Cancer Genetics 228–229 (2018) 184–196

Cancer Genetics

REVIEW ARTICLE

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

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Abstract

Background: Plasma cell neoplasms (PCNs) encompass a spectrum of disorders including monoclonal gammopathy of undetermined significance, smoldering myeloma, plasma cell myeloma, and plasma cell leukemia. Molecular subtypes have been defined by recurrent cytogenetic abnormalities and somatic mutations that are prognostic and predictive. Karyotype and fluorescence in situ hybridization (FISH) have historically been used to guide management; however, new technologies and markers raise the need to reassess current testing algorithms.

Methods: We convened a panel of representatives from international clinical laboratories to capture current state-of-the-art testing from published reports and to put forward recommendations for cytogenomic testing of plasma cell neoplasms. We reviewed 65 papers applying FISH, chromosomal microarray (CMA), next-generation sequencing, and gene expression profiling for plasma cell neoplasm diagnosis and prognosis. We also performed a survey of our peers to capture current laboratory practice employed outside our working group.

Results: Plasma cell enrichment is widely used prior to FISH testing, most commonly by magnetic bead selection. A variety of strategies for direct, short- and long-term cell culture are employed to ensure clonal representation for karyotyping. Testing of clinically-informative 1p/1q, del(13q) and del(17p) are common using karyotype, FISH and, increasingly, CMA testing. FISH for a variety of clinically-informative balanced *IGH* rearrangements is prevalent. Literature review found that CMA analysis can detect abnormalities in 85–100% of patients with PCNs; more specifically, in 5–53% (median 14%) of cases otherwise normal by FISH and cytogenetics. CMA results in plasma cell neoplasms are usually complex, with alteration counts ranging from 1 to 74 (median 10–20), primarily affecting loci not covered by FISH testing. Emerging biomarkers

Received March 13, 2018; received in revised form July 16, 2018; accepted July 30, 2018

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2210-7762/$ - see front matter
https://doi.org/10.1016/j.cancergen.2018.07.002

include structural alterations of *MYC* as well as somatic mutations of *KRAS, NRAS, BRAF,* and *TP53.* Together, these may be measured in a comprehensive manner by a combination of newer technologies including CMA and next-generation sequencing (NGS). Our survey suggests most laboratories have, or are soon to have, clinical CMA platforms, with a desire to move to NGS assays in the future.

Conclusion: We present an overview of current practices in plasma cell neoplasm testing as well as an algorithm for integrated FISH and CMA testing to guide treatment of this disease.

Keywords Plasma cell disorders, Plasma cell myeloma, Multiple myeloma, Cytogenetics, Chromosomal microarray testing, Next-generation sequencing, Guidelines, Recommendations.

Introduction

Summary statement

Plasma cell neoplasms (PCNs) are characterized by the uncontrolled clonal expansion of genetically altered plasma cells. These diseases include the precursor lesion monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma, plasma cell myeloma (or multiple myeloma), plasmacytoma, monoclonal immunoglobulin deposition diseases (amyloidosis), and PCNs associated with paraneoplastic syndromes. While karyotype and fluorescence in situ hybridization (FISH) analyses have been instrumental in determining prognosis and guiding therapy, the clinical significance of new and emerging molecular markers raise the need to explore expanded and alternative testing algorithms to guide care. In this article, we review the current state of clinically-informative markers and laboratory practices used to detect them using karyotype, FISH and chromosomal microarray (CMA) testing. We also review future directions in laboratory cytogenomics, and propose an algorithm for incorporating CMA testing into the current routine genomic work-up of plasma cell neoplasms.

Disease introduction, and known diagnostic, prognostic, and predictive markers

PCNs are progressive, debilitating, and incurable B-cell disorders arising from accumulation of malignant plasma cells predominately in the bone marrow. Risk factors for plasma cell myeloma include the presence of an MGUS, chronic antigenic stimulation, male sex, older age, and African–American ethnicity. Poorer prognosis at diagnosis is associated with elevated serum lactate dehydrogenase (LDH) and beta-2 microglobulin, and decreased albumin [1,2]. PCNs are particularly diverse at the genomic level, and numerous studies have shown that acquired genomic abnormalities carry prognostic significance (Table 1, Supplemental Table 1), albeit often on a background of substantial subclonal heterogeneity that may confer therapeutic resistance [3,4].

Plasma cell myeloma can be subdivided into two major categories based on karyotype. First, there is the hyperdiploid group that contains greater than or equal to 47 chromosomes with non-random gains of odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. Second, there is the nonhyperdiploid group that typically contains a hypodiploid, pseudodiploid or near-tetraploid karyotype, and often contains translocations involving the immunoglobulin heavy-chain locus (*IGH*) on 14q32. Favorable prognosis has been associated with hyperdiploidy or translocations involving the cyclin D gene family, particularly t(11;14) and t(6;14) [5]. However, recent evidence from a large series of 1095 patients suggests that outcomes of patients with t(11;14) myeloma are worse than standard risk patients [6]. Poor prognosis is associated with gain of chromosome arm 1q or loss of 17p containing the *TP53* gene, as well as *IGH* translocations t(4;14), t(14;16) and t(14;20) [5]. Monosomy 13/del(13q) is a secondary cytogenetic abnormality that is only considered a poor prognostic marker when detected by conventional cytogenetics or genome-wide methods that can rule out hyperdiploidy or association with an *IGH* translocation. Deletion of 16q has also been linked to worse overall survival in myeloma patients [7].

These prognostic markers provided a framework for risk-adapted therapy and led to the International Myeloma Working Group (IMWG) consensus statement [8], revised international staging system (R-ISS)[9], and the IMWG consensus criteria for response and minimal residual disease assessment [10]. The IMWG recently defined high-risk patients as those with an overall survival of < 2 years and harboring malignancies with 1q gain, t(4;14), or 17p loss. Low-risk patients were defined as those surviving > 10 years with cancers displaying hyperdiploidy, or t(11;14) or t(6;14) [11]. The IMWG R-ISS combines β2-microglobulin, serum albumin, LDH and interphase FISH findings to break myeloma patients into three prognostic stages with varying overall survival [9]. Patients in the low-risk R-ISS stage I have β2-microglobulin < 3.5 mg/L, albumin ≥ 3.5 g/dL, normal LDH, and lack high-risk chromosomal abnormalities del(17p), t(4;14), t(14;16), or 1q gain. High-risk R-ISS stage III have β2-microglobulin ≥ 5.5 mg/L and either high LDH or a high-risk chromosomal abnormality detected by interphase FISH. R-ISS stage II include all cases not classified as ISS stage I or III. An alternative staging system, the Mayo Clinic mSMART 2.0 classification [12], stratifies myeloma patients into three groups based primarily on genetic findings. High-risk includes del(17p), t(14;16), t(14;20), or a high risk gene expression profiling signature; intermediate-risk includes t(4;14), 1q gain, or a high plasma cell S-phase; and standard-risk includes trisomies, t(11;14), or t(6;14). Recently, the National Comprehensive Cancer Network (NCCN, guidelines version 4.2018) [13] recommended metaphase cytogenetic profiling of bone marrow, as well as FISH on plasma cells utilizing probes to detect 1q21 amplification, del(13q), t(4;14), t(11;14), t(14;16) and del(17p) at the time of diagnosis. These staging systems are largely consistent with our literature review (Supplemental Table 1) and survey of current practices for plasma cell neoplasm profiling (Table 2).

Table 1 Clinically significant cytogenomic alterations in plasma cell myeloma.

Evidence Level	Chromosomal Abnormality	Significance	Genes
Level 1	Hyperdiploidy (+3, +5, +7, +9, +11, +15, +21)	Good prognosis	
Well established evidence in NCCN guideline, WHO criteria,	t(4;14)	Poor prognosis, predicts bortezomib response	*IGH*
FDA-approved,	t(6;14)	Good prognosis	*IGH*
COG	t(14;16)	Poor prognosis	*IGH*
recommendation,	t(11;14)	Good prognosis	*IGH*
or based on large	t(14;20)	Poor prognosis	
body of	del(1p)	Poor prognosis	
publications.	1q+	Poor prognosis	
	del(13q)	Poor prognosis	
	16q	Poor prognosis	
	del(17p)	Poor prognosis (Level 1), predicts response (Level 2)	
Level 2	1p CN-LOH	Recurrent	
Emerging	+2	Recurrent	
evidence by one	del(4q)	Recurrent	
large study or	del(5p), 5q+, del(5q)	Recurrent	
multiple case	6p+	Recurrent	
reports	del(6q)	Recurrent	
	7q+	Recurrent	
	del(8p)	Recurrent	
	8q24.2+	Recurrent	*MYC*
	9p+	Recurrent	
	del(10q23.31)	Recurrent	*PTEN*
	11q+	Recurrent	
	del(12p) or 12p CN-LOH	Recurrent	
	del(13q32.2)	Recurrent	*TGDS*
	del(14q)	Good prognosis	
	14q CN-LOH	Recurrent	
	16 CN-LOH	Recurrent	
	17 CN-LOH	Recurrent	
	17q25+	Recurrent	
	+18	Recurrent	
	+19, 19q+	Recurrent	
	del(20p)	Recurrent	
	+20, 20q+	Recurrent	
	del(22)	Recurrent	
	22q21+	Associated with relapse	*PRAME*
	del(X), X+, X CN-LOH	Recurrent	
	Xq+ in males	Poor prognosis	

*See supplemental Table 1 for references and Level 3 alterations.

With increases in therapeutic options, new knowledge of predictive markers for therapy have also been identified. Patients with the favorable prognostic markers t(11;14) and hyperdiploidy have been identified as exceptional responders to lenalidomide-based therapy [14], while the poor prognostic t(4;14) has emerged as a positive predictive marker for response to bortezomib combination regimens and improved overall survival [15]. Pomalidomide plus low-dose dexamethasone has demonstrated efficacy in patients with del(17p) [16]. Secondary cytogenomic alterations also remove regulators of the NF-kB pathway genes [17], such as *BIRC2/3* (11q), *TRAF3* (14q), and *CYLD* (16q) and therefore may warrant treatment with inhibitors against this pathway [18–20]. Numerous targeted agents have emerged that exploit mutated proteins within the RAS/MAPK pathway including *BRAF, KRAS*, and *NRAS*, even within the same patient [3]. Therefore, integrated cytogenomic profiling of multiple types of cancer genome variation is the future course for directing care of patients with PCN.

Table 2 Survey results.

Total respondents	66	
What is your role in profiling of myeloma or other plasma cell disorders? (*n* = 66)		
Cytogeneticist, molecular geneticist, or pathologist	60	91%
Laboratory Technician	4	6%
Laboratory Supervisor	2	3%
When do you use plasma cell enrichment currently? (*n* = 66)		
at diagnosis	51	77%
follow-up testing after diagnosis	46	70%
upon relapse	41	62%
to detect residual disease	29	44%
never	10	15%
In a bone marrow aspirate, what is the tumour cell content required to trigger plasma cell enrichment for array or FISH analysis? (*n* = 66)		
<100% - we always perform enrichment	48	73%
<50%	1	1.5%
<40%	1	1.5%
<30%	0	0%
<20%	5	8%
<10%	0	0%
<5%	1	1.5%
0% - we never perform enrichment	10	15%
What methods do you use for plasma cell enrichment? (*n* = 59)		
RoboSep-S magnetic bead purification	29	49%
Miltenyi magnetic bead purification	17	29%
EasySep	6	10%
EpiSep by Wavesense	2	3%
Please provide a brief description of culture conditions and time lines you currently use to expand cells for testing: (*n* = 59)		
Direct bone marrow	13	22%
24 h	35	59%
48 h	6	10%
72 h	32	54%
96 h	6	10%
Marrow Max	17	29%
LPS	2	3%
What are your current FISH probes used for profiling plasma cell disorders? (*n* = 65)		
del(17p)	64	98%
t(4;14)	55	85%
t(11;14)	54	83%
1p/1q	54	83%
t(14;16)	53	82%
del(13q)	51	78%
IGH Breakapart	43	66%
t(14;20)	24	37%
MYC	22	34%
Enumeration	16	25%
del(6q)	13	20%
t(6;14)	11	17%
Please describe other non-FISH assays or algorithms in-place or soon to be available at your centre: (*n* = 39)		
Microarray	21	54%
NGS	5	13%
Gene expression (send-out test)	1	1.5%
None planned	8	21%

Existing testing methods for diagnosis and prognosis

To adequately profile the genomic landscape of PCN, bone marrow aspirates have been widely subject to karyotype and FISH analyses. The configuration of these assays differs widely across clinical laboratories, with scope and prioritization of these tests determined by quantity and quality of available material, requirements of clinical teams making use of cytogenomic information, and available infrastructure.

Karyotype analysis and culturing

Culture conditions vary between laboratories and often include a customization of culture times and media conditions. Despite the use of multiple culture times and conditions (direct, overnight, or long-term), obtaining metaphases is often unsuccessful or results in normal karyotypes, rather than yielding abnormal clones. Mature plasma cells frequently have an extremely low proliferation rate in vitro and multiple differing culture strategies are used to acquire or stimulate mitoses in malignant plasma cells. Successful karyotyping may also be dependent on plasma cell morphology and disease status, as cases associated with disease progression or relapse may be more mitotically active and may divide in direct or short-term cultures. In contrast, cases with a mature plasma cell morphology or following autologous stem cell transplantation with stable disease typically need long-term, cytokine or mitogen-stimulated cultures to obtain informative metaphases, as plasma cells represent an end-stage in B-cell development. Culture times vary from direct harvest to short-term or overnight unstimulated cultures, to long-term 72–120 h cultures using a variety of B-cell cytokines or growth factors in an attempt to obtain a higher yield of abnormal plasma cell metaphases for analysis. Commonly used cytokines or growth factors include IL2, IL4, IL6, GM-CSF, LPS, GCT, TPA, DSP30, CPG-ODN, PMA with lectin, and PBA, which may be added to general culture media [21], including commercially-available options MarrowMax, RPMI 1640 (both available from Thermo Fisher Scientific), and Chang BMC (Irvine Scientific). Overall, the success rate for identifying abnormal clones by conventional cytogenetics is between 30–40% [21–24].

Fluorescence in-situ hybridization

Given the low success rate of conventional cytogenetics and the fact that several prognostically-important abnormalities are cytogenetically cryptic or difficult to see by karyotyping, FISH is routinely utilized by most laboratories. To obtain accurate FISH results in bone marrow aspirates that often contain low numbers of plasma cells, many groups utilize either plasma cell enrichment or plasma cell staining techniques. Laboratories either establish an algorithm to determine which cases will undergo plasma cell enrichment or decide to perform plasma cell enrichment on all samples (Table 2). This algorithm may be based on the quantity of aspirate available, the percentage of plasma cells within the aspirate, or specific testing requested by the physician. FISH testing algorithms may vary between laboratories, but are often based on treatment guidelines including those from the IMWG R-ISS, mSMART and/or NCCN. In cases where a bone marrow aspirate is not available or diluted by blood, FISH may be performed on touch imprints from trephine biopsies that may have a greater percentage of plasma cells.

Plasma cell enrichment, staining, and flow cytometric sorting

FISH has traditionally been performed on cultured bone marrow cells, with the percentage of plasma cells in the aspirate varying from $< 1\%$ up to 100%. As the plasma cell percentage is often very low and less than optimal for FISH testing, either plasma cell enrichment or plasma cell staining is helpful to optimize the results. Magnetic microbeads conjugated with anti-CD138 antibodies represents a quick and easy way to concentrate plasma cells to increase sensitivity of FISH, CMA, or next-generation sequencing (NGS) by diminishing the number of non-malignant cells that may obscure the analysis. Flow cytometric sorting, while not available to most laboratories, is another effective method for plasma cell concentration but often results in low yield and poor plasma cell morphology. Both of these methods face the challenge of false negative results due to the loss of plasma-cell marker CD138 from the cell surface over time. Therefore, specimens should reach the laboratory as soon as possible (preferably within a few hours) for optimal plasma cell recovery. Additional risk of a false negative arises when a plasma cell neoplasm does not express CD138 which, while extremely rare, highlights the need to consider alternate strategies for profiling unenriched cells. For example, plasma cells can be identified by an intracellular immunoglobulin staining technique. However, while these co-staining procedures work for FISH, they are unsuitable for genomic studies such as CMA and NGS that have sensitivity limited when DNA is derived from a population of admixed cells.

Novel testing approach under review

The development and implementation of new molecular techniques, including CMA and NGS, have deepened our knowledge of genome alterations underlying PCNs. Large-scale genomic studies have uncovered substantial genetic heterogeneity, clonal evolution, and therapeutic selection in the context of disease diagnosis, progression, and relapse [3,4]. Given the diversity of molecular methods available to modern cytogenomic laboratories, we sought to assess the current state-of-the-art of profiling PCNs and to recommend best practice for testing these diseases. Herein, we provide a comprehensive review of the literature, especially focused on the clinical utility of these newer technologies for the diagnosis, prognosis, and therapeutic guidance of PCN.

Evidence base supporting clinical utility of genome-wide cytogenomic testing

Summary of literature review

We have reviewed 65 papers applying FISH, CMA, NGS, and gene expression profiling for PCN diagnosis and prognosis (study sample sizes range from 14–463, Supplemental Table 1). Chromosomal abnormalities were scored on a 3-level system: Level 1: well established evidence from NCCN

guidelines, WHO criteria, FDA-approved, ECOG/SWOG recommendation, or based on large body of publications; Level 2: emerging evidence supported by one large study or multiple case reports; and Level 3: presumptive evidence supported by case reports or expert opinion.

Plasma cell enrichment by CD138-coated magnetic beads was widely used prior to FISH testing, with frequent enrichment of plasma cell concentrations from $<5\%$ in the aspirate to as high as 99% post-enrichment. Overall, enrichment has been reported to result in >2X fold increase in cases with cytogenetic abnormalities detected by FISH [23,25,26]. CMA analysis further increased diagnostic yields, with detection of abnormalities in 5–53% (median 14%) additional cases otherwise normal by FISH and cytogenetics [23,27–30]. The overall detection rate with CMA testing was 85%−100% [22,23,27–34].

Prospective studies report the application and value of CMA testing in the clinical setting after plasma cell enrichment [23,28,29]. CMA results are usually complex, with alteration counts ranging from 1 to 74 (median 10–20), often affecting loci not covered by FISH testing. The most frequent myeloma markers are readily detected, especially numerical changes indicative of hyperdiploidy. CMA results for copy number alterations (CNAs) have shown excellent concordance with FISH findings except in the cases of low-level clonal aberrations present in $<30\%$ of cells. In these cases, FISH for del(13q) and del(17p) may remain necessary, as deletions in these areas may be present in subclones not detectable by CMA. However, in diagnostic myeloma cases that are typically rich in neoplastic plasma cells, abnormalities are detected in nearly every case by CMA, surpassing FISH in most studies. Even in smoldering myeloma, CMA analysis detected abnormalities in 85% of patients with an average of 7.5 aberrations per patient (range 1–23) [32]. Hyperdiploidy, an important marker in PCN risk stratification, is under-detected by both FISH and metaphase cytogenetics [23].

CMA also enables robust detection of bi-allelic deletions and copy-neutral loss-of-heterozygosity (CN-LOH) that may be present in a significant number of cases; up to 31% of cases reported in a prospective multicenter study [27]. The most frequently occurring CN-LOH regions involve 1p, 12p, 14q, 16, 17 and X (Table 1). Studies are ongoing to elucidate the definitive prognostic impact of these CN-LOH in PCNs. CN-LOH of 16q, encompassing the *CYLD* gene, a negative regulator of the NF-kB pathway, has been associated with poor prognosis in multiple myeloma [7]. LOH caused by either deletion or homozygous mutation of critical genes may result in the same pathogenetic consequence; hence, the diagnostic and prognostic significance of somatic CN-LOH may be equivalent to deletions of the same genomic region. This was partially corroborated by integrated analysis of copy number, LOH, and gene expression levels [35]. The frequency of CN-LOH is significantly higher in active multiple myeloma than in asymptomatic MGUS or smoldering myeloma [36]. More recently, homozygous recombination deficiency (HRD) detected by genome-wide LOH also showed progressively increasing frequency of HRD-LOH as PCN progresses; furthermore, the extent of HRD-LOH was correlated with high-risk markers [37]. These findings raised the possibility of LOH serving as a predictive marker for PARP inhibitors as therapy for PCNs.

Complex genomic rearrangements involving frequent and larger genomic aberrations (>5Mb) as well as chromothripsis are emerging risk markers that tends to cluster with ISS defined higher risk group, and can only be detected by a genome-wide approach [23,27]. As balanced rearrangements cannot be directly detected by CMA, FISH for *IGH* rearrangements will likely remain a mainstay for detection of these abnormalities until NGS becomes routine. However, 33–66% of patients with apparently balanced rearrangements have CNAs at the breakpoints detectable by CMA, suggesting these may be a useful surrogate to trigger targeted FISH analysis [23]. In our proposed testing algorithm (Fig. 1), we outline a strategy for combined CMA and FISH testing to maximize detection of both CNAs and translocations.

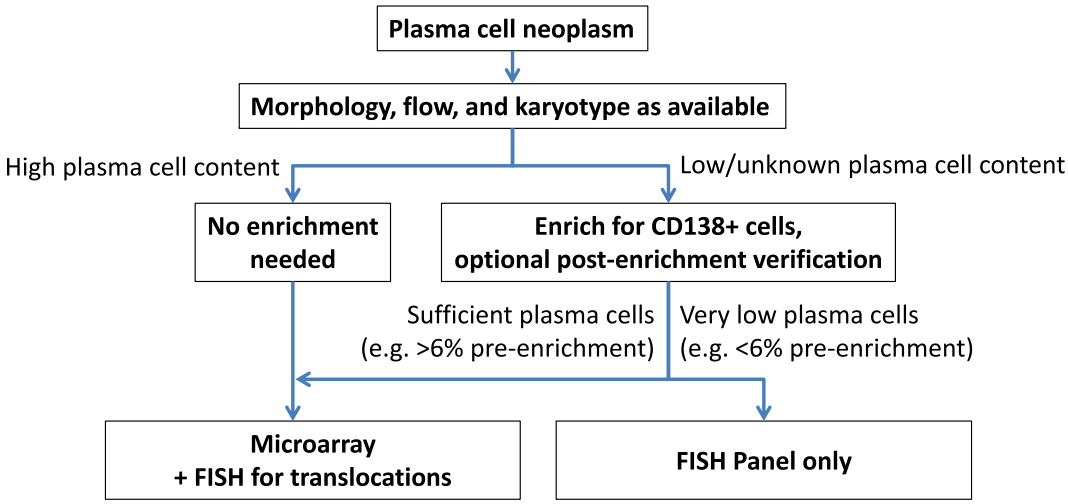
Genome and exome NGS can accurately recapitulate CNA profiles, structural variation, mutational profiles, and clonal heterogeneity underlying plasma cell neoplasms [3,17,38]. These methods have identified over 20 recurrently mutated genes, as well as many genes mutated at lower frequency, many with clinical implications [3,17,39]. Targeted DNA sequencing shows promise as a less-expensive and potentially more sensitive option for genomic profiling of multiple sources of genome variation, including mutations, CNAs, and chromosomal rearrangements [40]. Similar to CMA testing, NGS methods will benefit from plasma cell enrichment and are likely to be complementary to single cell assays such as FISH.

Summary of current practice from various clinical testing centers

To assess the current state of clinical molecular testing for myeloma, our working group conducted a 9-question survey of the Cancer Genomics Consortium membership (www.cancergenomicsconsortium.org) and the American Cytogenetics Forum List (cytogn-l@listserv.sc.edu) from March to April 2017 (Table 2). In total, we received 66 responses from respondents who self-identified as a cytogeneticist, molecular geneticist or pathologist (91%), laboratory technician (6%), or laboratory supervisor (3%). Plasma cell enrichment prior to testing is widespread with $>85\%$ of labs using CD138 + cell enrichment by a magnetic bead system (49% RoboSep-S, 29% Miltenyi, 10% EasySep, 3% epiSep). Across labs, 73% always perform enrichment while 12% restricted enrichment to specimens with lower plasma cell content. Laboratories that never use plasma cell enrichment were in the minority (15%) and several such groups stated the intention to establish enrichment as a standard protocol.

Culture conditions varied across groups with many reporting routine use of 24 or 72 h cultures (59% and 54%), although 48 and 96 h cultures were also reported (10% each). Direct FISH on bone marrow aspirates was reported by 20% of respondents. A variety of culture media was described, with MarrowMax the most frequently reported (29%).

The selection of FISH probes varied across respondents, with near consensus on testing for deletion of 17p (98%). Four additional abnormalities were reported as FISH tested by $>80\%$ of respondents: t(4;14) (85%), t(11;14) (83%), 1p/1q (83%), and t(14;16) (82%). Less frequently employed FISH assays include del(13q) (79%), *IGH* break-apart probes (66%), t(14;20) (37%), *MYC* rearrangements (34%), del(6q) (20%), and t(6;14) (17%). Enumeration probes for



Tier 1	t(4;14) t(14;16)	1p/1q t(4;14) del(17p) t(14;16)
Tier 2	IGH breakapart t(11;14)	IGH breakapart t(11;14) Monosomy 13 /del(13q)
Tier 3	MYC breakapart t(14;20) t(6;14)	MYC breakapart t(14;20) t(6;14)

Fig. 1 Proposed testing algorithm and tiered FISH panel design.

hyperdiploidy (most commonly chromosomes 5, 9, and 15) were reported by 25% of respondents, although this percentage may be higher as this panel was only reported in the comments section of the survey and not as a checkbox within this section.

When asked to describe other non-FISH assays or algorithms in-place or soon to be available in their lab for use in PCNs, CMA platforms were mentioned by 21 respondents. Of these, 13 reported offering CMA testing along with existing karyotype or FISH tests. NGS was reported by 5 labs although no details on specific platforms or configurations were provided. Gene expression profiling was reportedly offered by 1 lab as a send-out test.

New knowledge obtained from CMA and NGS testing has the potential to further refine clinical practice and patient management, particularly the interpretation of infrequent albeit recurrent genome alterations. One path to this analysis is engagement with emerging clinical data sharing initiatives, such as the AACR GENIE Project (http://www.aacr.org/genie) [41], that are aggregating clinically-annotated mutation and CNA profiles across numerous centers performing routine testing. Ultimately, leads stemming from these data will require prospective validation trials that incorporate these new testing approaches. Nevertheless, based on the evidence from our literature review and survey, the clinical utility of genome-wide CNA profiling is well established in several aspects. First, CMA testing clearly enhances diagnostic yield, and several labs are considering further augmentation of test offerings by NGS. In combination with existing testing modalities, these new approaches can identify additional patient-specific disease markers that may be used to monitor minimal residual disease after treatment. Second, less frequent abnormalities still have prognostic or predictive value and may guide therapeutic decisions. Third, costs may be reduced when a single genome-wide CNA profile is able to replace multiple FISH assays. For example, some laboratories currently employ an extensive multiple myeloma FISH panel that includes 1p/1q, aneuploidies for 5, 9, and 15, 13q-, 17p-, along with several *IGH* rearrangement probe sets. The cost of running a single CMA test covering all chromosomes may be comparable or less than running a panel of individual FISH probes for CNAs. Therefore, we encourage laboratories to work actively with payers to evaluate the cost effectiveness of CMA testing alongside clinical benefit.

Considerations for integration of the new testing to existing practice

Pre-analytical: specimen acquisition and triaging

The current state of genetic testing for PCNs varies across genetic laboratories, generally based upon clinical and research testing guidelines, differential diagnosis at specimen acquisition, specimen volume and quality, and laboratory capabilities. Laboratories attempting to follow clinical guidelines may be hampered by different clinical guidelines that recommend different testing algorithms for plasma cell myeloma patients. For instance, while most clinical guidelines specifically recommend FISH on plasma cells, the guidelines vary on which probes to use and whether or not routine karyotyping should

also be obtained. Additionally, some research protocols may also call for CMA testing or sequence analysis. As the number of independent assays grows, so does the demand on limited material available for testing.

In some centers, diagnosis of a PCN may not be made until several days after the laboratory has received and processed the specimen. This delay limits the ability to perform plasma cell enrichment and therefore requires laboratories to decide whether or not to perform FISH on unenriched specimens that may contain very few plasma cells. Further, a delay in the diagnosis of a PCN could also lead to cultures being set up for chromosome analysis that may be ultimately uninformative. Compounding this challenge, aspirate volumes can vary from as little as a few drops to greater than 5 mL of bone marrow. Since multiple aspirates are typically drawn for multiple assays including morphology, flow cytometry, cytogenetics/FISH, and other molecular testing, the order of the aspirate can often affect the percentage of plasma cells in the aspirate. Plasma cells typically do not aspirate well from the bone marrow, with the percentage of plasma cells often being much less in aspirates than in trephine biopsies. Sometimes, an aspirate will not be available (dry bone marrow tap), and in these circumstances, FISH can often be performed on trephine biopsy touch imprints. Decalcified trephine biopsies usually will not work for tissue FISH as the decalcification process usually destroys the chromatin. For these reasons, it is imperative that cytogenomic laboratories work closely with pathologists and the rest of the medical team to optimize the genetic/genomic results and testing algorithms. As genetic/genomic results are critical for determining risk groups for therapy and prognosis, we recommend cytogenetics laboratories obtain the second aspirate, with the first usually being reserved for morphology. Since flow cytometry often requires a minimal amount of the bone marrow aspirate, the leftovers from this test could be another resource for the cytogenetics laboratory to aid genomic analysis.

Analytical

The genetics of PCNS have been extensively studied and utilized for diagnostic and prognostic purposes. While karyotyping has been a mainstay of genetic profiling for decades, this technique has several major disadvantages compared to new technologies. First, despite the fact that almost all myeloma cells have cytogenetic abnormalities, routine karyotyping detects these abnormalities 30–40% of the time[22,23], compared to $>$ 90% by FISH or CMA. Second, karyotyping is relatively slow and labor-intensive. Lastly, karyotyping has a limited resolution that will miss small CNAs and cryptic rearrangements known to occur in plasma cell neoplasms that are readily detected by CMA and/or FISH. However, the focused nature of FISH limits the ability to accurately determine polyploidy and still incurs the labor and technical costs of karyotyping, particularly for larger probe panels.

CMA by single nucleotide polymorphism (SNP) and copy-number array combines benefits of karyotyping with the focused nature of FISH. High-resolution microarrays can contain millions of markers distributed across the genome to enable CNA analysis at resolution greater than karyotyping and FISH. An additional advantage of SNP-containing microarrays includes the ability to detect CN-LOH evident by the absence of signal from one of two possible alleles across large segments of the genome. Disadvantages of microarray testing include the loss of signal when profiling unselected samples that may obscure the signal from plasma cells, subclones due to tumor heterogeneity, as well as the inability to detect balanced rearrangements. Therefore, combining several technologies in a single testing algorithm may be a more effective approach to assay molecular markers necessary for guiding therapy and prognosis.

Post-analytical: reporting and integration with other testing results

Reporting is a critical step for the accurate communication of molecular profiling results to ensure appropriate interpretation and subsequent clinical decisions by physicians. Therefore, a clear, unambiguous report is needed that accurately conveys the molecular markers detected by the assay while highlighting specific features that are clinically informative. In Appendix A, we provide an example of a simple report of CMA findings that emphasizes alterations linked to clinical action while providing complete profiles compatible with published checklist requirements and guidelines such as those from the College of American Pathologists (CAP) and the American College of Medical Genetics (ACMG). The presence of malignancy and reporting of high-risk markers are the top priority of the report, and therefore these are highlighted at the top of the document. In a separate section further down the report, we provide detailed results of all alterations, including the International System for Human Cytogenomic Nomenclature (ISCN 2016). Interpretation includes diagnosis, prognosis, correlations with other laboratory testing results and concurrent or historical genetic testing results. Comments include description of the test limitations as validated as well as sample-specific issues such as low cell content or quality. At the bottom of the report, we provide methodological details and disclaimers based on CAP requirements. We also recommend a section "Relevant Cytogenetics History" that gives background of the patient's disease and enables comparison with prior test results.

Recommendations for best practice based on the evidence

Integration of novel testing approach into clinical use

Triaging of the specimen will often depend on multiple factors including the quantity and quality of the aspirate, the time the specimen has spent in transit, testing being requested, whether or not the laboratory performs plasma cell enrichment, and whether it is a new diagnosis or a follow-up study. If multiple studies are requested such as karyotype, FISH, CMA and/or NGS, the laboratory will need to decide how to aliquot the specimen for each study. Because of the low success rate in karyotyping myeloma cells, the knowledge that greater than 90% of myeloma cases have abnormalities detectable by FISH and CMA, and because the cytogenetic laboratory often receives a small volume of aspirate with a low percentage of plasma cells, many laboratories must decide how to prioritize handling of limited specimens. A close communication with ordering oncologists and pathologists is highly recommended to prioritize testing needed.

In addition to diagnostic cytogenetics and FISH, some laboratories are exploring the use of newer technologies such as CMA, NGS, and gene expression profiling, technologies that also may require enriched plasma cells. Follow-up studies are taking on increasing importance in plasma cell myeloma. The IMWG recently published a consensus document for monitoring response to therapy and assessing minimal residual disease by multi-parametric flow cytometry or molecular methodology such as NGS and qPCR. Additional biomarkers include structural alterations (e.g. *MYC*) and somatic mutation (*KRAS, NRAS, BRAF,* and *TP53*).

Testing algorithm example

Based on our literature review and survey of current clinical practice in cytogenomic laboratories, we propose a testing algorithm for PCNs with and without integration of CMA testing (Fig. 1). This algorithm is primarily focused on PCNs at diagnosis and relapse, but may apply for other stages of the disease as well. For example, patients with asymptomatic smoldering myeloma could be tested for genetic markers of high likelihood of progression using this algorithm in combination with annual MRI and/or CT surveillance tests as per current NCCN guidelines [13].

Morphologic evaluation and flow cytometric analysis should be performed on all bone marrow aspirates to confirm diagnosis and to estimate plasma cell content for genetic studies. Karyotype is recommended by the NCCN Guidelines Version 4.2018 [13], even though it has much lower detection rate than FISH and CMA. Karyotyping is still the only clinically-available test that can clearly delineate independent clones and definitively ascertain clonal evolution. Monosomy 13/del(13q) is only considered high-risk when it is observed by karyotype analysis and detection by FISH or CMA does not yet carry the same prognostic significance. Therefore, we have still included karyotyping in our recommended testing algorithm, although some laboratories may forego this test based on local physicians' preference.

To maximize diagnostic yield and detection of subclonal populations, we recommend CD138 + plasma cell enrichment in cases with low plasma cell content prior to FISH or CMA analysis. For cases with a high plasma cell content, CD138 enrichment is not necessary. Either CMA and FISH testing for *IGH* rearrangements, or FISH testing for CNAs and *IGH* rearrangements, could be performed. The combination of CMA and FISH has a reported detection rate of 98–100% and has been recommended by several papers [27,30,31], and proposed as a cost-effective front-line assay for PCN diagnosis [23,29,30]. For cases with a low percentage of plasma cells, CD138 enrichment is recommended. We do not recommend doing CMA testing on specimens with low quantities plasma cells prior to enrichment, as even post-enrichment, these specimens often do not allow for detection of CNAs by CMA. In these cases, FISH may still be informative but well established cut-offs for normal and abnormal FISH patterns is required. As a benchmark, we propose a low plasma cell content threshold of 6%, based on the reported 3.4 to 74-fold enrichment factor for CD138 + enrichment process [25] and the reported CMA limit of detection of 20%. Hence, a conservative estimate using the 3.4-fold enrichment factor requires at least 6% plasma cell content for clonal abnormalities to be detectable by CMA (6% x 3.4 = 20.4%). During validation studies, we expect that individual laboratories may set their own plasma cell thresholds for CMA profiling, particularly if methods (such as post-enrichment flow cytometry [30]) are available to determine the fraction of this population that corresponds to abnormal plasma cells. Naturally, specimen age, cell viability, and CD138 expression level may further modulate this threshold in individual labs.

We hereby present a recommendation of tiered FISH testing for risk stratification (Fig. 1). Tier 1 FISH testing includes all high-risk markers important for R-ISS risk stratification. Concurrent CMA testing can easily identify hyperdiploidy associated with low-risk and complex karyotype associated with high-risk. Tier 2 FISH testing includes high-prevalence markers for further classifying plasma cell neoplasms with additional probes necessary when CMA is not employed. Tier 3 FISH testing includes other less frequent, but prognostically significant markers. Because deletion of 17p is both prognostic and predictive, some laboratories may consider including FISH for del(17p) even when CMA testing is performed so as to avoid missing low-level del(17p). However, the greatest prognostic value of del(17p) comes when it is found in > 60% of plasma cells, so dual-testing may not be warranted in pre-treatment samples [31]. Post-treatment samples may benefit from CMA and FISH analysis of 17p, especially when plasma cell content is low and enrichment is not possible. Naturally, we encourage labs to refine priority and reflex strategy based on provider preference, sample volume, other tests performed, required turn-around-time, and cost effectiveness.

Future directions

Alternative and more robust markers of malignant plasma cells have been reported for plasma cell enrichment, as alternatives to CD138, especially in CD138-negative PCNs. CD319/CS1 (*SLAMF7*) and CD269/BCMA (*TNFRSF17*) have been reported to be highly expressed specifically in multiple myeloma cells and have consistent expression over 40 hours in delayed and frozen specimens [42]. CD54, CD229 and CD319 have also been evaluated as alternatives to CD38 and CD138 markers in MM patients receiving anti-CD38 and anti-CD138 therapy for detection of minimal residual disease. While CD229 was found to be the most robust marker, none of these molecules are cell-type specific, with some level of expression noted in B-cells, T-cells, NK cells and dendritic cells [43]. It remains to be assessed by larger studies whether these new markers represent a potential alternative to CD138 magnetic bead selection or are restricted to flow sorting applications in conjunction with other markers.

Thousands of patients with PCNs continue to be profiled by large-scale genomics projects such as the Multiple Myeloma Research Foundation's CoMMpass study [44] (https://research.themmrf.org/). Over 20 significantly mutated genes have been reported in multiple myeloma including those encoding pathways for MAP kinase signaling (*KRAS, NRAS, BRAF*), DNA repair (*TP53, ATM*), NF-kB signaling (*TRAF3*), histone modification, coagulation, cell cycle regulation, and RNA processing [3,17,45,46]. As the number of profiles continue to grow, the significance of less frequent genomic alterations and overall mutational load are becoming clear [47], particularly with the advent of new immunothera-

pies that mobilize the immune system in new ways to combat cancer. Larger NGS panels will likely be necessary to capture this expanded mutational footprint and our survey data suggest a significant proportion of clinical labs are investing in these platforms. In addition to DNA-based assays, gene expression profiling can provide additional prognostic value [48] as a high risk signature has been observed in about 15% of newly diagnosed patients. These complementary assays have the potential to further refine genomic signatures and nominate predictive markers in individual patients. Key to understanding these patterns is longitudinal collection of clinical data and correlation with treatment response and outcome. Emerging genome technologies to probe single cells [38,49,50] and cell-free DNA [51,52] from these patients will further our understanding of the cytogenomic make-up of this disease and enable clinical laboratories to offer assays to track shifts in these patterns over time. As with current karyotyping, FISH and CMA assays before them, broad adoption of new genomic assays will require demonstration of clinical utility and engagement of the laboratory genetics and genomics community to establish guidelines and recommendations for best practices.

Acknowledgments

We gratefully thank the survey participants from the Cancer Genomics Consortium Membership and American Cytogenetics Forum ListServ.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.07.002.

Appendix A: example chromosome genomic array testing report

RESULT HIGHLIGHT: **Abnormal Chromosomal Microarray** consistent with the diagnosis of multiple myeloma and with **high-risk markers** including **1q+** (IMWG2015 and NCCN2017) and **17p-** (IMWG2015 and mSMART2.0)

INTERPRETATION: These results are consistent with the diagnosis of multiple myeloma. Findings include a female genotype with multiple poor prognosis indicators including gain of 1q, deletion of 17p, and chromothripsis. The gain of chromosome 11 with a breakpoint at the *CCND1* locus is consistent with the concurrent FISH finding of *IGH/CCND1* rearrangement [t(11;14)] with an extra fusion (see Relevant Cytogenetics History).

COMMENTS: Of note, the abnormal percentages reported on CD138+ enriched cells are not representative of the actual disease burden.
This study is not meant to be quantitative. It cannot detect balanced rearrangements (including balanced translocation, inversion, or insertions), point mutations, small insertion/deletions (indels), or low-level mosaicism. Constitutional variants or small CNAs of uncertain clinical significance are not reported.

RESULT DETAILS: **ISCN:** arr[hg19] (1q,7,9q,15)x3, 11pterq13.3(1_69,399,649)x3, (16q)x2 hmz, 17p13.2p12(6,228,166_15,932,898)x1, 19pterp13.11(1_18,827,380)cth, (X)x1
CNAs: -X, +1q, +7, +9q, +11pterq13 (breakpoint at *CCND1*), +15, 17p- (*TP53*), and 19p chromothripsis
CN-LOH: 16q CN-LOH
List of segmental abnormalities:

Chr Band	Event	Size (Mb)	Estimated level	Cancer genes covered (known markers, poor prognosis)
11pterq13.3	Gain	69	~80%	*HRAS, NUP98, CCND1*
16q arm	CN-LOH	44	~80%	*CDH11, CBFB, MAF*
17p13.2p12	Deletion	9.7	~80%	***TP53***
19pterp13.11	Chromothripsis	19	~80%	*SH3GL1, MLLT1*

METHODS: Plasma cell enrichment – Company Product Name (beads, instrument)
DNA extraction – Company Blood/Bone Marrow Kit
Array Platform – Company Product Name (2.5 million total probes)
Filter size/ resolution – 500 Kb for CNAs and 10 Mb for CN-LOH
Reference databases – DGV, OMIM
NCBI Build – GRCh37 (hg19)

Disclaimer: This test was developed and its performance characteristics determined by the XXX Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing. This test is used for clinical purpose. The information provided with these array studies should be used in conjunction with cytogenetics and other clinical laboratory tests.

Relevant Cytogenetics History

XX/XX/2017 Lab Name	46,XX[20].nuc ish(CDKN2C2 × 2,CKS1Bx3)[18/200],(FGFR3 × 2,IGHx4)[17/200], (MYBx2,ATMx2,TP53 × 1)[19/200], (CCND1,IGH)x4(CCND1 con IGHx3)[20/200], (IGHx4,MAFx2)[17/200] Myeloma FISH panel results: Abnormal with + 1q (9.0%), *IGH/CCND1* rearrangement [t(11;14)] with an extra fusion (10%), and 17p- (9.5%); No evidence of t(4;14) and t(14;16)

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