormalities in myeloma have been cytogenetics, FISH, and gene expression profiling. As our knowledge of genetic alterations increased, so have the methodology to detect new genetic information evolved. Cytogenetics of myeloma specimens is often noninformative, and primarily seen in aggressive phase of the disease. FISH is also limited to a small set of common abnormalities. While single nucleotide polymorphism (SNP)-microarray can detect small copy number changes, such as gene deletion, chromothripsis, complex copy number changes, and amplification, throughout the entire genome at a much higher resolution (<200 kb) than chromosome analysis and provide a genome-wide view rather than selected regions as with FISH at a lower cost. Multiple studies have detected copy number abnormalities in up to 98% of myeloma cases analyzed.[8,49,50] Arrays are also capable of identifying copy neutral loss of heterozygosity (CN-LOH) which is an important mechanism in tumor development. Chromothripsis and chromoanasynthesis are also important mechanisms in cancer development and progression only detected by microarray. Chromothripsis has been seen in most cancer types and is associated with more aggressive tumors and possibly therapy resistance.[8,9]

Another advantage of microarray testing is that it can be performed on DNA from cells that have been enriched for CD138$^+$ plasma cells. The use of enriched plasma cells enables microarrays to be performed when plasma cells are present in a low percentage of cells in the blood or bone marrow, increasing the purity to >80% after sorting with anti-CD138 antibodies. This is particularly valuable when the abnormal cells have a low mitotic index or are below the level of detection by other methods. Microarray performed on plasma cells can identify secondary abnormalities in small populations due to clonal evolution. The most common of these secondary aberrations include complex *MYC* 8q24 rearrangements or amplification, gain of 1q, loss of 1p, and 17p13 all of which are associated with more aggressive disease.[8,50,51]

As with both cytogenetics and FISH studies, patients can be divided into 2 groups. Group 1, comprising approximately 45%-50% of patients, demonstrates hyperdiploidy with gain of odd number chromosomes, or t(11;14) or t(6;14) and is associated with standard risk.[8,51] Group 2 patients have complex copy number changes, often with *IGH* rearrangements t(14;16), t(14;20) and chromosome loss such as del(17p). The risk for these patients is high, based on the *IGH* rearrangement partner and specific deletions. Table 2 summarizes the copy number variations seen in 11 studies in which microarray were performed.

4.1 | *IGH* rearrangement

IGH rearrangement/translocations are present in about 40% of myeloma cases most of which are balanced translocations which cannot be detected by microarray. Most of the patients with *IGH* translocations contain a multiple numerical and structural rearrangement that can be detected by microarray. These include focal losses in 4p16.3 containing *FGFR3* and *WHSC1* and 4p15.2 with t(4;14)(p16;q32), as well as loss of 16q with t(14;16)(q32;q23).[8,51]

4.2 | Gains

The most common gains for Group 1 myeloma patients observed by microarray are gain of the odd number chromosomes seen by both chromosomes and FISH. However, in Group 2, gain of 1q, 3q, 6p, 9q, and 11q are more common. These gains can span large regions which are readily seen by chromosomes or FISH, or more focal gain that may be missed if the region is not included in the standard myeloma FISH panel. A recent review by Manier et al[52] describes some of the focal gains seen in two studies. Gains of 1q spanning the region,1q21.2-1q23 including *CKS1B* and *ANP32E* have been associated with poor outcome. Other recurring smaller regions of gain include 2p14, 3p24.3-26.2, 5q35.2, 6p24.3, 8q24.2 (containing *MYC*), 9q34.13, and 11q13.2 (containing *CCND1*).[52]

4.3 | Loss/Deletions

As with FISH, the most common chromosomal losses occur in 1p, 13q, and 17p. Although Group 1 is characterized by hyperdiploidy, the few losses usually include chromosome X, 13, and 22. However, multiple losses are seen in Group 2. There are two common deleted regions on 1p, 1p22.1, and 1p32.3, which contain multiple cell cycle genes including *CDKN2C* and *FAF1*. Chromosome 13 is most often seen as monosomy, but deletion 13q includes 13q14.2-q21.3 containing *RB1* and *miR-15q/mir-16-1* genes are also seen. Additional recurring abnormalities seen in Group 2 by SNP arrays include loss of 6q, 8p, 11q, 12p, 14q, 16p, 16q, 20p, and 22. Common focal deletions within these chromosomes include 6q25.3, 8p22, 12p13.1,

TABLE 2 Summary of SNP microarray studies

Reference	Sample size	Technique	Loss	Gain	Other abnormalities	Clinical information (Implications)
Agnelli 2009[56]	n = 45	SNP array	1p22.2-21.2, 4p, 6q22.32-22.33, 6q24.1-25.2, 6q25.3-27, 8p21.2-23.3, 13/ del13q21.2-22.1, 14/14q31.1-31.2, 16q12.1-22.1, 16q23.1-qter, 17p with *TP53*,22q11.2 at *IGLλ*	3, 5, 7, **9**, 11, 15, **19**, 21	CN-LOH 1p12-31.1, 13, 16q12.1-23.2	Reported only abnormalities seen in ≥15%.
Avet-Loiseau 2009[51]	n = 192	SNP array	Group 1: whole chromosome: 13, 22, X in female	3, 5, 7, 9, 11, 15, 18, 19, 21		Gain of 5q31 has a better outcome in hyperdiploid, where as del(12p), del(17p), and t(4;14) have a poor prognosis.
			Group 2: chromosome segments: 1p, 6q, 8p, 12p, 14q, 16p, 16q, 20p,	1q, 6p, 11q,	CN- LOH/loss 16q12, 16q23	CN-LOH/del16q12 and 16q23 have an adverse outcome.
Berry 2017[9]	n = 58	All CGH, 9 by SNP arrays			Chromothripsis 1q, 14, 16	16% with complex stepwise events and chromothripsis, both may have a poor prognosis
Chretien 2015[18]	n = 965	SNP array	1p32, 17p	3, 5, 7, 9, 11, 15, 18, 19, 21		Patients with t(4;14), trisomies 3 and/or 5 seemed to overcome the poor prognosis.
Kamada 2012[57]	n = 39	SNP array	1p, 6q, 8p, 9p, 11q, 13q, 16q, 22q	1q, 3q, 5, 6p, 7q, 9q, 11q, 15, 18, 19p, 19q, 21q	CN-LOH 1p, 1q, 2p, 2q, 6q, 8q, 9p, 13q, 16q, 22q	Loss of a single chromosomal arm was frequent at 1p, 8p, 13q, and 22q in more than 10% of both the hyperdiploid and the nonhyperdiploid patients. Hyperdiploidy with CN-LOH has a poorer prognosis than those without CN-LOH. del 13q has a poorer prognosis in nonhyperdiploid cases.
Kim 2015[54]	n = 35	CGH array	X, 1p, 1p22-21, 1p32, 6q, 12q, 14q	1q, 3q/3q21-23, 5p, 5q, 7q, 9p, 9q, 11q, 15q, 19q		CNV's at diagnosis significantly correlates with not achieving a very good partial response to VMP treatment, as well as, occurrence of progressive disease, suggesting that stronger or multicombinational therapeutic modalities might be required to eliminate resistant in MM cells.
Kjeldsen 2016[8]	n = 123	aCGH	Group 1: hyperdiploidy X, 13 Group 2: near diploid 1p22p21.1, 4q35.1, 5q13.2, 8p23, 14q24, 14q32q33, 16p11.2, 22q11, Xp21	Group 1: hyperdiploidy 3, 5, 7, 9, 11, 15, 19, 21 Group 2: near diploid 6q11q13, 7p15.2, 7q34, 15q24.1, 19p13.2, 19q13, Xq27q28	Chromothripsis 22% of new diagnoses, 16 most common, 1, 6, 8, 20 seen in both hyperdiploid and near diploid cases	Array failed to detect abnormalities seen by FISH clones present in <30% of cells, and detected a few translocations that were unbalanced. FISH for balanced rearrangements must be included in testing. Ploidy calculation by array may need to be adjusted by FISH.
Magrangeas 2011[58]	n = 764	SNP array	1q, 16q	1q, 16q	Chromothripsis 1q, 16q	Chromothripsis is seen in multiple chromosomes, primarily 1q and 16q, and is associated with poor prognosis.

(Continues)

TABLE 2 (Continued)

Reference	Sample size	Technique	Loss	Gain	Other abnormalities	Clinical information (Implications)
Mikulasova 2016[59]	n = 123	CGH+SNP array	1p, 6q, 8p, 12p, 13q, 16q	3, 5, 7, 9, 11, 15, 18, 19, 21	Structural abnormalities of 1q	Chromosome abnormalities are in two groups, hyperdiploidy and multiple structural abnormalities. Patients with gain 5q31 appear to have the best prognosis.
Smetana 2014[50]	n = 91	CGH array	1p, 6q16.3-q25, 8p, 11q22, 12p13, 13q, 14q, 16q, 17p13, 20p, 22	1q, 11, 17q21.33,20		100% of patients had abnormal microarray results.
Walker 2010[46]	n = 114	SNP array	1p, 6q, 8p, 8q, 12p, 13q, 14q, 16q, 17p, 20, 22 most common, 1p22.1-p21.3, 1p12, 1p31.1, 6q25.3, 13q14.11-q14.3, 16q22.1	X, 1q, 3, 5, 6p, 7, 9, 11, 15, 19, 21	CN-LOH 1q, 16q, X, *MYC* and *IGH* unbalanced rearrangements	CN-LOH is associated with regions of both gain and loss.

Copy number studies with a minimum of 30 patients were included in the table. These studies utilized either SNP microarray or comparative genomic hybridization (aCGH) (does not test for CN-LOH). In these studies, CN-LOH greater than 5-10 Mb was reported, unless the change is known to be clinically significant for myeloma.

12q21.33, 14q32.32, and 16p13.3.[51-53] Two of these losses, 12p and 16q containing the genes *CDKN1B* and *WWOX*, respectively, have been associated with a poor prognosis.[51] These regions are usually not included in the standard myeloma FISH panels and are not likely to be identified by standard cytogenetics until later in disease progression.

Homozygous loss of tumor suppressor genes such as *FAF1*and *CDKN2C* on 1p32.3, *BRCA2* and *RB1* on 13q14, *TRAF3* and *RCOR1* on 14q32.32, and *BIRC2/3* and *MMP* cluster on 11q22 have been observed in multiple studies and are associated with a poor outcome.[50,52,54,55]

4.4 | Loss of heterozygosity

CN-LOH is created by duplication of a chromosome or chromosomal regions following a deletion, recombination, gene conversion, or mitotic nondisjunction or loss. SNP microarray is particularly effective in identifying CN-LOH which may expose primary mutations that become homozygous or hemizygous, such as loss of tumor suppressor genes. At present, microarray is the only technique that can detect large regions (>10 Mb) of CN-LOH. This is particularly important as many of the regions are similar to the losses that are seen by FISH and chromosome analysis in myeloma such as 1p and 13q.[8,53] The most common regions of CN-LOH detected in myeloma are X, 1p, 6q, 8p, 13, 14q, 16q12-q23, and 17[56-59] (Table 2).

At present, there is not enough follow-up information on CN-LOH in myeloma for specific clinical prognoses, but in general, the clinical prognosis of CN-LOH is similar to that of loss of the same region. These include Xq in males, 1p, 6q, 16q, and 17p, which are all likely to indicate a poor prognosis.

Small stretches of homozygosity (usually <3 Mb) are seen in the normal constitutional karyotypes of outbred populations. Both the number and size of these regions are variable. These regions are most often interstitial stretches of homozygosity which may have arisen due to normal evolution, close common ancestry within an isolated population, consanguinity, or uniparental disomy. Acquired stretches of homozygosity are more often seen on terminal regions of the chromosomes. Care should be taken in the interpretation of CN-LOH, reporting only regions greater than 10 Mb as acquired genetic abnormalities related to myeloma, unless comparing results of normal tissue from the same individual.[60]

4.5 | Chromothripsis, chromoanasynthesis, and complex intrachromosomal copy number changes

Chromothripsis and chromoanasynthesis are phenomena that are localized to a chromosome, chromosome arm, or region and occur in a one catastrophic event, rather than being acquired over time. These complex patterns can be only detected by DNA based microarrays. In chromothripsis, the end result seen by microarray is complex DNA rearrangements of oscillating copy number states with loss of heterozygosity (LOH)[8,9,58]. Chromoanasynthesis is characterized by gained or amplified segments that retain heterozygosity. Similar complex copy number changes can also occur over time with clonal evolution. It may be difficult to separate the origin to these copy number changes to determine if the mechanism affects the progression or therapeutic response of the disease,

Although chromothripsis, chromoanasynthesis, and similar step-wise changes have been seen in many solid tumors, there have been very few studies related to myeloma. In the few studies utilizing copy number microarray, the most common chromosome regions that have been reported to have oscillating copy number variations are 1q, 14, and 16q, which were associated with a poor outcome.[8,9] It is highly likely that the consequences of complex gains, loss, and new gene rearrangements affect both expression

and function of many genes at once leading to aggressive disease progression. However, the clinical implications of complex copy number changes are limited to only a small number of cases. Similar changes in other chromosomes may not have the same prognostic indications.

4.6 | Conclusions

Multiple studies have reported that microarray testing performed on enriched plasma cell preparations yield abnormalities in more than 98% of cases. Most of the cases harbor over 20 alterations including biallelic losses, CN-LOH, and complex gains and losses, primarily in regions not covered by FISH[8,52]. Such complex genomic abnormalities as chromothripsis and chromoanasynthesis, which often cluster with high-risk factors, along with CN-LOH can only be detected with microarray. Although many of the aberrations identified, at present, do not have known predictive indications, there may be markers that can be used to follow residual disease, and still add value to treatment. Focal deletions and amplifications, along with minimum overlapping regions derived from larger copy number changes, can be surveyed for possible candidate genes involved in the clinical course of myeloma, and the development of targeted therapies.

Chromosome analysis and FISH have been the primary genetics tools for determining prognosis and guiding treatment, but with the emerging use of microarray, new routine workup strategies need to be considered. Abnormal karyotypes are usually seen only during aggressive disease, whereas almost all of cases reveal abnormalities by FISH together with microarray. As laboratories often receive small samples with varying percentages of plasma cells, it is important to focus on the clinical utility and cost-effectiveness of each test performed. In most laboratories, a single microarray is less expensive than a large myeloma FISH panel. However, the quality and quantity of a specimen rather than cost often directs the testing performed. If sufficient plasma cells have been enriched, FISH for *IGH* rearrangements and microarray are likely to have the highest detection rate. However, if the percentage of plasma cells is <6%, a myeloma FISH panel may be more likely to detect abnormalities. In cases without plasma cell enrichment, the microarray should only be performed when the percent of plasma cells is over 30%, as the sensitivity for most microarray platforms is 20%.

5 | CONCLUSION AND FUTURE PERSPECTIVES

In this report, we have reviewed G-band cytogenetic analysis, FISH, and molecular genetic studies for the genetic characterization of MM patients. This review summarized that cytogenetic evaluation is important for all patients with newly diagnosed MM and includes interphase FISH with enrichment of $CD138^+$ cells especially in samples having less plasma cells. As the published risk stratification algorithms in MM include rearrangements such as t(4;11) and t(14;16) that are best detected by FISH, FISH testing is often the first-tier test performed in clinical laboratories and remains an important part of the cytogenetic workup of MM patients. However, microarray-based genomic profiling is increasingly used to detect copy number alteration throughout the genome, as it is more cost-effective than performing multiple FISH tests for the detection of gains and losses. Conventional G-banding can also be performed to further characterize the ploidy level and help with the integration of clonal heterogeneity in some patients. The advent of even newer assays such as high throughput sequencing will provide additional diagnostic and prognostic markers for an era of genome-guided target therapies in the near future. In summary, it is important to understand the limitation of each detection technologies. While the next generation sequencing (NGS) is still largely unavailable as a routine laboratory testing, conventional karyotyping, interphase FISH, and microarray analysis will remain for now the most reliable method of cytogenetic profiling in MM patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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