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Guidelines for the testing and reporting of cytogenetic results for risk stratification of multiple myeloma: a report of the Cancer Genomics Consortium Plasma Cell Neoplasm Working Group

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Fluorescence in situ hybridization (FISH) remains the gold-standard clinical assay to detect genetic abnormalities in multiple myeloma (MM). However, FISH panel design, use of conventional chromosome banding analysis and reporting practices have been reported to vary among laboratories. Therefore, standardization in FISH testing and reporting practices is needed to improve report clarity and avoid misinterpretation. The recommendations in this paper represent a consensus of our Cancer Genomics Consortium Plasma Cell Neoplasm Working Group, comprising a joint panel of cytogenetic laboratory directors and clinical investigators with expertise in the diagnosis, risk stratification, and treatment of multiple myeloma. Prior to developing these consensus recommendations, we performed a full literature review and conducted a survey of 102 oncologists to assess current variations and challenges in MM cytogenetic/FISH testing and reporting. Our guidelines establish best practices for the optimization of FISH panel selection, and recommendations for standardized reporting of cytogenetic results to align with the 2025 International Myeloma Society (IMS)/International Myeloma Working Group (IMWG) Updated Risk Stratification.

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INTRODUCTION

Multiple myeloma (MM) is a plasma cell (PC) neoplasm representing ~10% of all blood-related cancers [1]. Nearly all cases of MM can be characterized by either recurrent immunoglobulin rearrangements (*IGH-r*) and/or hyperdiploidy defined by gains of odd-numbered chromosomes (trisomies) [1]. Approximately 40% of MM can be defined by five generally mutually exclusive *IGH-r* including t(4;14)(p16;q32), t(14;16)(q32;q23), t(14;20)(q32;q12), t(11;14)(q13;q32) and t(6;14)(p21.1;q32). Approximately 50% can be characterized by hyperdiploidy with or without a primary *IGH-r* [2–4]. These recurring acquired cytogenetic abnormalities within the PC clone represent known biomarkers of disease prognosis and response to therapy [3]. Given the prognostic and therapeutic value, these cytogenetic data have been incorporated into risk stratification guidelines including 1) the International Myeloma Society/International Myeloma Working Group (IMWG) [5]; 2) the National Comprehensive Cancer Network (NCCN) [6]; and 3) the Mayo Stratification for Myeloma and Risk-Adapted Therapy (mSMART) [7]. All guidelines consider deletion and/or mutation of *TP53* as a high-risk cytogenetic entity [8, 9]. Most guidelines now consider either t(4;14), t(14;16) and t(14;20) in combination with gains or amplifications of 1q21 or deletions of 1p32 as high-risk. All other abnormalities such as hyperdiploidy, t(11;14) or t(6;14) when occurring without a *TP53* deletion or mutation or biallelic 1p32 deletion are considered standard risk.

Risk-defining cytogenetic abnormalities are currently detected using fluorescence in situ hybridization (FISH). Although these abnormalities can also be identified by conventional chromosome banding analysis, chromosomal microarray analysis (CMA) or next-generation sequencing (NGS), FISH remains the current gold-

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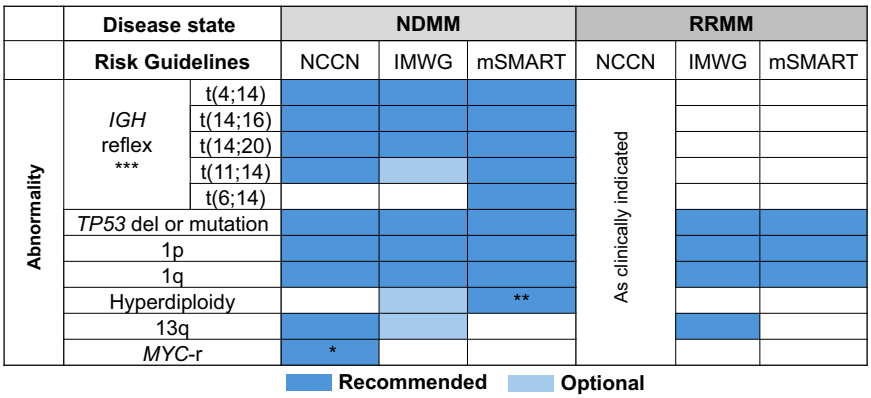

Fig. 1 Previous FISH panels proposed by various expert groups. FISH panels incorporated into the major risk stratification guidelines (NCCN, IMWG and mSMART). * While not on the recommended diagnostic minimal panel, the NCCN lists *MYC*-r as a factor considered high-risk for progression/relapse. ** Hyperdiploidy defined by multiple trisomies can be detected by FISH or an equivalent method like flow cytometry. *** Consider implementing a reflex strategy. Many laboratories use an *IGH* BAP probe to determine rearrangement status. If an *IGH* BAP signal pattern is detected, laboratories will perform a reflex panel including pertinent *IGH* partners. However, rare *IGH* translocations may occur resulting in a "false-negative" using *IGH* BA; therefore, the decision to perform sequential testing is at the discretion of the individual laboratory. If a *TP53* deletion is identified, reflex to evaluate for *TP53* mutation can be considered, but is not required.

standard clinical assay in the genetic evaluation of MM. However, MM clinical workup is heterogeneous among numerous clinical laboratories worldwide, often with variations in FISH panel design, PC enrichment methodologies, use of conventional chromosome banding analysis and reporting practices [10, 11]. This lack of standardization has resulted in confusion and may increase the probability of incorrect integration of cytogenetic results in risk stratification [12]. To improve the standardization of cytogenetic testing in MM, we formed the Cancer Genomics Consortium (CGC) Plasma Cell Neoplasm Working Group composed of board-certified cytogenetic laboratory directors from a variety of clinical laboratory settings from the United States and Canada to propose clear solutions to improve standardization and establish best MM FISH panel design and reporting practices aimed to improve the care of patients with MM.

CANCER GENOMICS CONSORTIUM PLASMA CELL NEOPLASM WORKING GROUP

The CGC Plasma Cell Neoplasm Working Group was formed by a professional group of 12 clinical cytogenetic laboratory directors from the CGC, an organization formed in 2009 with the goal of promoting best practices in clinical laboratory cancer genomics. The workgroup members represent small to medium academic laboratories and large reference commercial laboratories within the United States and Canada. The workgroup convened monthly for 1-hour via teleconference for 1.5 years. We also included three clinical MM oncologists from Mayo Clinic (SK and RF) and U of Washington (RB). We collected peer-reviewed literature of large case series or clinical trials concentrating on the clinical significance of cytogenetic abnormalities in MM. We searched the National Institute of Health National Library of Medicine's "PubMed" database for studies in humans published in English between 2013 to 2024 with an emphasis on publications focusing on cytogenetics within the last 5 years. The role of each abnormality in diagnosis, prognosis, and therapy, and methods for detection was discussed. The primary literature was also evaluated to identify best practices for the cytogenetic evaluation of MM including the value of conventional chromosome banding analysis, optimization of FISH panel selection, and standardization of clinical reporting of cytogenetic results. Articles which focused on pre-malignant conditions such as monoclonal gammopathy of undetermined significance or smoldering MM were excluded as the clinical significance of different abnormalities in these contexts may vary from MM. To identify the status and aid the development of our recommendations, we conducted a survey interrogating numerous aspects of clinical MM FISH testing and reporting. We also ensured that our recommendations for testing and reporting are fully aligned with the 2025 IMS/IMWG Updated Risk Stratification [5].

CONSENSUS RECOMMENDATIONS FOR STANDARD FISH PANEL DESIGN AND TESTING ALGORITHM

Newly diagnosed MM (NDMM) are often characterized by two major primary genomic events including *IGH* rearrangement at chromosomal level (*IGH-r*) which accounts for ~40% of NDMM cases [3, 13] and hyperdiploidy, which is often defined as gain of at least two or more odd-numbered chromosomes including chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, and accounts for ~50% of the NDMM cases [14–16].

Our recommendations are based on a literature review of data from existing original research papers as well as current risk-stratification guidelines (Fig. 1) to ensure that we have evidence to support the clinical significance of each recommended FISH probe. We propose two standard FISH panels, an initial diagnostic panel, and a relapsed MM panel (Fig. 2). For all newly diagnosed MM (NDMM), a minimal evaluation for the following primary *IGH-r*, is recommended: t(4;14), t(14;16), t(14;20), t(11;14) if an *IGH-r* has been detected in the initial screen. Detection of an *IGH-r* occurs when the *IGH* break-apart (BAP) FISH probe is abnormal. Both the diagnostic and relapsed MM panels should include evaluation for the following abnormalities: 17p deletion including the *TP53* gene, 1p deletion and 1q gain or amplification.

Translocation t(4;14) is present in ~15% of NDMM and results in overexpression of *FGFR3* and/or *NSD2* [17]. The t(14;16) is present in 3–5% of NDMM and leads to increased expression of *MAF* [18] while the t(14;20) is present in 1–2% of NDMM and leads to increased expression of *MAFB* [3, 19]. These subtypes are often associated with other high-risk secondary abnormalities [18, 20], a high mutation burden and hyper-APOBEC activity characterized by single base substitution (SBS2 and SBS13) signatures [21–24]. The t(11;14) involves *IGH* and *CCND1* resulting in increased *CCND1* expression, is present in 15–20% of NDMM and in 50% primary plasma cell leukemia, representing the most common primary *IGH-r* in MM [25, 26]. Patients with t(11;14) have been reported to be more sensitive to BCL2 inhibition [27–29].

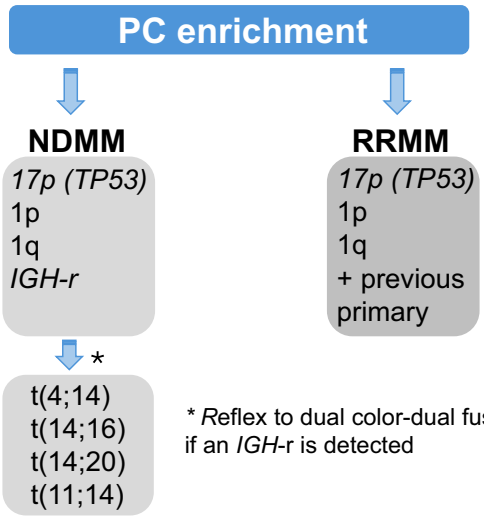

Fig. 2 Minimum FISH panel proposed to conform to the 2025 IMS/IMWG risk stratification. Consensus recommendation for minimum FISH panel design includes detection of *TP53* deletion using a probe targeting 17p13, 1q gain or amplification using a probe targeting 1q21 (typically *CKS1B*), 1p deletion using a probe tageting 1p32 (typically *CDKN2C*) and *IGH*-r for all NDMM. * If an *IGH*-r is identified, reflex to DC-DF probes targeting t(4;14), t(14;16), t(14;20) and t(11;14) should be performed. Detection of an *IGH-r* occurs when the *IGH* break-apart (BAP) FISH probe is abnormal. For RRMM, a minimal FISH panel that includes assessment for *TP53* deletion, 1p deletion, 1q gain or amplification, and at least one probe targeting the primary abnormality observed at diagnosis is recommended. Inclusion of a previous primary abnormality serves as a positive control for detection of the patient's previously described MM clone.

Detection of each of these *IGH-r* can be achieved using specific dual-color, dual-fusion (DC-DF) probes. Laboratories often use an *IGH* break-apart (BAP) probe as an initial screen. If abnormal, including deletions of either 5′ or 3′ *IGH*, the laboratory typically performs reflex testing with t(4;14), t(14;16), t(14;20) and t(11;14) DC-DF probes (Fig. 2) in a labor and cost-conscious approach enabling the judicious use of limited enriched PC samples. The approach is supported by evidence indicating that nearly all recurrent *IGH-r* result in an abnormal *IGH* BAP result [30] and primary *IGH-r* are generally mutually exclusive [3, 13]. Of those cases with an abnormal *IGH* BAP result, approximately 80% have a recurrent *IGH*-r, while the remaining 20% have an *IGH* rearrangement not involving *CCND1, CCND3, FGFR3, MAF, MAFB* or have deletions of either 5′ or 3′ *IGH* [3]. While the specific reflex design or ordering may vary between cytogenetic laboratories, all laboratories must have a system to identify all recurrent *IGH-r*.

Deletion of *TP53*, located at 17p, is present in 7–10% of NDMM [31] and up to 80% of patients with relapsed and/or refractory multiple myeloma (RRMM) and in secondary plasma cell leukemia, [32]. *TP53* biallelic inactivation can be observed as a biallelic deletion, or a heterozygous deletion with concurrent mutations detected by sequencing, sometimes resulting in aberrant splicing [33, 34]. *TP53* deletion can be detected by FISH using enumeration and locus-specific probes targeting the centromere of chromosome 17 and 17p (*TP53*).

Chromosome 1 abnormalities and their associated clinical implications in MM have been historically difficult to define due to the use of different FISH probe targets and variability in reporting of copy states of chromosome 1. Deletion of 1p is present in 8–12% of NDMM [35–37] and the most significant region of interest is reported to be 1p32.3 (*CDKN2C, FAF1*) [11, 38, 39]. 1p deletion can be detected using FISH probes targeting *CDKN2C*. Gain or amplification of 1q is present in 30–45% of NDMM [37, 40] and in 55–70% of MM patients at relapse [37, 40]. Gain of 1q is defined as 3 total copies of 1q and amplification is defined as 4 or more total copies of 1q (relative to a diploid genome). Standardization of reporting practices of 1q have been particularly challenging in the cytogenetics community [12]. Although gain/amplification of 1q is typically extensive and involves multiple genes in large regions of chromosome 1 [11, 38, 39, 41], it is typically detected using FISH probes targeting *CKS1B*. Probes targeting 1p and 1q are often combined.

For RRMM, a minimal FISH panel that includes assessment for *TP53* deletion, 1p deletion, 1q gain or amplification, and at least one probe targeting the primary abnormality observed at diagnosis is recommended (Fig. 2). Including a previously abnormal probe allows for confirmation that abnormal PCs have been identified minimizing the risk of a "false-negative" FISH study. Prioritization of FISH probes associated with high-risk disease, such as *TP53*, t(4;14) and 1p/1q can be performed in cases with limited PCs. Following treatment, FISH testing is not recommended to evaluate for minimum residual disease. In cases where the initial FISH test at diagnosis was incomplete or insufficient, a comprehensive diagnostic panel may be warranted.

OPTIONAL FISH PROBES FOR MULTIPLE MYELOMA

For all NDMM, additional probes to evaluate for ploidy including hyperdiploidy or hyperhaploidy, t(6;14), *MYC*-r, or 13q deletion may be considered. Hyperdiploidy is found in approximately 50% of NDMM and is typically associated with 47–57 chromosome count [14–16]. As the classic definition of hyperdiploidy was derived from a genome-wide chromosome perspective, the identification of hyperdiploidy is challenging when using a limited FISH panel. However, many clinical studies have evaluated the prognostic significance of FISH-based hyperdiploidy considering ≥2 odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 [42]. If ploidy assessment is desired, FISH targeting odd-numbered chromosomes using tricolor probe sets of chromosomes can be utilized. Enumeration of chromosome 15 may have reduced sensitivity in association with African ancestry [43]. Flow cytometry [44] or CMA can also be used to assess hyperdiploidy.

Hyperhaploidy is an infrequent subtype associated with a genome wide loss of numerous chromosomes typically with a 24–34 chromosome count. The disomic chromosomes retained in hyperhaploidy are the same odd-numbered chromosomes found in hyperdiploidy. *IGH-r* are infrequently observed, but mutations of *TP53* are common and often biallelic [45, 46]. Although hyperhaploidy is not a specific entity in risk stratification guidelines, *TP53* deletion and mutation status is included. Use of probes targeting t(4;14), t(14;16), and *TP53* allows detection of loss of chromosomes 4, 14, 16, and 17 enabling identification of potential hyperhaploidy.

Another rare *IGH*-r that can be considered for evaluation includes the t(6;14), present in 1–2% of NDMM leading to increased expression of *CCND3* [3, 21, 47]. Using gene expression analysis, the profile of PCs with t(6;14) is similar to t(11;14) [48–50]. Both t(6;14) and t(11;14) can be represented by 2–3 distinct expression groups (termed CD-1, CD-2a and CD2b) with CD-1 having increased expression of KLHL4 and INHBE and CD-2 having increased expression of CD20, VPREB and PAX-5) [48–50]. Similar to t(11;14), patients with t(6;14) may also be sensitive to BCL2 inhibition [51]. The t(6;14), detected using DC-DF FISH probes for *IGH* and *CCND3*, can be considered reflex *IGH* testing.

Secondary rearrangements involving *MYC* (located at 8q24.21) are associated with increased *MYC* expression, and are present in 30–45% of NDMM and 50% of RRMM when detected by genomic sequencing [52–54]. *MYC-r* have been associated with progression of MM [55, 56], can be complex and involve numerous genomic mechanisms and partner genes [57]. *MYC-r* have not yet been incorporated into many risk guidelines but are discussed in the

current 2024 NCCN guidelines [6]. Detection of *MYC-r* can be achieved using a *MYC* BAP or specific DC-DF probe sets, although only about half of *MYC-r* can be identified by FISH [53, 58].

Deletion of 13q or monosomy 13 is present in approximately half of NDMM [38, 59–62] and is frequently associated with other MM subtypes t(4;14) and deletion of *TP53* [60, 62–64]. The prognostic significance of del(13q) has been debated and may be a surrogate marker or a weak contributor to disease risk. Thus, del(13q) is not currently included in most risk stratification guidelines. Del(13q) is most often detected by FISH using probe sets targeting the *RB1* locus at 13q14.2 or *DLEU1/MIR15A/MIR16-1* (D13S319 or D13S25) at 13q14.2q14.3, with a control locus in distal 13q34, allowing differentiation of interstitial deletions from presumed monosomies [11].

INTERPRETATION OF ABNORMAL FISH RESULTS

The most common FISH patterns are indicated in Supplementary Table 1. Hyperdiploidy is suggested when ≥2 odd-numbered chromosomes are identified, however identification of hyperdiploidy can be a challenge to precisely define with limited FISH panels. Distinguishing between hyperdiploidy or near-tetraploidy may not be possible. Tetraploidy or near-tetraploidy, which typically refers to the doubling or endoreduplication of a diploid genome, can be found between 5–10% of MM cases, an estimate dependent on the method of ascertainment. Near-tetraploidy can be present as a secondary clone, sometimes at low level [65, 66]. Overall, ascertainment of the ploidy status of a clone by FISH presents a challenge.

Caution should be taken when interpreting gains and amplifications of 1q and deletion of *TP53* in the context of a tetraploid/near-tetraploid genome. When 2 copies of *TP53* are observed in the context of a tetraploid (four copy) genomic state, interpretation of a deletion of 17p may be appropriate if there is evidence of a diploid clone with a *TP53* deletion. However, in the context of limited ploidy assessment, minimization of the term "relative deletion" is advised and inclusion of a statement indicating that it is unclear if *TP53* function is impacted could be helpful to underscore the uncertainty of the finding. Correlating with *TP53* mutation status is recommended. Similarly, when 6 copies of 1q are observed in the context of a near-tetraploid genomic state, interpretation of a relative gain of 1q (not amplification) may be appropriate if there is evidence of a diploid clone with a 1q gain.

Our survey showed that clarity of FISH testing and interpretation is needed (Supplementary Results and Supplementary Fig. 1A–E). Only 60–65% of respondents were satisfied with the clarity, summary, and interpretation, respectively, of the FISH reports they currently receive (Supplementary Table 2). When clinicians were challenged with a difficult-to-interpret FISH result (Supplementary Material-Survey question 11) where an *IGH*-r with an unknown partner was listed as "atypical positive" for all listed FISH probes, 22% interpreted the report correctly with the remaining unsure of the interpretation (25%) or misinterpreted the report (53%) demonstrating a need for improved FISH report clarity.

CONSENSUS RECOMMENDATIONS FOR REPORTING FISH RESULTS FOR RISK STRATIFICATION OF MYELOMA

In addition to the general FISH result reporting recommendations from the American College of Medical Genetics and Genomics (ACMG) Technical Standards for Clinical Genetics Laboratories (2021 revision) and in the College of American Pathologists (CAP) Cytogenetics Checklist we suggest the inclusion of the following additional elements.

- **Highlighting critical results**. This may include a concise summary sentence at the top of the report in bold. For example, "Gain of 1q (*CKS1B*) detected", "Deletion of *TP53* detected", "t(11;14)/*IGH::CCND1* rearrangement detected", "t(14;20)/*IGH::MAFB* rearrangement not detected".
- **Table of results**. A table of results can be included to document FISH probes being tested, the laboratory's established cut-off value for each probe and the abnormality being detected or not detected. However, we urge caution regarding the interpretation of the percent positivity particularly regarding secondary abnormalities, as this percentage will depend on the PC burden and enrichment. Historic results can also be included for comparison for specific abnormal signal patterns in follow-up studies.
- **The use of plain language**. Some may recognize cytogenetic abnormalities by the chromosomal aberrations while others may recognize them by their gene names. It is recommended to include both gene names and the associated chromosomal aberration in the report. For example, "t(11;14)/*IGH::CCND1* rearrangement detected".
- **The use of standardized language**. The use of standardized language beyond the use of the international system for human cytogenomic nomenclature (ISCN) is critically important to improve clarity of result. We further recommend:
  - Use of "**rearrangement**" to describe for example, a t(11;14)/*IGH::CCND1*
  - Use of "**gain**" to describe an additional copy number signal and "**amplification**" to describe gain of two or more signals.
  - Use of "**deletion**" to describe a copy number loss (heterozygous or biallelic).
  - Avoid the term "**atypical abnormal**" when a FISH signal pattern displays a technically abnormal result but does not detect an expected abnormality. For example, a gain of an additional *IGH* signal may be observed in the context of a t(4;14)-r when using the t(11;14) FISH probe set. Rather, "**not detected**" for t(11;14) and "**detected**" for t(4;14) is suggested to avoid confusion.
  - **Clearly defined methods**. It is important to indicate whether PC enrichment was performed with the following comment: "Interphase FISH analysis was performed on cultured or uncultured bone marrow cells" or "Interphase FISH analysis was performed on plasma cells enriched from the bone marrow sample".
  - **Clear language that interprets the FISH results and accurately classifies patients into standard-risk and high-risk based on the 2025 IMS/IMWG Risk Stratification guidelines** [5] (Figs. 3–4).

A sample report incorporating improvements highlighted above can be found in (Fig. 4). When we enhanced the clarity and improved the result summary and interpretation implementing the recommendations described above, about 80% of the clinicians were now satisfied with the improved reporting format and interpretation (Supplementary Table 3).

OPTIONAL INCLUSION OF *TP53* MUTATION TESTING

The IMS/IMWG Risk Stratification guidelines now recognize *TP53* mutation as a high-risk entity [5]. Mutation in *TP53* occurs in approximately 6% of NDMM [67]. Notably, *TP53* mutations are predominantly associated with *TP53* deletions, with studies indicating that about 37% of patients with a *TP53* deletion harbor a *TP53* mutation while patients without a *TP53* deletion were not reported to have a *TP53* mutation [68]. Thus, if sample is available, evaluation for *TP53* mutation may be considered particularly in cases without a *TP53* deletion, but it may not be feasible to perform mutation testing in most laboratories globally. Although bi-allelic *TP53* inactivation (double hit) has been recognized as an

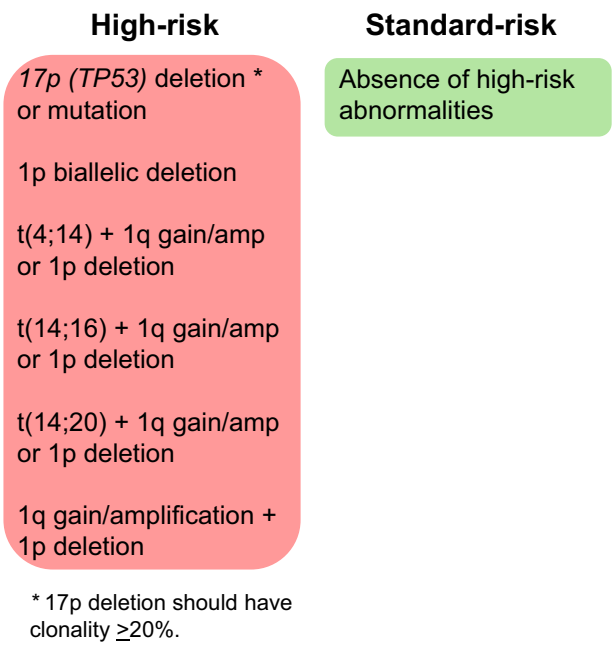

Fig. 3 Cytogenetic risk assessment and reporting for MM according to the 2025 IMS/IMWG risk stratification. Following FISH detection, laboratories should provide a report using clear language that interprets the FISH results with accurate classification into standard-risk and high-risk based on the 2025 IMS/IMWG Risk Stratification guidelines. Cytogenetic high-risk includes the presence of at least one of the following cytogenetic abnormalities: *TP53* deletion or *TP53* mutation, 1p biallelic deletion, t(4;14) plus 1q gain or amplification or 1p deletion, t(14;16) plus 1q gain or amplification or 1p deletion, t(14;20) plus 1q gain or amplification or 1p deletion, 1q gain or amplification plus 1p deletion. 17p deletion should have clonality of ≥20%. Standard-risk includes the absense of a high-risk cytogenetic abnormality and can include isolated hyperdiploidy, t(11;14) or t(6;14). Other features such as β2 microglobulin and creatinine also impact the overall IMS/IMWG risk stratification-please refer to Avet-Loiseau, et al. [5] for a complete risk stratification incorporating these variables along with the cytogenetics.

important driver of prognosis when compared to mono-allelic *TP53* inactivation [67], the IMS/IMWG considers *TP53* deletion or *TP53* mutation as high risk. Thus, testing for *TP53* mutation in the context of a *TP53* deletion is not currently necessary for the IMS/IMWG risk stratification. If *TP53* mutation testing is desired, such testing can be performed by NGS using DNA extracted from a PC-enriched sample using an MM-targeted sequencing panel designed, as described by others [69–72]. Caution is advised when interpreting *TP53* mutation status from NGS performed on non-PC-enriched samples, as admixture with non-MM cells may affect accuracy. Over time, we anticipate these capture-based NGS tests, or even WGS or other technologies, when performed on PC-enriched samples will eventually replace FISH, as discussed by Akkari, et al. [73].

TECHNICAL RECOMMENDATIONS

Special requirement or consideration for FISH studies for multiple myeloma

PC enrichment. A challenge in performing FISH on PCs is the generally low proportions of PCs in bone marrow aspirates, in comparison to other malignancies such as acute leukemias. Therefore, it is recommended that all samples undergo PC enrichment to minimize the potential for "false-negative" test results. Of interest, about 20% of the surveyed MM clinicians were unaware of (12.9%) or did not use (5.9%) PC enrichment by their FISH laboratory (Supplementary Fig. 1F). Enrichment of PCs is typically achieved using several technologies, typically utilizing antibodies that are relatively specific for PCs, i.e. CD138. The majority of laboratories utilize CD138 magnetic beads for enrichment [10], while some laboratories enrich PCs using a combination of antibodies including CD138, CD38, CD319, or CD229 by flow sorting [74–80]. However, flow cytometry may be less efficient at PC recovery in comparison to magnetic-based enrichment [76, 81]. Enrichment is subject to a variety of factors including sample volume, PC concentration and time to processing (where CD138 and other cell surface markers can be internalized over time) [82]. When possible, 1–2 ml of bone marrow aspirate from the first or second draw should be used. Samples should be processed within 24 h, but no more than 120 h after collection, depending on each laboratory's validation of sample stability [82]. Sodium heparin and EDTA are acceptable anticoagulants. Bone marrow is preferred for PC enrichment, though peripheral blood can be used in cases of blood involvement such as in plasma cell leukemia. Confirmation of adequate enrichment is necessary to ensure an appreciable increase of PCs during validation [77, 83]. Samples with differing PC levels should be included in the verification process to evaluate the effectiveness of enrichment relative to PC burden [84]. Additionally, multiple samples with known aberrations should be tested with standard MM probes to ensure that the enrichment process did not affect the ability of cells to produce correlative FISH results. Each laboratory must establish its own cutoff value for MM FISH using the ACMG technical standards [85]. The preferred number of enriched PCs assessed by FISH should be 50–100 [75, 86]. If PC enrichment is not possible, FISH on direct bone marrow aspirate or cultured sample when flow supports >10–20% abnormal PCs may be possible.

Bone marrow smear and touch preparation utilization for FISH. Bone marrow smears and touch preparation slides can be utilized for FISH testing, when the aspirate has low cell count with patchy PCs by morphology [87]. Communication with the hematopathologist and evaluation of smears or touch preps should be performed prior to FISH to ensure the presence of adequate abnormal PCs. When FISH is performed, 200 cells should be assessed, but 50–100 cells may be evaluated in cases with low cellularity. We recommend the following disclaimer is included: "The sensitivity of FISH analysis of unstained smears is lower as compared to the analysis of $CD138^+$ enriched plasma cells, and the negative FISH results do not preclude the presence of abnormalities associated with plasma cell neoplasms. If clinically indicated, a repeat FISH study on $CD138^+$ enriched PCs is recommended." Occasionally, FISH studies can be performed on paraffin-embedded tissue, if appropriate validation studies are in place.

G-banded karyotype studies in multiple myeloma. Except for the cryptic t(4;14), nearly all cytogenetic subtypes of MM can be identified by conventional chromosome banding analysis when there is an abnormally dividing PC clone in cell culture. However, due to the low-proliferative nature of PCs, only about 20% of karyotypes in MM are abnormal, even following IL-4 stimulation [88, 89]. Therefore, some laboratories do not perform conventional chromosome banding analysis, instead assessing proliferative capacity with S-phase by flow cytometry [90]. ACMG guidelines continue to recommend karyotyping [84, 90], since a complex karyotype (≥ 3 clonal abnormalities) indicates poor risk [4, 91] and often reflects high PC proliferation associated with an aggressive PC clone [2, 4, 90]. The IL-4 stimulated cell pellet can be used for FISH if needed. Conventional chromosome banding analysis in unstimulated cultures may detect concurrent therapy-related myeloid neoplasms, particularly when a monosomy 5 or monosomy 7 (or del(5q) or del(7q)) is identified [89]. The addition of myelodysplastic syndrome (MDS) FISH panels could also be considered in scenarios of cytopenias unexplained by MM or in

Sample Report

Case Details
Cytogenetics Sample Number: 24NM-1234
Sample Type: Bone marrow aspirate
Associated Case Number: BM24-5678
Collection date: 02/11/2024 10:20
Reason For Referral: Multiple myeloma
Clinical Status: Diagnosis

Result Summary

FISH performed on CD138+ enriched plasma cells: t(11;14)/*IGH*::*CCND1* rearrangement detected and hyperdiploidy detected.

1q21 gain or amplification (*CKS1B*) not detected, gain of chromosome 11 not detected, deletion of chromosome 17 (*TP53*) not detected, deletion of 1p (*CDKN2C*) and *MYC* rearrangement not detected.

Interpretation

Interphase FISH analysis performed on **enriched plasma cells** identified an *IGH* rearrangement (61%) that was confirmed to be a t(11;14)/*IGH*::*CCND1* rearrangement (61.3%), gain of chromosomes 3, 5, 7, 9, and 15 (7.2-13.2%) indicative of hyperdiploidy. FISH analysis was within normal limits for all other loci tested (see methods).

In multiple myeloma, this finding represents a standard-risk cytogenetic abnormality based on the 2025 International Myeloma Society/International Myeloma Working Group Risk Stratification.

A complete description of the signal configurations observed, as well as results from control studies, are on file in the cytogenetics Laboratory.

Results

Test Performed	*Results*	*Cut-off values*
Gain of chromosome 3	**Detected (13.2%)**	6.1%
Gain of 5p15.2 or chromosome 5	**Detected (8.1%)**	2.0%
Gain of chromosome 7	**Detected (7.7%)**	2.0%
Gain of chromosome 9	**Detected (7.9%)**	2.0%
t(11;14)(q32.3;q13.3)/*IGH*::*CCND1* rearrangement	**Detected (61.3%)**	1.1%
IGH (14q32.3) rearrangement	**Detected (61.8%)**	7.1%
Gain of chromosome 15	**Detected (7.2%)**	3.3%
Gain of 1q/*CKS1B* (1q21.3)	Not detected	2.2%
Deletion of 1p/*CDKN2C* (1p32.3)	Not detected	6.1%
MYC (8q24.2) rearrangement	Not detected	1.5%
Gain of chromosome 11	Not detected	3.3%
Deletion of chromosome 17	Not detected	3.0%
Deletion of 17p/*TP53* (17p13.1)	Not detected	5.1%

FISH history (Previous results are tableted if reported by the same lab)

ISCN

nuc ish(*CDKN2C*,*CKS1B*)x2[100]
nuc ish(D3Z1x3)[13/100]
nuc ish(D5S23/D5S721x3)[8/100]
nuc ish(D7Z1x3)[7/100]
nuc ish(*MYC*x2)[100]
nuc ish(D9Z1x3)[7/100]
nuc ish(D11Z1)x2[100]
nuc ish(*CCND1*,*IGH*)x3(*CCND1* con *IGH*x2)[61/100]/(*CCND1*x2,*IGH*x3)[11/100]
nuc ish(*IGH*x2)(3'*IGH* sep 5'*IGH*x1)[61/100]
nuc ish(D15Z4x3)[7/100]
nuc ish(*TP53*,D17Z1)x2[100]

Methods

Specimen: Bone Marrow Aspirate, CD138+ enriched

Methods: Interphase fluorescence in situ hybridization with probes from (Vendor)

1. *CDKN2C* (1p32.3, SpectrumGreen) and *CKS1B* (1q21.3, SpectrumOrange)
2. D3Z1 (SpectrumOrange), D7Z1 (SpectrumGreen), D11Z1 (SpectrumAqua) (centromere of chromosomes 3, 7, and 11)
3. D5S23/D5S721 (5p15.2, SpectrumGreen), CEP 9 (SpectrumAqua), D15Z4 (SpectrumOrange) (centromere of chromosomes 9 and 15)
4. 5'*MYC* (SpectrumOrange) and 3'*MYC* (SpectrumGreen), (8q24.2), break-apart
5. *CCND1* (11q13.3, SpectrumOrange) and *IGH* (14q32.3, SpectrumGreen)
6. 5'*IGH* (SpectrumGreen) and 3'*IGH* (SpectrumOrange) (14q32.3), break-apart
7. *TP53* (17p13.1, SpectrumOrange), and D17Z1 (centromere of chromosome 17, SpectrumGreen)

Quality control: A negative control or a positive control, if applicable, is performed in parallel with the patient sample in the same hybridization process.

Scoring and analysis: A total of 100 interphase cells were evaluated for each probe independently by two technologists. An abnormal result is determined if the percentage of cells with abnormal FISH signal patterns is above the relevant cut-off values at 95% confidence.

Assay Disclaimer: This FISH test was developed, and its performance characteristics determined by this laboratory. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvements Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.

the context of transplant or T-cell redirecting therapy. The value of conventional chromosome banding analysis was further reflected in the MM clinician survey where conventional chromosome banding analysis was always utilized by 59.8% of clinicians, never utilized in 17.6% and utilized depending on the clinical context (concern for MDS) in 22.5% of clinicians (Supplementary Fig. 1G). We recommend laboratories validate a workflow which prioritizes PC enrichment for FISH. If needed, the remaining sample can be used for conventional chromosome banding analysis after 24 hr and/or 72 hr stimulated culture. Correlation of conventional chromosome banding analysis and FISH results is recommended.

Fig. 4 Recommended format and components of sample report to conform to the new 2025 IMS/IMWG Risk Stratification of Myeloma. Sample of a FISH report reflecting the critical results highlighted in bold at the top of the report under "result summary" (example in blue shading). In this case, there is a t(11;14)/*IGH::CCND1* rearrangement and hyperdiploidy detected. Also indicated are the results for cytogenetic abnormalities not detected in this sample. An interpretation written in plain and standardized language is provided which indicates that FISH analysis was performed on enriched plasma cells and identifies specifically which abnormalities were detected and not detected (example in purple shading). A comment about whether the FISH findings represent a standard or high-risk abnormality according to the 2025 IMS/IMWG risk stratification is also indicated. A table of results is provided (example in green shading), which indicates the FISH probes being tested, the laboratory's established cut-off values for each probe and the abnormality being detected or not detected. Finally, additional technical details including previous FISH results if available, the ISCN nomenclature and the methods are also included (example in grey shading).

SUMMARY

FISH testing on enriched PCs has long been recognized as the standard-of-care assay to detect recurrent cytogenetic aberrations that can be used to incorporate into risk stratification algorithms. Here, we summarize the clinical value of recurrent chromosomal abnormalities, recommend optimal MM FISH panel design, analysis, interpretation, and clear reporting practices with the goal to provide solutions that can be implemented by other laboratories to improve the quality of their MM FISH testing practices.

We recommend close communication with the clinical management team including MM oncologists/providers, cytogeneticists, molecular geneticists and pathologists to ensure maximal diagnostic benefit. We observed increased utilization of in-house FISH testing among providers within the academic setting in comparison to the community setting. This suggests additional challenges may exist among the community providers, who are more likely to utilize reference laboratories, for their FISH testing. Ease and frequency of communication with the cytogeneticist at the reference laboratory may differ in comparison to in-house cytogenetics laboratories. Reduced access to the reference laboratory may be reflected by the increased frequency of providers who reported a lack of knowledge of PC enrichment practices among their reference laboratory services. Further, the impact of increased transit times of the PC specimen when evaluated by the reference laboratory is unknown. A direct comparison of PC FISH failure rates between reference and in-house FISH laboratory could be of value.

MM FISH interpretations remain a challenge even among the cytogeneticists within the workgroup. Identification of hyperdiploidy or even overall ploidy status in the context of small FISH panels and whether optimal quantification of 1q gain should be determined relative to the overall ploidy of the clone or determined based on total copies of 1q independent of clonal ploidy status can be a challenge. Implementation of MM risk stratification systems based on FISH data is imperfect as cases can harbor multiple high-risk and standard-risk factors [92]. Studies have shown a synergistic negative impact of high-risk abnormalities including *TP53* deletion in combination with other high-risk *IGH-r* such as in cases with "double hit" high-risk abnormalities [67]. Some evidence suggests that the combination of trisomies may reduce the risk of high-risk abnormalities [88]. Thus, a comprehensive genomic scoring system, ideally genome-wide assessment of cytogenetic aberrations, genomic mutations, and PC proliferation index may improve risk stratification in the future.

A limitation to FISH is the interrogation (albeit on a single-cell level) of only a limited region where FISH probes are applied and, in some instances, low sensitivity to detect copy number abnormalities. CMA has shown clinical utility in detecting recurrent copy number aberrations across the entire genome and to potentially replace FISH testing for 1p and 17p deletions, 1q gain and for the assessment of trisomies, when PC content is >20% PC [10, 93], if not enriched. However, since CMA cannot detect balanced rearrangements and cannot distinguish mixed ploidy populations, this testing modality cannot be used to replace all FISH testing. CMA-based copy number detection is often less sensitive for copy number detection than FISH and in addition having adequate specimen to perform all this testing is often challenging. NGS could be implemented to detect all recurrent genetic abnormalities in MM, which may replace FISH in the future [73]. While still within the research domain, a combination of genome sequencing-based testing for the identification of recurrent rearrangements and gene expression of enriched PC using RNA-sequencing may be performed in the future clinically [94, 95]. A limitation of this study is the relatively small sample size of the survey respondents primarily representing a North American perspective, which may impact the generalizability of the findings globally. Overall, FISH remains a vital standard-of-care assay in the evaluation of patients with MM. We propose improvements in FISH testing and reporting with the goal to reduce variability and increase clarity to benefit patient care in MM.

DATA AVAILABILITY

Raw survey data are provided in supplemental results.

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AUTHOR CONTRIBUTIONS

XL, EFA, CCE, PRG, AML, PMM, TP, FQR, VCT, DJW, JZ, LBB contributed to survey creation, literature data review, survey data analysis, and manuscript preparation and approved the final content. XL and LBB wrote the original manuscript draft. RB, SK, RF reviewed survey data, reviewed the manuscript and approved the final content.

COMPETING INTERESTS

EA reports consultancy AbbVie. RB reports consultancy Adaptive Biotech, BMS, Caribou Biosciences, Genentech, Janssen, Karyopharm, Legend Biotech, Pfizer, Sanofi, SparkCures; Research: Novartis, Pack Health. RF reports consultancy for AbbVie, Adaptive Biotechnologies, AMGEN, AZeneca, Bayer, Binding Site, BMS (Celgene), Millenium Takeda, Jansen, Juno, Kite, Merck, Pfizer, Pharmacyclics, Regeneron, Sanofi; scientific advisory boards for Adaptive Biotechnologies, Caris Life Sciences, Oncotracker; board of directors for Antegene, AZBio; and patents for FISH in myeloma. SK reports consultancy from BMS/Celgene, Takeda and Janssen and research funding from BMS (Celgene), Takeda, Novartis, AbbVie, Janssen and Amgen. LBB reports consultancy Genentech. The remaining authors have no interests to disclose.

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