

Array-Based Karyotyping in Plasma Cell Neoplasia After Plasma Cell Enrichment Increases Detection of Genomic Aberrations

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Abstract

The discovery of genomic abnormalities present in monoclonal plasma cells has diagnostic, prognostic, and disease-monitoring implications in plasma cell neoplasms (PCNs). However, technical and disease-related limitations hamper the detection of these abnormalities using cytogenetic analysis or fluorescence in situ hybridization (FISH). In this study, 28 bone marrow specimens with known PCNs were examined for the presence of genomic abnormalities using microarray analysis after plasma cell enrichment. Cytogenetic analysis was performed on 15 of 28 samples, revealing disease-related genomic aberrations in only 3 (20%) of 15 cases. FISH analysis was performed on enriched plasma cells and detected aberrations in 84.6% of specimens while array comparative genomic hybridization (aCGH) detected abnormalities in 89.3% of cases. Furthermore, aCGH revealed additional abnormalities in 24 cases compared with FISH alone. We conclude that aCGH after plasma cell enrichment, in combination with FISH, is a valuable approach for routine clinical use in achieving a more complete genetic characterization of patients with PCN.

Plasma cell neoplasm (PCN) can be divided into several subgroups, of which monoclonal gammopathy of undetermined significance (MGUS) and plasma cell myeloma (PCM) are the most frequent. MGUS is found in 3% of the elderly population and is characterized by the presence of less than 30 g/L monoclonal immunoglobulin (also known as M protein) in the serum. No other myeloma-related tissue impairment is observed, and less than 10% clonal bone marrow plasma cells are present.¹ MGUS is considered a precursor lesion, with about 1% per year converting to PCM.² PCM comprises approximately 10% to 15% of all diagnosed hematopoietic neoplasms in the United States.¹ In 2010, more than 20,000 new cases were identified.³ The disease is characterized by the accumulation of monoclonal plasma cells in the bone marrow.^{1,2} Although a number of different treatment regimens have been established,⁴ PCM is still considered to be incurable, with a median survival of 3 to 4 years.¹

As a result of its genetic heterogeneity, a large number of different genomic aberrations are associated with PCM. Despite this, from a genetic perspective, PCM can be divided into 2 main categories: a hyperdiploid group with typically multiple numerical gains (trisomies of chromosomes 3, 5, 7, 9, 11, 15, and 21) and few translocations involving the *IGH* gene locus on chromosome 14, and a nonhyperdiploid group consisting of hypodiploid, pseudodiploid, and near-tetraploid cases.⁵ The nonhyperdiploid group also tends to have more structural abnormalities including translocations involving *IGH*. Hyperdiploidy has been reported in a high percentage of patients, and in the absence of a TP53 deletion, is a good prognostic indicator.^{6,7} Translocations involving the *IGH* locus on chromosome 14 occur in 55% to 70% of patients with PCMs, commonly involving partner genes *CCND1* (11q13), *FGFR3*/

MMSET (4p16), and *MAF* (16q23).¹ The genomic aberrations observed in PCNs are prognostically significant. The International Myeloma Working Group (IMWG) and others have established guidelines for clinical testing of these genomic prognostic indicators.⁷⁻⁹

PCM has an inherently low proliferation rate, resulting in the failure of conventional cytogenetic analysis to detect genomic abnormalities in PCM because of the lack of dividing plasma cells in a significant number of cases.^{5,10} Interphase fluorescence in situ hybridization (FISH) analysis, on the other hand, is hampered by the generally low proportions of plasma cells in bone marrow aspirates and can thereby result in false-negative test results, especially in the early stages of disease or after treatment. Therefore, in cases with a low monoclonal plasma cell percentage, the IMWG states that FISH analysis should be performed in combination with plasma cell targeting or enrichment strategies as described previously.^{7,11-13} Nevertheless, the detection of aberrations using FISH is limited to the number of genomic loci chosen for testing by a given laboratory and results in interpretations limited to those probes used.

Array comparative genomic hybridization (aCGH) provides a powerful analytic tool for clinical and genetic assessment of PCNs because it circumvents the major disadvantages of both cytogenetic analysis and FISH. aCGH is not only independent of the mitotic activity of malignant plasma cells but is also able to evaluate the entire genome in 1 assay at a submicroscopic level. Further, analysis is at a higher resolution than that obtained with conventional cytogenetics or FISH. In addition to large deletions and duplications, single nucleotide polymorphisms (SNPs) and loss of heterozygosity can be detected with the appropriate array platform.¹⁴ A large percentage of patients with PCN carry genomic gains and losses as clonal abnormalities easily detected with genomic arrays. This method can provide genetic information early in the course of a patient's disease, a major advantage over cytogenetic analysis, which is often normal until disease progression.⁵ The identification of genomic abnormalities that are undetected on FISH or cytogenetic analysis, using aCGH, provides not only previously inaccessible prognostic information¹⁵ but also valuable monitoring markers for assessing tumor evolution and detecting residual disease after treatment.

A limitation of aCGH is the inability to detect abnormalities present in a small clone in the background of normal cells in a specimen. In this study, plasma cell enrichment and aCGH were combined to analyze specimens with a low tumor burden. The combined technologies revealed the presence of additional genomic aberrations in 26 of 28 specimens. Compared with plasma cell-enriched FISH and conventional cytogenetic analysis, this approach extends the detection of genetic abnormalities, even in cases with

minimal disease burden, to facilitate prognostic assessment and monitoring of more patients.

Materials and Methods

Specimens

Bone marrow aspirate specimens (sodium heparin) with known PCNs were evaluated for genomic abnormalities. In the first cohort, results from 80 PCM FISH-positive specimens were compared with the results from conventional cytogenetic evaluation. In the second cohort of 28 specimens, results from FISH, aCGH, and conventional cytogenetic analysis were compared. The percentage of monoclonal plasma cells in the second group of specimens (identified with flow cytometry in 22 of 28 specimens) varied between 1.2% and 67% (median, 9.65%). The second patient cohort was composed of 15 women and 13 men with a median age of 68 years (range, 52-85 years). All specimens were deidentified and the study was performed in accordance with review exemption issued by the Western Institutional Review Board (Olympia, WA).

Cell Separation

Magnetic cell separation (MACS) of plasma cells was performed using the whole blood CD138 microbeads, whole blood column kit, and QuadroMACS separation unit (Miltenyi Biotec, Auburn, CA) according to the manufacturer's protocol and as described previously.¹³

FISH

FISH was performed on CD138+ enriched cells obtained from 26 of the 28 samples as described previously.¹³ The PCM FISH panel was designed to detect deletion of 13q/monosomy 13, t(4;14)(p16.3;q32.2) translocation, or alternate *IGH* gene rearrangements; and loss of TP53/monosomy or gain of 17 and 11q abnormalities (loss or gain) (LSI D13S319[13q14.3]/LSI 13q34 [LAMP1], *IGH*/FGFR3 dual color dual fusion translocation, LSI TP53/CEP17, and MLL dual color break-apart rearrangement, respectively [Abbott Molecular, Abbott Park, IL]). Hybridization was performed on enriched plasma cells.

A total of 200 cells per sample were evaluated with fluorescence microscopy. Touching and overlapping cells were excluded. Cells were analyzed regardless of their shape or size. During previous validation studies, sensitivity cutoff values were calculated using the Microsoft Excel statistical function CRITBINOM (n, P, α ; Microsoft, Redmond, WA) with a confidence level of 95%.¹⁶ A specimen was considered "abnormal" if the scores of 2 technicians independently exceeded the sensitivity cutoff values for 1 or more signal patterns.

Array-Based Genomic Analysis

Genomic DNA was isolated from CD138+ MACS-enriched cell populations using the QIAamp DNA mini kit (Qiagen, Gaithersburg, MD) according to the manufacturer's instructions. Three different platforms were used to obtain virtual karyotypes: whole genome bacterial artificial chromosome (BAC) microarray, oligonucleotide microarray, and SNP array.

Samples MA-A01 and MA-A05 were evaluated using the CytoScan HD array (Affymetrix, Santa Clara, CA), an SNP array including more than 2.6 million copy number markers. DNA specimens were obtained as described before and sent to a reference laboratory as part of a "proof of principle" study. Array processing and data analysis were performed according to the manufacturer's recommendations.

For the other 26 cases (MA-MMHL-01 to MA-MMHL-54), aCGH analysis of the tumor genome was performed using the DNAarray heme profile test (Combimatrix Molecular Diagnostics, Irvine, CA). A first set of samples (MA-MMHL-01 to MA-MMHL-13) was tested using a 3039 probe whole genome BAC microarray. Tumor genomic DNA (test DNA) and reference DNA of the opposite sex (used as an internal control) were differentially labeled with Alexa Fluor 555 and Alexa Fluor 647 fluorescent dyes (Life Technologies, Carlsbad, CA), respectively, and hybridized to the BAC arrays. Hybridized microarray slides were scanned and quantified with GenePix 4000B scanner and GenePix Pro (Molecular Devices, Sunnyvale, CA). The normalized Alexa Fluor 555/647 intensity ratios were computed and plotted for each chromosome using BlueFuse software (BlueGnome, Cambridge, England). The second set of samples (MA-MMHL14-MA-MMHL-54) was tested using an oligonucleotide 180K high-density microarray featuring 20,000 probes designed by the Cancer Cytogenomics Microarray Consortium to target 500 genes and other cancer-specific loci. Tumor-genomic DNA and reference DNA of the same sex were labeled with Cy5 and Cy3, respectively, and hybridized to the oligonucleotide arrays (Agilent Technologies, Santa Clara, CA). Hybridized microarray slides were scanned with an Agilent scanner, and the data were analyzed with Agilent feature extraction and Nexus copy number software (BioDiscovery, El Segundo, CA). For both sample sets processed at Combimatrix, a ratio plot was assigned such that gains in DNA copy number at a particular locus were observed as the deviation of the ratio plots from a modal value of 0.0 (log2). The ratio plot showing a positive deviation above the zero reference was observed as a gain in copy number change and the ratio plot showing a negative deviation below the zero reference as a loss in copy number change. Copy number changes were determined by ratio plot visualization and objectively determined by fluorescence intensity ratios determined with the BlueFuse and Nexus software.

Results

FISH vs Conventional Cytogenetics Only

Over a 12-month period from 2010 to 2011, 80 specimens submitted to our laboratory tested positive with PCM FISH analysis using CD138+ enriched cell populations. These specimens were also analyzed with conventional cytogenetics (Table 1). Abnormalities were detected in 11 specimens using cytogenetic analysis and only 5 (6.3%) of 80 cases had detectable PCM-related abnormalities. The remaining 6 cases revealed genomic abnormalities of possible myeloid or other origin (2 cases with del[9], 1 case with +21, as well as 3 cases with -X or -Y) that did not correlate with the FISH findings. The average percentage of plasma cells in 34 of 80 of these bone marrow aspirates detected with flow cytometry was 13% (range, 0.3%-72%).

Conventional Cytogenetics in Cases Analyzed With aCGH

The low detection rate of conventional cytogenetics for myeloma-related genetic abnormalities triggered a quest for alternative methods to probe more of the genetic aberrations in this disease. The potential role of aCGH in a second cohort of patients was therefore assessed. Cytogenetic studies were performed in 15 of 28 specimens, including analysis of one 24- and two 72-hour cultures, with one interleukin 2-stimulated specimen. Chromosome abnormalities were detected in 6 of 15 specimens evaluated. In 3 cases, the aberrations were not consistent with PCM. Cases 2 and 4 demonstrated loss of 1 sex chromosome, most likely an age-related phenomenon. Case 11 had trisomy 8, consistent with a myeloid disorder. For cases 15, A01, and A05, cytogenetic analysis revealed complex hyperdiploid clones with several structural aberrations (see Supplementary Table at www.ajcp.com). Clonal evolution was evident in case 15 because 2 abnormal cells also had deletion of the distal short arm of the third copy of chromosome 5.

FISH in Cases Analyzed With Plasma Cell aCGH

Bone marrow aspirates with known monoclonal plasma populations ranging from 1.2% to 67% (median, 9.65%) as detected with flow cytometry in 26 of 28 specimens were evaluated for genomic aberrations using both FISH and

Table 1
Summary of Patient Population With Positive PCN FISH Results Compared With Conventional Cytogenetic Analysis*

Abnormal PCN FISH specimens	80
Abnormal with conventional cytogenetics	11/80 (13.8)
Abnormality PCN related with conventional cytogenetics	5/80 (6.3)

FISH, fluorescence in situ hybridization; PCN, plasma cell neoplasms.
* Data are number (percentage).

plasma cell aCGH. FISH performed on plasma cell-enriched samples detected genomic abnormalities in 22 of 26 specimens (Table 2). A total of 58 abnormal events were observed with FISH, with a mean of 2.6 abnormalities (median, 2) per case. The most common aberrations were deletion of 13q/monosomy 13 (50% of all cases) and gain of 11q (38.5%). In addition, 8 cases had chromosome 17 abnormalities, 3 cases had a 17p (TP53) deletion, and 5 cases had a gain of chromosome 17.

In 7 cases, FISH discovered balanced translocations involving the *IGH* locus. These findings were not detected with aCGH. However, the vast majority of numerical abnormalities revealed by FISH were concordant with aCGH findings. Furthermore, aCGH revealed 113 additional genomic aberrations compared with FISH.

Plasma Cell aCGH Analysis

Genomic abnormalities were observed in 25 of 28 samples analyzed with plasma cell aCGH. A total of 171

aberrations were detected, with a mean 6.8 abnormal events per case (median, 5). The detected events consisted of 100 gains and 71 losses. This is a much higher abnormality detection rate compared with either FISH or conventional cytogenetics (Figure 1).

Hyperdiploidy was detected in 10 cases (35.7%). The most frequent chromosome gains were observed as follows: 9>15>11>3;5;7;21>19. According to several studies, hyperdiploidy is associated with more favorable clinical features and is more common in elderly patients.^{6,7}

Deletions of 13q and monosomy 13 were identified in 5 (17.9%) and 15 (53.6%) cases, respectively. These findings were consistent with the results of FISH. Deletions of 13q or monosomy 13 (which are detected in almost 50% of PCM cases) had previously been associated with poor prognosis and early stages of the disease^{17,18}; however, recent studies show that in the presence of t(4;14) and/or deletion of *TP53*, 13q aberrations do not add additional prognostic significance.^{7,19}

Table 2
Summary of Cytogenetic, FISH and aCGH Findings and Risk Stratification

Summary of Results			
Patient	Conventional Cytogenetics	FISH	aCGH
MA-MMHL-01	Normal	t(4;14), -13	G: 1q, 3, 11p, 15, 21; L: 8p, 13, 22
MA-MMHL-02	45,X,-X [3/30]	Putative hyperdiploidy	L: 16, 6q, 8p, 13, 14
MA-MMHL-03	Normal	11q+, 4p+	Hyperdiploidy; L: 8p
MA-MMHL-04	45,X,-X [4/30]	t(11;14)	G: 11q, 14q, 19
MA-MMHL-05	Not tested	Putative hyperdiploidy, 13q-	G: 1q, hyperdiploidy; L: 6q, 13q, 16p
MA-MMHL-06	Normal	17p-, -13, 11q+	G: 1q, hyperdiploidy; L: 1p, 8p, 13, 17p
MA-MMHL-07	Not tested	t(11;14), 11q+	G: 11q, 14
MA-MMHL-08	Normal	17p-, -13, 11q+, 14q32+	G: 11q, 14q; L: 1p, 12q, 13, 16q, 17p, 20q
MA-MMHL-10	Normal	-13, +17	Hyperdiploidy; L: 1p, 13, 14q, 16q
MA-MMHL-11	45,X-Y[7], 47,XY,+8[5/20]	-13	L: 13, 14q
MA-MMHL-12	Not tested	Normal	Normal
MA-MMHL-13	Not tested	t(11;14)	Normal
MA-MMHL-15	Complex hyperdiploid cell clone with chromosome 1 abnormalities	Normal	Hyperdiploidy; L: 1p, 13, 19q, 22q
MA-MMHL-16	Not tested	Putative hyperdiploidy	Hyperdiploidy
MA-MMHL-17	Normal	Normal	Normal
MA-MMHL-20	Normal	t(4;14), -13	L: 9p, 2q, 6p, 13q, 20q
MA-MMHL-21	Not tested	11q+, 13q-	G: 11q; L: 2q, 6q, 13q, 20q
MA-MMHL-34	Normal	t(14;16), putative tetraploidy	G: 1q; L: 13, 16q
MA-MMHL-47	Not tested	<i>IGH</i> gene rearrangement, -13, -14	G: 9q; L: 13, 14
MA-MMHL-48	Not tested	17p-	Chromothripsis of chromosome 17; L: 12, 13, 22
MA-MMHL-49	Not tested	14q32- *	Hyperdiploidy; L: 13, 14, 16q
MA-MMHL-50	Not tested	-13	L: 13, 16q
MA-MMHL-51	Normal	t(14;16), -13	G: 1q, 9q; L: 1p, 13, 14q, 16q
MA-MMHL-52	Not tested	Normal*	G: 11q
MA-MMHL-53	Not tested	Not tested, previously: -13	G: 1q; L: 10q, 13, 22
MA-MMHL-54	Not tested	13q-, 11q+	Hyperdiploidy; L: 13q-
MA-A01	Complex hyperdiploid cell clone with chromosome 1 abnormalities	Not tested	G: 1q, hyperdiploidy; L: 8p, 13, 16q, 17p
MA-A05	Complex hyperdiploid cell clone	Not tested	G: 16p, hyperdiploidy; L: 8p, 16q

aCGH, array comparative genomic hybridization; FISH, fluorescence in situ hybridization; IND, indeterminate; MA-A, samples using the Affymetrix CytoScan array;

MA-MMHL, samples using the oligonucleotide or bacterial artificial chromosome array; NA, not available.

* Limited FISH data available, samples only tested for *IGH* gene rearrangement (chromosome 14) and deletion of *TP53* (chromosome 17).

Using aCGH and FISH, deletions of 17p (TP53) were detected in 3 specimens (10.7%). Deletion of 17p13 is considered to be the most important molecular cytogenetic prognostic factor. The deletion results in the loss of the locus for the tumor suppressor gene *p53* and is linked with overall shorter survival and more aggressive disease.^{1,8,15}

Two categories of aberrations on chromosome 1 have been identified as conferring prognostic significance in PCM, and both are associated with shorter survival: gain of 1q and 1p loss.^{15,20,21} In this study of 28 patients, 1q gain and 1p loss were discovered in 25% and 17.9% of patient samples using aCGH analysis.

In 39.3% of cases, a deletion of 16q was identified. Deletions of 16q have been found in a high number of PCM cases and linked to adverse outcome.^{20,22}

We found gain of chromosome 5 in 7 (25%) of 28 specimens. Avet-Loiseau et al²⁰ reported gain of 5q31 as the most favorable prognostic marker in hyperdiploid PCM cases. In contrast, another group was not able to replicate this finding when analyzing survival data of a different patient cohort.²³

Other frequent alterations included 11q gain (46.4%), chromosome 3 gain (35.7%), 8p loss (25%), and 22q loss (14.3%).

Risk Stratification

Cytogenetic abnormalities detected with FISH and aCGH are used for risk stratification according to several guidelines.^{4,7,9,19} Abnormalities associated with standard risk included hyperdiploidy and t(11;14) and t(6;14) translocations. Patients with aberrations that correlate with a shorter overall survival or poor outcome (deletions of chromosome 16 and 12p13 deletion) were assigned to the intermediate-risk group. Deletion of 17p, chromosome 1 abnormalities, and translocations t(4;14), t(14;16), and t(14;20) were considered adverse indicators for patients in the high-risk disease group.

FISH findings alone identified 6 standard and 7 high-risk patients; risk stratification was not possible for 13 (50%) of 26 patients. With findings from aCGH, the same patient cohort was classified into 11 standard, 6 intermediate, and 11 high-risk cases. Combining aCGH results with FISH findings for *IGH* gene rearrangements, we were able to stratify 27 (96%) of 28 patients (Table 2).

Case Studies

The relationships between the different techniques are complex and best illustrated using specific cases. We observed various discrepancies when we compared FISH and aCGH results for case 34. Initial FISH analysis alone showed gains of 11q (MLL), 4p (FGFR3), 14q32 (*IGH*), and chromosome 17. Results of each marker were suggestive of

Prognostic Stratification

Cytogenetic Results	FISH Results	aCGH Results	aCGH and <i>IGH</i> FISH Combined
IND	High	High	High
IND	Standard	Intermediate/poor	Intermediate/poor
IND	IND	Standard	Standard
IND	Standard	Standard	Standard
NA	Standard	High	High
IND	High	High	High
NA	Standard	Standard	Standard
IND	High	High	High
IND	IND	High	High
IND	IND	Standard	Standard
NA	IND	Standard	Standard
NA	Standard	Standard	Standard
High	IND	High	High
NA	Standard	Standard	Standard
IND	IND	Standard	Standard
IND	High	Intermediate/poor	High
NA	IND	Intermediate/poor	Intermediate/poor
IND	High	High	High
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NA	High	High	High
NA	IND	Intermediate/poor	Intermediate/poor
NA	IND	Intermediate/poor	Intermediate/poor
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NA	IND	High	High
NA	IND	Standard	Standard
High	NA	High	High
High	NA	Intermediate/poor	Intermediate/poor

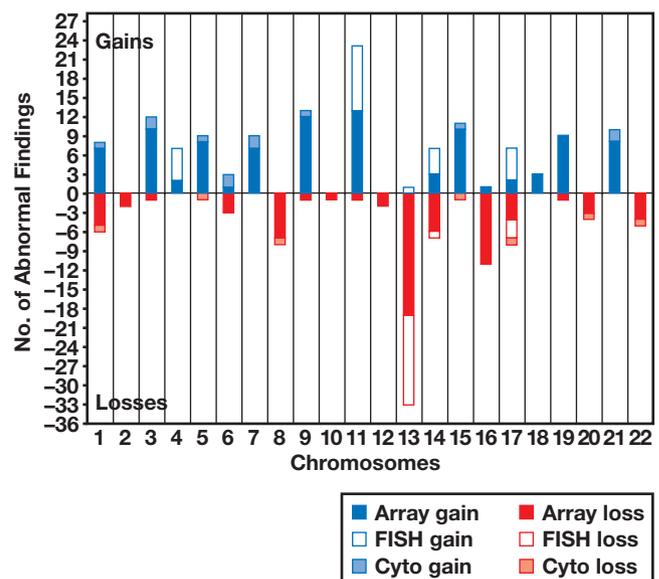


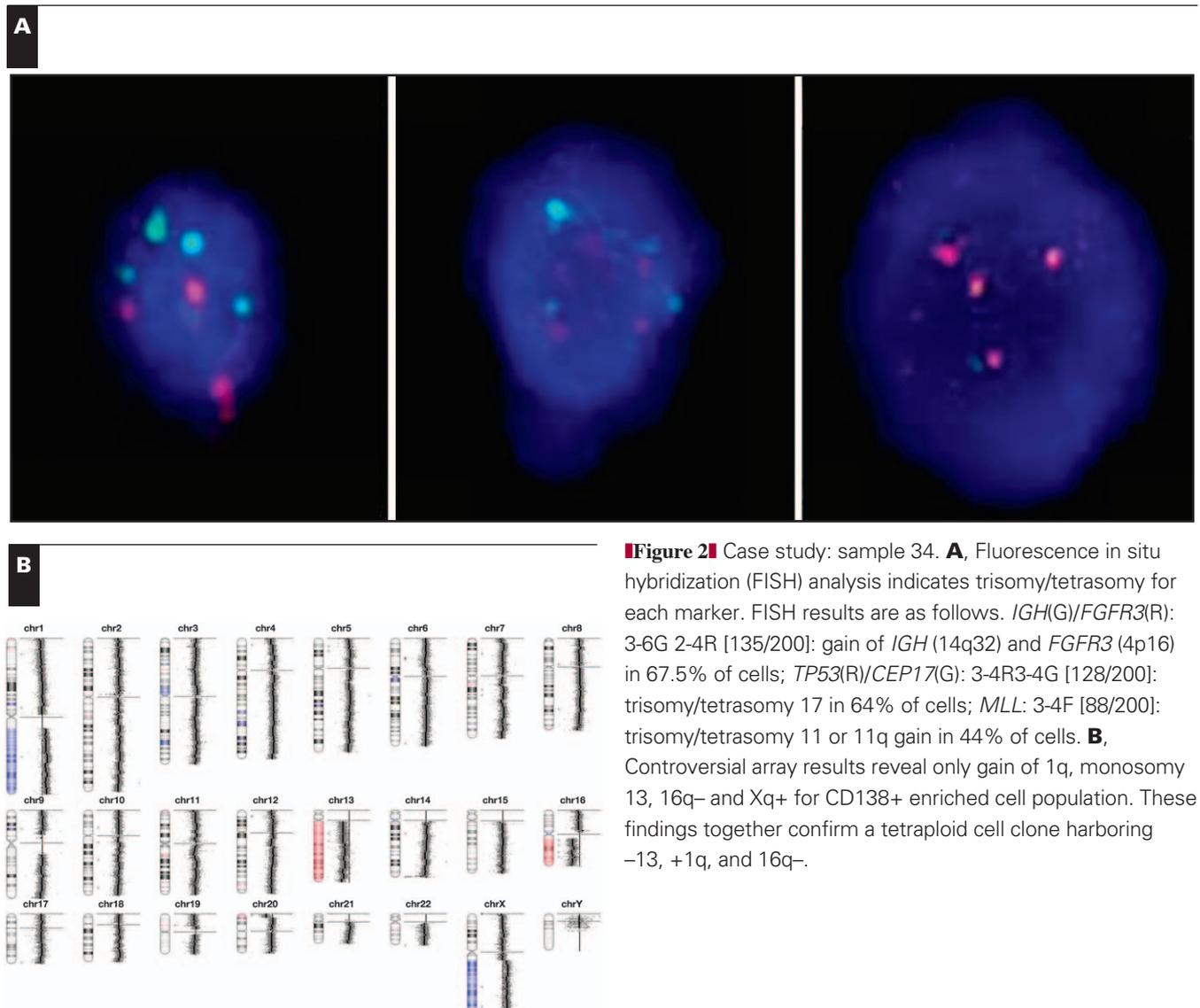
Figure 1 Summary of detected gains and losses by chromosome.

trisomy/tetrasomy **Figure 2A**. aCGH revealed a 1q gain, monosomy 13, 16q loss, Xq gain, without any specific gains of loci identified with FISH **Figure 2B**. Combining FISH with aCGH results revealed a tetraploid plasma cell clone with $-13;1q+,16q-$. With a better understanding of the tumor's ploidy, we reanalyzed the initial FISH data. Up to 6 copies of the IGH signal were observed, indicating the presence of an alternate *IGH* gene rearrangement in the tetraploid cell clone. As a result, FISH studies were performed and the t(14;16) translocation was identified, which is associated with a poor prognostic category and high-risk disease for PCM.

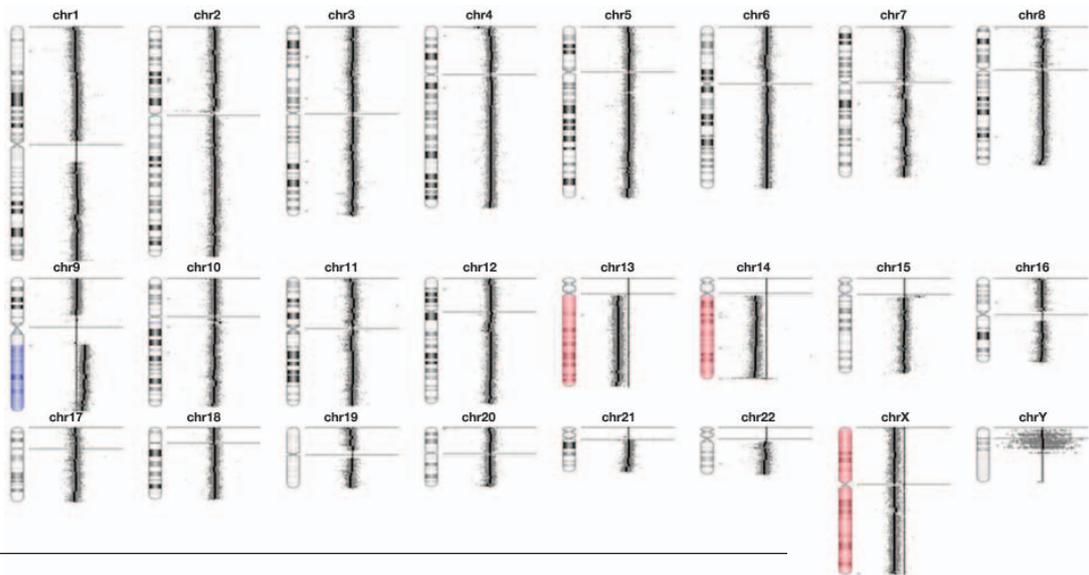
For case 47, aCGH detected gain of 9q, monosomy 13, monosomy 14, and X **Figure 3A**. FISH studies alone yielded conflicting results with a normal signal pattern and no loss of 14q32 (*IGH*) **Figure 3B**. To investigate this result further, we performed FISH using *CCND1/IGH* and *MAF/IGH* probes but again observed normal signal patterns. Therefore,

no evidence of t(11;14), t(4;14), or t(16;14) could be identified with FISH. We further investigated the *IGH* locus using a break-apart FISH probe to rule out a cryptic *IGH* translocation potentially undetectable with dual color/dual fusion probes. An abnormal signal pattern (one fusion signal, one 5'*IGH* signal, but no 3'*IGH* signal) was observed, confirming the loss of the derivative chromosome 14 from an *IGH* gene rearrangement with an unknown partner gene **Figure 3C**.

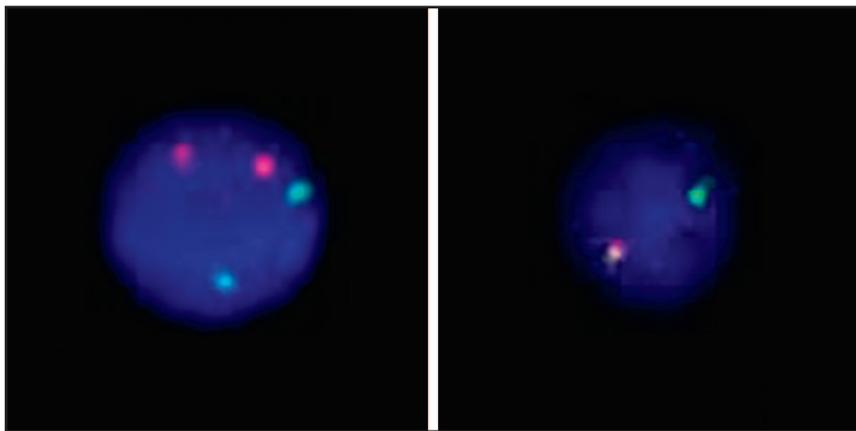
A final case study illustrating disparate results between FISH and aCGH involves the detection of chromothripsis (case 48, **Figure 4**). In this case, FISH only detected loss of 17p whereas aCGH identified a high number of additional gains and losses on chromosome 17, indicative of chromothripsis. Chromothripsis detected with microarray analysis defines genomic instability featuring a large number of chromosome rearrangements involving a localized genomic region. Chromothripsis has been postulated to be a novel



A



B



C

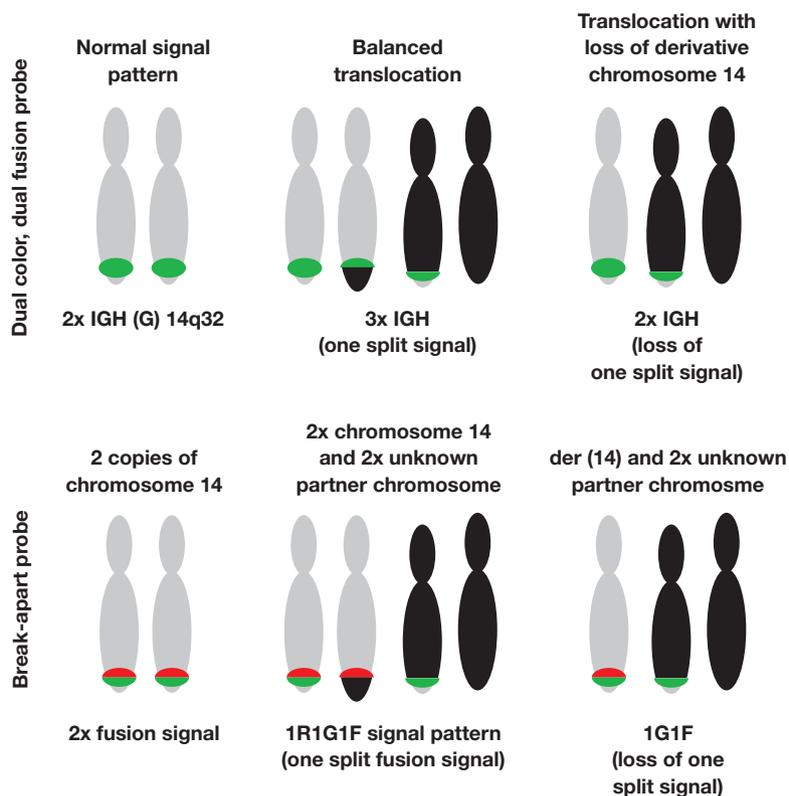


Figure 3 Case study: sample 47.

A, Array results demonstrate monosomy 14 on sorted plasma cells.

B, FGFR3/IGH fluorescence in situ hybridization (FISH) studies (left pane) do not confirm loss of the 14q32 (IGH) signal while the IGH break-apart probe set (right pane) indicates an alternated IGH gene rearrangement with unknown partner gene in addition to loss of the derivative chromosome 14. FISH results are as follows. IGH(G)/FGFR3(R): normal signal pattern [200]; 5'IGH(G)/3'IGH(R) 1F1G [79/100]: IGH rearrangement and loss of derivative chromosome 14 in 79% of cells.

C, FISH signal patterns for dual fusion (top) and break-apart probes (bottom).

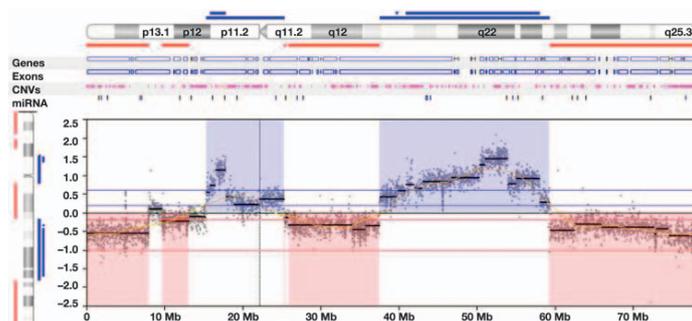


Figure 4 Array comparative genomic hybridization revealed chromothripsis of chromosome 17, featuring a large amount of chromosomal rearrangements.

clonal evolution process occurring as a single event, with a single or a limited number of chromosomes shattering into pieces and subsequently being spliced back together, producing highly complex derivative chromosomes.^{24,25}

Before and After Plasma Cell Enrichment

To demonstrate the importance of plasma cell purification before DNA extraction, we compared aCGH results of 2 specimens before and after MACS enrichment. Flow cytometric analysis identified 3.6% and 22% monoclonal plasma cells in the unpurified bone marrow aspirates (cases 20 and 21, respectively). Array results for case 21 were positive for the bone marrow aspirate as well as the corresponding plasma cell purified sample. The abnormal findings were identical for both DNA preparations (2p-, 6q-, 11q-, 13q-, 20q-). In contrast, no numerical aberrations were identified in the unpurified sample of case 20 (3.6% plasma cells) whereas the MACS-separated specimen revealed a total of 5 abnormalities: 2q-, 8p-, 9p+, -13, 20p- **Figure 5**.

Discussion

Identification of genomic abnormalities in patients with PCN has a major effect on prognosis and disease risk stratification of these patients. In this study, we performed aCGH after plasma cell enrichment and demonstrated the benefit of this approach compared with FISH and conventional cytogenetics.

Conventional Cytogenetics Compared With FISH and Microarray Analysis

Conventional cytogenetic analysis is a standard test for clinical assessment of genomic aberrations in hematopoietic malignancies. Abnormal cytogenetic results can reveal numeric abnormalities but also structural rearrangements and clonal evolution. However, metaphase analysis is often uninformative in PCM cases because of the lack of dividing tumor cells in vitro. Over a course of 1 year, we observed PCN-related chromosomal abnormalities in only 6.3% of cases,

which in contrast, were positive on FISH analysis of CD138+ enriched cell populations. This demonstrates that cytogenetic analysis as a sole test is not sufficient and other technologies have to be used to evaluate genomic anomalies in PCN.

Of the 28 specimens evaluated with aCGH, 15 were also tested using conventional cytogenetics. In 12 of 15 cases, cytogenetic analysis did not identify myeloma-associated chromosome abnormalities. In 1 specimen (case 15), a complex karyotype and clonal evolution were detected. Flow cytometric analysis revealed 51% monoclonal plasma cells in this bone marrow aspirate specimen, suggesting a correlation between tumor load and the detection sensitivity of cytogenetic testing. However, it has been reported that the ability of cytogenetics to identify an abnormal myeloma-associated cell does not correlate with the percentage of monoclonal plasma cells in the marrow but instead reflects the proliferative status of the tumor.⁵

FISH Compared With Microarray Analysis

Standard FISH analysis of plasma cell-enriched cell populations was able to identify 58 genomic aberrations in 22 of 26 specimens tested, with an average detection rate of 2.6 alterations per case. In contrast, aCGH detected 171 genomic abnormalities, with an average of 6.8 per specimen. In addition, cases with putative hyperdiploidy noted on FISH could be confirmed with aCGH because FISH analysis alone can often only suggest gains of entire chromosomes. aCGH, however, can distinguish between a partial or complete chromosomal aneuploidy, thus conferring prognostic value. In addition to a higher detection rate of abnormalities, microarray analysis was also superior in disease risk stratification. Clinically significant abnormalities not evaluated with FISH (eg, loss of chromosome 16 or 1q/1p abnormalities) were identified with aCGH in 13 cases. Risk stratification according to FISH results was not possible in 50% of cases because the abnormalities detected did not bear prognostic significance (eg, 11q+ or 13q-). In contrast, aCGH results enabled risk classification for all cases analyzed (Table 2). The combination of both aCGH and FISH results for *IGH* gene rearrangement probes led to a more comprehensive stratification but

resulted in 1 indeterminate case (MA-MMHL-47). Although aCGH findings were indicative for standard risk group, FISH analysis revealed the presence of an *IGH* gene rearrangement. The translocation partner was not identified with FISH, therefore it is not possible to stratify this patient because the classification depends on the involved partner gene.

aCGH Enables a Better Comprehension of Genomic Abnormalities in PCN

As we demonstrated in cases 34, 47, and 48, aCGH allows a more comprehensive assessment of genomic changes in PCN.

At first view, FISH and microarray findings in case 34 seem contradictory because aCGH did not reveal the abnormalities detected with FISH. However, the conflicting test results can be explained when considering the main principle of aCGH. Because the most frequent ratio level was set as “normal,” aCGH did not provide information regarding the ploidy. Having a balanced DNA content, a tetraploid clone without further copy number variations would appear normal in aCGH. Only the combined analysis of both aCGH and at least 1 FISH target enabled a full understanding of the existing abnormalities, confirmed tetraploidy, and led to the discovery of an alternate *IGH* translocation. In addition to the prognostic value of this finding, the discovery of the t(14;16) is also very valuable for further disease-monitoring purposes because dual color/dual fusion probes are more sensitive to detect residual cell clones than enumeration probes.

Although aCGH is in general not able to detect balanced rearrangements, the presence of a translocation involving the *IGH* gene on chromosome 14 would not have been identified without the use of microarray analysis for case 47. All FISH studies were normal for available *IGH* dual color/dual fusion probe sets. Only the detection of monosomy 14 (location of the *IGH* gene) with aCGH led to further investigation using a break-apart probe (Figure 3) and discovery of an alternate *IGH* gene rearrangement with loss of the derivative chromosome 14.

Microarray analysis also revealed a case of chromothripsis, a clinically relevant finding that is not detectable with FISH. So far, chromothripsis has been reported in only a few patients with PCM (1.3% in a study by Magrangeas et al²⁴) but clinical outcome data suggest that chromothripsis is associated with an aggressive malignant phenotype and rapid disease progression. Consequently, the identification of chromothripsis as seen in case 48 is of therapeutic significance.

Plasma Cell Enrichment

One technical limitation of aCGH is the inability to identify low-level mosaicism. Tumor cells must comprise approximately 20% of the sample to allow the detection of abnormalities. Therefore, plasma cell isolation is required before aCGH analysis in cases of low tumor cell burden. Our study

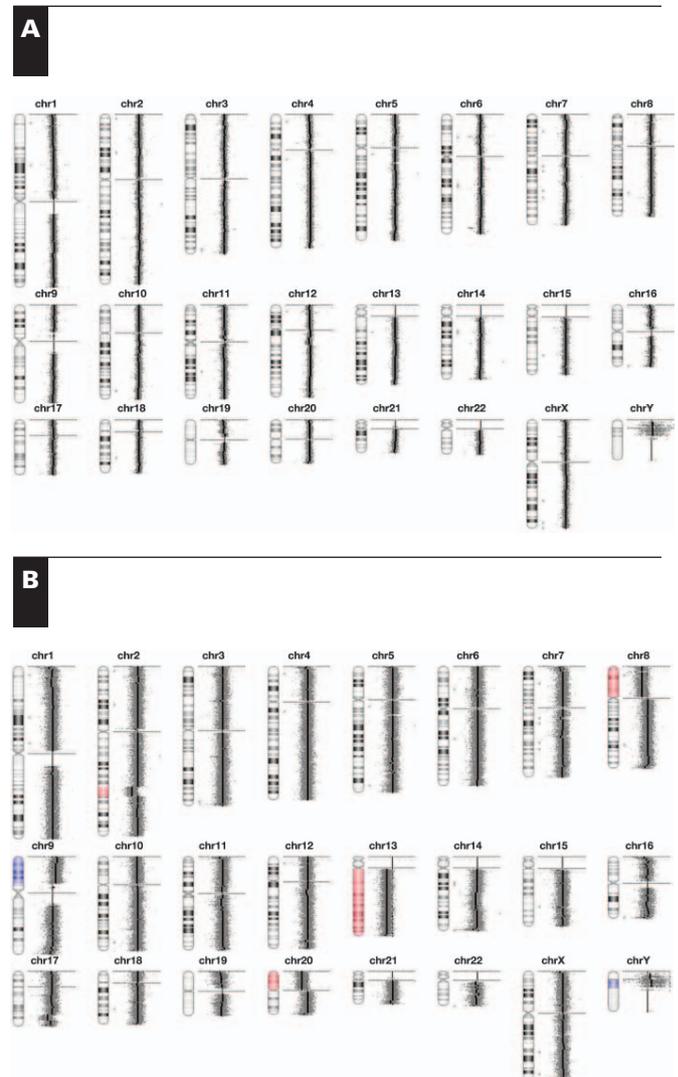


Figure 5 Benefit of plasma cell enrichment for abnormality detection by array comparative genomic hybridization. **A**, Array results of the unsorted specimen (3.6% plasma cells [PCs]) are normal. **B**, In contrast, array results for the PCs enriched sample of the same patient reveal several abnormalities.

and those of others previously demonstrated that plasma cell enrichment using immunomagnetic beads may lead to low purity, especially when the enrichment is performed several days after the bone marrow aspirate is obtained.¹³ According to Jourdan et al,²⁶ plasma cells rapidly lose the CD138 marker once they are separated from the bone marrow environment. Plasma cell enrichment should be performed within 48 hours to ensure sufficient plasma cell yield. In addition, flow cytometric quantification of CD138- and CD38-expressing plasma cells is necessary to provide a quality control step and should therefore be implemented before and after each plasma cell collection.

Gene Expression Profiling for Diagnostic Workup

Interest has recently focused on gene expression profiling (GEP) for risk stratification in PCM.²⁷⁻²⁹ Transcriptome analysis of CD138+ cells is accomplished using a microarray-based approach. Several studies have used GEP data to generate transcriptome signatures that correlate with patients' outcome and treatment response.³⁰⁻³³ Zhan and colleagues³⁴ developed a GEP-based classification system that divides patients into 7 molecular subtypes and 3 risk groups (low, moderate, and high).²⁹ Interestingly, it was shown that the molecular groups are linked with known genetic lesions such as hyperdiploidy or translocations involving the *IGH* gene locus. A 15-gene model developed by Decaux et al³⁰ also showed significant association between the established risk groups and underlying genomic abnormalities such as deletion on 13q, 17p, or hyperdiploid status. Prediction of underlying cytogenomic aberrations according to GEP patterns has been reported with a mean accuracy of 0.89 (range, 0.74-1.00).³⁵ However, prediction models might be less suitable for the evaluation of disease progression and/or minimal residual disease detection because it is imperative to be certain about existing genomic abnormalities to monitor persistent cell clones with FISH or real-time polymerase chain reaction.

GEP also has certain technical limitations potentially hampering diagnostic workup of patients with PCM. First, it has to be considered that GEP analyzes RNA, which is known to be much less stable than DNA. Degradation of RNA during sample transport and processing might bias the transcriptome analysis. More important, GEP requires a very pure plasma cell isolate. In one study, GEP signatures of 27% of newly diagnosed patients could not be determined because tumor cell isolations were contaminated with myeloid and normal plasma cells.³⁴

As a consequence, it is open to discussion whether GEP is an adequate substitute for technologies such as FISH, cytogenetics, and aCGH, which directly detect genomic aberrations and thereby provide prognostic classifications in correlation to previously established GEP categories.

Future Importance of aCGH

Little is known about the oncogenetic events leading to a transformation of MGUS to PCM.^{2,36} From a clinical perspective, it is of high importance to identify genetic lesions responsible for disease progression. With this information, the risk for individual patients with MGUS to undergo a conversion to PCM could be assessed and a more tailored treatment facilitated.³⁶ Because aCGH is an explorative approach for investigating genome-wide aberrations, this technique is potentially helpful in achieving a better understanding of MGUS and PCM with regard to underlying genetic abnormalities responsible for disease progression. Because of limited access to comprehensive clinical information

for samples investigated in this study, we cannot compare genomic aberrations found in MGUS and PCM. Future aCGH studies evaluating clinical progression and diagnostic criteria for MGUS and PCM are imperative.

In conclusion, this study has shown that the implementation of aCGH into the diagnostic workup of patients with PCNs allows full characterization of chromosome gains and losses, and is consequently a powerful tool for prognostic assessment and risk stratification of this disease. The high cost of FISH tests and, often, small sample size limits the feasibility of this technique to the evaluation of all current and still-to-be-established prognostic markers for MGUS/PCM. Conversely, aCGH allows genome-wide detection of numeric aberrations but cannot detect balanced translocations. Therefore, microarray analysis of plasma cell-enriched samples, in combination with FISH evaluation for *IGH* rearrangements, is recommended to detect all genomic aberrations in PCM and MGUS. The superior abnormality detection rate demonstrated in this study and those of others justifies the routine use of microarray analysis for the clinical workup of PCN cases. The cost associated with microarray analysis is equivalent if not lower than the cost of a comprehensive FISH panel, which provides only limited abnormality detection. Furthermore, new prognostic categories may emerge from accumulating follow-up data incorporating loss of heterozygosity findings. Consequently patient-specific monitoring targets can be designed for follow-up FISH analysis for individual patients.

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