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Plasma Cell Enrichment Enhances Detection of High-Risk Cytogenomic Abnormalities by Fluorescence In Situ Hybridization and Improves Risk Stratification of Patients With Plasma Cell Neoplasms

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Abstract

Context—Methods for plasma cell enrichment in bone marrow (BM) specimens can increase the sensitivity of fluorescence in situ hybridization (FISH) for detecting cytogenomic abnormalities, but there is no published report using these methods to evaluate high-risk cytogenomic abnormalities in patients with treated plasma cell neoplasms (PCN) and clinicopathologic data in follow-up.

Objective—To evaluate the utility of plasma cell enrichment combined with FISH and follow-up data for high-risk cytogenomic abnormalities in post-therapy PCN patients.

Design—twenty-eight PCN patients with 22 treated were included in this study. Plasma cells were enriched in BM aspirates using a magnetic cell-sorting procedure to select CD138+ cells. Probes were chosen to assess for del(17p13/TP53), del(13q14/RB1), 1q21/CKS1B gain, IgH/FGFR3 and IgH/MAF. Clinicopathologic data were collected during clinical follow-up after plasma cell enrichment.

Results—Plasma cells in non-enriched specimens ranged from 1%–28% (median, 8%) compared with 28%–96% (median, 73%) in enriched specimens ($p<0.0001$). In a subset of treated-patients in clinical remission, FISH detected high-risk cytogenomic abnormalities only in plasma cell

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enriched samples. This approach also detected abnormalities in cases of solitary plasmacytoma and monoclonal gammopathy of undetermined significance.

Conclusions—Plasma cell enrichment of BM samples increases FISH sensitivity to detect high-risk cytogenomic abnormalities, particularly in treated-patients, and these results, in combination with data from clinical follow-up, can be of value to improve risk stratification and patient management.

Plasma cell myeloma (PCM) is the most common type of plasma cell neoplasm, diagnosed in approximately 20,000 patients each year in the United States. In patients with monoclonal gammopathy of undetermined significance (MGUS), approximately 10% of progress to PCM each year.^{1,2} Plasma cell infiltration of bone marrow (BM) is one of the diagnostic criteria for PCM, in addition to other clinical features and laboratory findings, including elevated serum monoclonal (M)-protein, lytic bone lesions, elevated serum β 2-microglobulin, and a plasma cell immunophenotypes.^{3,4} Plasma cells are typically positive for CD38 and CD138, express monotypic cytoplasmic immunoglobulin light chain, and are negative for CD20.⁵

Plasma cell myeloma is molecularly heterogeneous. A variety of cytogenomic abnormalities contribute to its pathogenesis and affect risk stratification and treatment. Others have proposed that at least 3 cytogenomic pathways are commonly associated with development of plasma cell neoplasms, include the aneuploidy, *IgH*, and the del(13q)/-13 pathways.^{1,2,6-9} These pathways often coexist and interact during the development or progression of PCM. Almost all MGUS cases are aneuploid, showing chromosome gains that typically involve the odd-numbered chromosomes.^{2,9} Rearrangements of *IgH* occur in up to 65% of plasma cell neoplasms. These rearrangements can involve more than a dozen partners, including *CCND1-XT* (chromosome 11), *FGFR3* (chromosome 4), *MAF* (chromosome 16), *CCND3* (chromosome 6), and *MAFB* (chromosome 20), as well as unknown partners.^{1,7,9} Translocations of *IgH* are believed to be primary or secondary genetic events in the course of plasma cell neoplasms.^{1,6,7} Several cell-cycle regulators are involved in the *IgH* pathway, including *CCND1*, *CCND2*, and *CCND3*.^{1,10-13} Interstitial del(13q)/-13 has been observed in up to 70% of PCM cases and indicates disease progression.^{1,14,15}

In general, patients with t(11;14)(q13;q32) as the sole abnormality or who lack high-risk cytogenomic abnormalities are considered to be in the standard-risk group, whereas patients with t(4;14)(p16;q32) or t(14;16)(q32;q23) alone or in combination with hyperdiploidy, are considered to be high-risk.^{1,16} The presence of *MYC* rearrangement resulting from t(8;14)(q24.1;q32) or its variants can indicate progression to late-stage plasma cell myeloma.^{17,18} Abnormalities of chromosome 1, in particular, del(1p21/*CDC14C*) and 1q21 abnormalities [amp(1q21/*CKS1B*) or gain of 1q21/*CKS1B*], observed in up to 45% of PCM patients, are usually considered markers of disease progression, and their presence indicates a need for more intensive chemotherapy.¹⁹⁻²¹ It has been reported that gain of 1q21/*CKS1B* has an adverse effect on progression-free survival and overall survival of PCM patients treated with bortezomib.²² Results from recent studies indicate that cyclin kinase subunit 1B nuclear expression is also an adverse prognostic factor for PCM patients treated with bortezomib.²³

Del(17p/*TP53*) is considered the most unfavorable prognostic marker in patients with PCM.²⁴

Although the initial choice of therapy for patients with plasma cell neoplasms is based on clinical criteria, data on cytogenomic abnormalities in plasma cells are valuable for determining prognosis, as well as in planning long-term therapy.^{9,25} Interphase fluorescence in situ hybridization (FISH) has been proven to be a powerful method for detecting cytogenomic abnormalities in PCM. Thus, sensitive and specific methods to detect cytogenomic abnormalities by FISH are needed.²⁶ The use of samples enriched for plasma cells also has been shown to improve the sensitivity of FISH for detecting cytogenomic abnormalities in PCM.²⁷ To our knowledge, however, this approach has not been used to assess patients after therapy, nor has the detection of abnormalities in this setting been correlated with clinicopathologic data.

The purpose of this study is to use a method to enrich plasma cells in bone marrow (BM) specimens of patients with PCM, before or after therapy, and then use interphase FISH to detect cytogenomic abnormalities. Our results show that plasma cell enrichment methods greatly increase the sensitivity of FISH for detecting cytogenomic abnormalities in patients with plasma cell neoplasms, particularly in the post-therapy setting, and have clinical relevance.

MATERIALS AND METHODS

Study Group and Clinicopathologic Data

A total of 28 BM aspirate samples from patients with plasma cell neoplasms who were treated at The University of Texas MD Anderson Cancer Center were collected with approval from the institutional review board. A waiver of informed consent was obtained to collect clinicopathologic, follow-up, and cytogenomic data. According to diagnostic and prognostic criteria for plasma cell neoplasms and their clinicopathologic status,²⁸ the 28 patients were divided into three groups: (1) remission, including cases with no evidence of residual disease by morphologic or clinical evaluation (n=11), (2) persistent disease, including persistent disease, or progressive disease (n=11), and (3) new diagnosis (n=6). A total of 22 (78%) patients were receiving treatment at the time a BM specimen was obtained and used in this study for plasma cell enrichment.

Clinicopathologic data were collected retrospectively from the medical records and included: (1) BM status, including plasma cell percentage and presence or absence of plasmablastic morphology, (2) biochemical markers, including serum $\beta 2$ microglobulin, creatinine, immunoglobulin (such as IgG, IgA, IgM) levels, serum calcium, and urine protein levels, (3) bone lesions, (4) therapy and response, including chemotherapeutic agents, relapse, and refractoriness to therapy, and (5) immunophenotypic markers, including expression assessment of cytoplasmic immunoglobulin light chains, CD20, CD38, CD56, CD138, lambda, or kappa.

Samples and Plasma Cell Enrichment

Bone marrow aspirate samples from each patient in this study were divided into two aliquots. One aliquot was handled in a routine fashion for karyotyping and FISH analysis in clinical cytogenetics laboratory; it is referred to as the “routine sample” throughout the manuscript. The second aliquot was used for this study; it is referred to as the “study sample”. The study sample was further divided into two parts: one part was not enriched for plasma cells (non-enriched sample) and the second underwent plasma cell enrichment (enriched sample). Both the non-enriched and enriched samples underwent FISH analysis.

Specimens were enriched for plasma cells using a magnetic cell-sorting procedure (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) with minor modifications. Briefly, BM aspirate samples were first passed through a 0.2-mm filter to remove debris. Samples were then incubated with CD138 magnetic microbeads, allowing for positive selection of plasma cells (surface CD138+), and were then loaded onto a MACS column, to which the CD138+ plasma cells bind, located within a MACS separator (magnet). The enriched plasma cells were eluted from the column with elution buffer (phosphate-buffered saline, 0.5% bovine serum albumin, 2 mol/L EDTA).

We calculated a plasma cell enrichment factor for this study. Each BM aspirate sample underwent CD38 immunofluorescent staining. Briefly, small aliquots of non-enriched and enriched samples were incubated with a secondary CD38-biotin antibody, followed by incubation with anti-biotin-FITC antibody and counterstained with DAPI. A total of 200 cells were analyzed to obtain the percentage of CD38+ plasma cells in samples before and after plasma cell enrichment. The plasma cell enrichment factor was calculated as the percentage of CD38+ plasma cells in the enriched sample divided by the percentage of CD38+ cells in the non-enriched sample.

Conventional Cytogenetic and Interphase FISH Analyses

Conventional cytogenetic analysis was performed on the routine sample. Interphase FISH analysis was performed on the routine and study (non-enriched and enriched) samples, as previously described.^{29,30} FISH analysis was performed using a panel of probes specific for t(11;14)(q13;q32)-*IgH/CCND1-XT*, t(4;14)(p16;q32)-*IgH/FGFR3*, t(14;16)(q32;q23)-*IgH/MAF*, del(17p13)/*TP53*, del(13q)/*RBI*, and *IgH* (Vysis/Abbot Molecular, Inc., Abbot Park, IL) and gain of 1q21/*CKS1B* (Cytocell/Rainbow Scientific, Windsor, CT). *IgH* rearrangement with an unknown partner and/or those demonstrating loss or gain of 3'-*IgH* or 5'-*IgH* was classified as u*IgH* in this study. Two hundred interphase nuclei were evaluated for each sample. The cutoff values used to define positivity were: 7.6% for u*IgH*, 2.8% for *IgH/CCND1-XT*, 1.0% for *IgH/FGFR3*, 1.3% for *IgH/MAF*, 4.6% for del(13q)/*RBI*, 6.2% for del(17p13)/*TP53*, and 2.2% for gain of 1q21/*CKS1B*, which were used in clinical cytogenetics laboratory, with an exception of the 2.2% for gain of 1q21/*CKS1B*.

Flow Cytometric Immunophenotyping and BM Morphologic Evaluation

We reviewed BM aspirate smears or touch imprints stained with Wright-Giemsa, and performed differential 500 cell counts in all cases. Bone marrow aspirate clot and decalcified core biopsy specimens were routinely processed and stained with hematoxylin

and eosin. Immunophenotypic analysis was performed on BM aspirate samples by multicolor flow cytometry as previously described.³¹ The antibody panels included: CD19, CD20, CD38, CD45, CD56, and CD138.

Statistical Analysis

Since the differences in plasma cell counts between the initial BM sample used for routine karyotypic analysis and the research aliquot were not normally distributed, the Wilcoxon rank sum test was used. To compare plasma cell counts in the non-enriched and enriched plasma cell aliquots, the paired t-test was applied. We used the Pearson chi-square test or Fisher's exact test to compare the FISH results between samples before and after plasma cell enrichment. Results were considered statistically significant with 95% confidence interval, if the p -value was < 0.05 .

RESULTS

Clinicopathologic Findings

The clinical and pathology findings are summarized in Table 1. There were 18 men and 10 women, ranging in age from 36 to 78 years (median, 59.9 years). The initial diagnoses were PCM in 24 cases, MGUS in 3 cases, and solitary plasmacytoma of bone in 1 case. At the time BM was obtained on which plasma cell enrichment was performed, 6 patients were newly diagnosed and 22 were previously treated. The treated cohort included 11 patients who were in clinical remission and 11 with persistent disease.

Morphologic and Immunophenotypic Findings

Morphologic examination of BM aspirate smears demonstrated plasma cells, either increased in number and/or morphologically abnormal in 27 patients. In 1 patient (case 1) who was diagnosed with a right femur solitary plasmacytoma of bone, BM smears obtained from the posterior iliac crest were negative for plasma cells. In all cases the plasma cells were strongly positive for CD38, CD138, and monotypic immunoglobulin light chain (kappa in 18, lambda in 10). The plasma cells were negative or dimly positive for CD45, and negative for CD19, CD20, and CD56.

Comparison of Plasma Cell Counts in Routine, Plasma Cell Non-enriched, and Plasma Cell Enriched BM Aliquots

In the routine BM samples, plasma cells constituted from $<1\%$ to 63% (median, 3%) of total cells. In the non-enriched samples, the plasma cell percentage ranged from 1% to 28% (median, 8.0%) (Table 2). The difference between the initial BM and the non-enriched BM research aliquot was not significant and supports the interpretation that the research aliquot was similar to the initial BM sample treated routinely. Henceforth, we refer to the research aliquot as the non-enriched BM sample.

In samples enriched for plasma cells, the percentage of plasma cells ranged from 28% to 96% (median, 72.5%), significantly different from non-enriched BM samples ($p < 0.0001$) (Figure 1). The plasma cell enrichment factor ranged from 3.4 to 74.0-fold.

Detection of Cytogenomic Abnormalities

Results from concurrent routine cytogenetic study using G-banding chromosomal analysis were available in 26 cases. Normal diploid was revealed in 21 cases whereas a hyperdiploid karyotype was revealed in 5 patients (cases 5, 8, 11, 15, and 21) showing both numerical and structural changes. With an exception of case 8 in that a del(13)(q14) clone was detected in metaphases, the structural abnormalities were not associated with any of the cytogenomic abnormalities targeted by any of the FISH probes in this study.

The percentage of cells positive for cytogenomic abnormalities by FISH in plasma cell enriched samples ranged from 19% to 92% (Table 3), well above the cutoff for each of the probes tested. Cytogenomic abnormalities by FISH were detected more frequently in plasma cell enriched than in non-enriched BM samples, 14 (50%) versus 5 (17.9%), respectively ($p=0.0111$) (Table 4), and these abnormalities in plasma cell enriched samples included *IgH/CCND1-XT* (3 cases), del(17p/*TP53*) (6 cases), del(13q/*RBI*) (4 cases), *IgH/MAF* (1 case), *uIgH* (3 cases), and gain of 1q21/*CKS1B* (2 cases). The frequency of positive tests for individual abnormalities between plasma cell enriched and non-enriched BM samples was statistically significant ($p=0.0143$) (Table 4). High-risk abnormalities were confirmed by FISH in 10 cases, including del(17p/*TP53*), *IgH/MAF*, del(13q/*RBI*), and gain of 1q21/*CKS1B*. Of these, 7 patients (cases 3, 5, 7, 9, 10, 13, and 14) had received therapy and 3 patients (cases 1, 4 and 8) were newly diagnosed (Tables 1 and 3).

Correlation of FISH Results with Clinical Follow-up

Clinicopathologic follow-up was available for 25 (89.3%) patients, and ranged from 4 to 24 months (median 17 months). Three patients (cases 2, 4, and 26) were lost to follow-up shortly after plasma cell enrichment; therefore correlation of FISH findings with clinical follow-up was not available in these 3 cases.

Twelve patients had FISH-positive plasma cell enriched BM samples (Table 1 and Table 3). In this group, 3 were newly diagnosed and showed del(17p/*TP53*) in case 1, an *uIgH* and del(13q/*RBI*) as well as del(17p/*TP53*) in case 8, and an isolated *uIgH* in case 11. Four patients after therapy had persistent disease and demonstrated *IgH/MAF* and del(13q/*RBI*) in case 5, *IgH/CCND1-XT* in case 12, del(17p/*TP53*) in case 13, and del(13q/*RBI*) in case 14. Five patients after therapy were clinically considered to be in remission, and showed del(17p/*TP53*) in case 3, *IgH/CCND1-XT* in case 6, del(17p/*TP53*) and gain of 1q21/*CKS1B* in case 7, *uIgH* and gain of 1q21/*CKS1B* in case 9, and del(13q/*RBI*) in case 10.

Thirteen patients were negative by FISH for cytogenomic abnormalities in plasma cell enriched BM samples: 2 patients (cases 16 and 21) were newly diagnosed, 5 patients (cases 18, 19, 23, 25, and 27) had persistent disease after therapy, and 6 patients (cases 15, 17, 20, 22, 24, and 28) were in clinical remission after therapy. The 2 patients with newly diagnosed PCM received appropriate therapy, responded accordingly, and achieved remission. Of the 5 patients with persistent disease, 4 were well controlled with appropriate therapy, remaining in either remission or standard risk disease, and 1 patient (case 25) had progressive disease, relapsed and became consistently refractory to therapy 55 months post-stem cell transplant. Of the 6 patients in clinical remission after therapy, 4 remained in remission without, or with

maintenance therapy (case 24). One patient (case 17) was in remission under standard therapy, but the patient died of graft-versus-host disease 6 months later. One patient (case 28) developed progressive PCM, relapsed, and became refractory to therapy during the 7-month follow-up.

Of the 3 patients lost to clinical follow-up, 2 were positive by FISH: *IgH/CCND1-XT* in case 2 and *del(17p/TP53)* in case 4. These abnormalities were only detected in plasma cell enriched BM samples. In case 2, the patient had persistent PCM after therapy. In case 4, the patient had newly diagnosed MGUS. One patient (case 26) was negative by FISH, but had persistent PCM.

COMMENT

In this study we show that plasma cell enrichment results in a three-fold increase in sensitivity of FISH for detecting high-risk cytogenomic abnormalities in patients with plasma cell neoplasms. Further, plasma cell enrichment led to detection of cytogenomic abnormalities by FISH in patients that had been treated and were judged to be in clinical remission.

The BM aspirate samples in this study were collected from patients who were actively undergoing diagnosis, many of whom were receiving treatment. Therefore, we needed to divide the BM samples, using half for clinical routine cytogenetic analysis, as requested by the clinicians as a part of patient care. For the aliquot of BM not used for routine workup, we divided each of these samples into two. One sample was not manipulated and is the non-enriched plasma cell sample. The other half was enriched for plasma cells using CD138+ magnetic beads. There was no significant difference in plasma cell counts between the initial BM sample and the research sample that was not enriched for plasma cells ($p=0.34$). In contrast, the plasma cell counts in plasma cell enriched BM samples were significantly greater ($p<0.0001$). The relative low of 28% plasma cell in the enriched sample in case 7 was likely affected by the few plasma cells (<1%) and small size of its original bone marrow sample. The increase in plasma cells was accompanied by an increased rate of detection of cytogenomic abnormalities in plasma cell enriched BM samples compared with non-enriched plasma cell BM samples ($p=0.0143$). Using plasma cell enriched samples, in 10 patients high-risk cytogenomic abnormalities were detected by FISH, including patients with *del(17p/TP53)*, *del(13q/RB1)*, *IGH/MAF*, or gain of *1q21/CKS1B*.

In case 13, the plasma cell count in the non-plasma cell enriched sample was 47% versus 96% in the plasma cell enriched sample. The percentage of interphase cells positive for *del(17p/TP53)* also correspondingly increased from 34% to 84%. Similarly, the percentage of interphase cells positive for different probes in plasma cell enriched BM samples was well above the cutoff for the corresponding cytogenomic abnormalities, ranging from 19% to 92%, and therefore false-positive results seem unlikely. *del(17p/TP53)* defines a group of PCM patients associated with short survival, and none of the currently available therapies have consistently shown an ability to overcome this adverse prognosis. At the time of plasma cell enrichment, the patient had fully developed PCM that had progressed from smoldering myeloma over 6 months. Moreover, after 8 months of follow-up, only a 50%

clinical response was observed despite combination therapy consisting of bortezomib, cyclophosphamide, and dexamethasone. This aggressive clinical behavior further supports the likelihood that the del(17p/TP53) was indeed present, and did not represent a false-positive result.

In this study probes for *IgH/MAFB*, *IgH/MYC*, and *IgH/CCND3* were not included in the FISH panel, and therefore a signal pattern showing a split-apart *IgH* with or without a loss and/or gain of either 3'-*IgH* or 5'-*IgH* in a plasma cell neoplasm could indicate the presence of other unknown *IgH*-partners associated with high- or standard-risk abnormalities. Such an *IgH* signal pattern in this study was classified as u*IgH*. In case 11, a patient who had newly diagnosed PCM and 43% plasma cells in BM after plasma cell enrichment responded well to standard therapy and achieved remission at 7-months clinical follow-up. In addition, the patient had no 14q32/*IgH* rearrangement confirmed by FISH although a hyperdiploid karyotype, 55,XY,+3,+5,+7,+9,+9,der(11)t(11;11)(p15;q13),+15,+15,+19,+mar was present. In this case, the u*IgH* most likely resulted from *IgH* rearrangement involving a non high-risk partner, such as *CCND3*,^{1,7,32} or others that were undetectable by the FISH probes used in this study.

As a sole abnormality, isolated *IgH/CCND1-XT* was detected by FISH in plasma cell enriched BM samples in 3 patients (cases 2, 6, and 12), each of which had a diploid karyotype. In case 12, the patient was diagnosed with PCM in 2007. Despite therapy the patient had persistent disease. In the plasma cell enriched sample the plasma cell count was 45%. *IgH/CCND1-XT* was also detected as the sole abnormality after 7-months clinical follow-up, however, the patient had progressive disease that became refractory to therapy on the seventh cycle of lenalidomide, thalidomide, and dexamethasone. It seems possible that a high-risk cytogenetic abnormality was present in this neoplasm, but was undetectable by the FISH probes used in this study. Similarly, the poor prognosis of other patients in this study (cases 25 and 28) may be explained by the presence of high-risk cytogenomic abnormalities not detectable using the FISH probes tested in this study.

The results of this study support the hypothesis that del(13q/*RBI*) is one of the common cytogenomic pathways associated with the development of plasma cell neoplasms. In case 7, the patient was initially diagnosed with PCM in September of 2009, and had a diploid karyotype with an isolated del(13q/*RBI*) in 19% of nuclei detected by FISH. A BM sample obtained 8 months after diagnosis underwent plasma cell enrichment as part of this study showed a diploid karyotype. FISH was negative for del(13q/*RBI*) but positive for two newly detected high-risk markers, del(17p/*TP53*) and gain of 1q21/*CKS1B* in 56% and 49% of nuclei, respectively. Similar to case 7, in case 13, a patient with smoldering myeloma, del(13q/*RBI*) was detected as an isolated change at initial diagnosis, but isolated del(17p/*TP53*) was detected at the time of progression to PCM. In case 14, a patient had PCM with del(13q/*RBI*) as a sole abnormality detected by FISH and this patient's disease remained active over the following 17-months.

Gain of 1q21/*CKS1B* is often associated with an unfavorable prognosis or disease progression, and patients with this abnormality commonly require intensive therapy.^{19–22} As shown in Table 2, 2 patients (cases 7 and 9) had BM smears with very few plasma cells and

non-plasma cell enriched BM samples similarly has few plasma cells. These two cases were diploid. However, analysis of plasma cell enriched samples by FISH showed gain of 1q21/*CKS1B*, in 59% and 49% of nuclei, respectively (Table 3). Owing to the presence of gain of 1q21/*CKS1B* and its negative effect on bortezomib therapy in treated PCM patients,²² in case 9 bortezomib was replaced with lenalidomide and dexamethasone. In addition, the patient was considered to be in remission at the time gain of 1q21/*CKS1B* was detected by plasma cell enrichment, but the disease subsequently relapsed and became refractory to therapy over the next 5 months. This observation suggests that gain of 1q21/*CKS1B* might have modified the sensitivity of bortezomib during the patient's treatment in this case.

del(17p/*TP53*) was detected in 6 cases (cases 1, 3, 4, 7, 8, and 13), accounting for approximately 20% of cases in this study, a higher frequency than that reported by others.^{1,9,24} In this patient subset, 4 had PCM, 1 had solitary plasmacytoma (case 1), and 1 had MGUS (case 4). To our knowledge, these are the first cases of MGUS and solitary plasmacytoma in which an isolated del(17p/*TP53*) has been detected at initial diagnosis. In case 4, the patient presented with newly diagnosed MGUS who had 4% BM plasma cells, a serum β 2 microglobulin of 1.9 mg/L, and serum creatinine of 0.7 mg/dL. In case 1, del(17p/*TP53*) was detected in the plasma cell enriched sample when the patient was newly diagnosed with solitary plasmacytoma. Morphologic evaluation and flow cytometry analysis revealed no evidence of plasma cell neoplasm involving the BM. Findings from these cases suggest that using FISH on plasma cell enriched samples provides enhanced ability to detect specific cytogenomic abnormalities in association with different plasma cell diseases. Moreover, such findings could be of great utility to clinicians, since solitary plasmacytoma or MGUS patients with high-risk features may be candidates for more intensive surveillance, although this hypothesis would need to be tested in the setting of prospective observational trials.

Deletion of chromosome 17p/*TP53* has been consistently reported to be a high-risk abnormality that defines a patient group with unfavorable prognosis.^{1,9,24} This concept is supported by the 4 patients (cases 3, 7, 8, and 13) with del(17p/*TP53*) in this study. As an isolated change, del(17p/*TP53*) was detected in cases 3 and 13. In case 3, the patient had PCM and was in remission at the time BM was obtained. The plasma cell enrichment of this sample showed del(17p/*TP53*) and the patient's disease progressed with development of a plasmacytoma involving the T11 vertebra within 22 months. In case 13, a patient with smoldering myeloma, a BM sample was obtained when the patient's disease progressed to PCM. Plasma cell enrichment of this BM sample showed del(17p/*TP53*). During a follow-up period of 8 months, the patient achieved only a 50% clinical response with therapy consisting of bortezomib, cyclophosphamide, and dexamethasone, and proceeded on to stem cell transplant. In case 8, the patient had newly diagnosed PCM and FISH performed on the plasma cell enriched BM aliquot showed a del(17p/*TP53*) in addition to the del(13q) which was also detected by chromosomal analysis. The patient was treated with high-dose cyclophosphamide, bortezomib and dexamethasone for 4 cycles, and then autologous stem cell transplant, but relapsed within a 17-month follow-up period. The effect of the del(13q) in metaphases on overall survival in this case needs to be further investigated. Deletion of 13q and/or -13 in both metaphases and interphases have been reported recently to be

associated with a significant shorter overall survival compared to the del(13q) detected solely by FISH.³³

In summary, we show that plasma cell enrichment of BM samples improves sensitivity of interphase FISH and can be used to assess for high-risk cytogenomic abnormalities in patients with treated plasma cell neoplasms. We also show the potential clinical relevance of this approach as PCM patients treated and thought to be in remission had cytogenomic abnormalities, as did one patient with newly diagnosed MGUS and another patient with solitary plasmacytoma. In the latter patient, FISH performed on a plasma cell enriched BM sample detected an abnormality in morphologically normal BM. In combination with follow-up clinicopathologic data, detection of these abnormalities has utility in allowing improved risk stratification and prognostication and may influence choice of therapy.

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Figure 1.

Comparison of plasma cell percentage in plasma cell enriched and non-enriched bone marrow samples.

The bone marrow was from a patient with plasma cell myeloma in case 5, and showed 85%, and 7% plasma cells in the enriched (A), and non-enriched sample (B), respectively.

Table 1
Clinicopathologic Status of 28 Patients at Plasma Cell Enrichment and Thereafter Clinical Follow-up

Case	Age	Sex	Initial diagnosis	Clinical status at PCE	Pathology diagnosis at PCE	Clinical status In follow-up
1	49	M	SP	New diagnosis	NED	Remission
2	46	M	PCM	Persistent disease	PCM	LTFU
3	52	M	PCM	Remission	NED	Progressive disease
4	60	F	MGUS	New diagnosis	MGUS	LTFU
5	58	F	PCM	Persistent disease	PCM	Progressive disease
6	39	F	PCM	Remission	NED	Remission
7	62	M	PCM	Remission	NED	Progressive disease, Deceased due to tMDS/tAML
8	38	F	PCM	New diagnosis	PCM	Relapsed disease
9	59	M	PCM	Remission	NED	Progressive disease
10	66	M	PCM	Remission	NED	Relapsed disease
11	71	M	PCM	New diagnosis	PCM	Remission
12	67	M	PCM	Persistent disease	PCM	Progressive disease
13	63	F	PCM	Persistent disease	PCM	PCM
14	60	M	MGUS	Persistent disease	MGUS	MGUS
15	57	M	PCM	Remission	Residual disease	Remission
16	50	M	PCM	New diagnosis	PCM	Remission
17	60	F	PCM	Remission	NED	Remission, but deceased due to GVH disease
18	72	M	PCM	Persistent disease	PCM	Standard risk disease
19	62	M	PCM	Persistent disease	PCM	Remission
20	58	M	PCM	Remission	Residual disease	Remission
21	42	F	PCM	New diagnosis	PCM	Remission
22	36	F	PCM	Remission	NED	Remission
23	37	M	MGUS	Persistent disease	MGUS	Stable disease
24	66	M	PCM	Remission	NED	Remission, in maintenance therapy
25	60	M	PCM	Persistent disease	PCM	Progressive disease
26	64	F	PCM	Persistent disease	PCM	LTFU

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Case	Age	Sex	Initial diagnosis	Clinical status at PCE	Pathology/ diagnosis at PCE	Clinical status In follow-up
27	78	M	PCM	Persistent disease	No results	Standard risk disease
28	55	F	PCM	Remission	NED	Progressive disease

Abbreviations FISH, fluorescence in situ hybridization; M, male; SP, solitary plasmacytoma; NED, no evidence for diagnosis; PCM, plasma cell myeloma; F, female; LTFU, lost to follow-up; MGUS, monoclonal gammopathy of undetermined significance; tMDS, therapy-related myelodysplastic syndrome; tAML, therapy-related acute myeloid leukemia; GVH, graft-versus-host

Table 2

Comparison of Plasma Cell Counts in Initial Bone Marrow Specimens and in Aliquots Before and After Plasma Cell Enrichment

Case	Routine PC count	Study PC count		EF
		non-PCE	PCE	
1	2%	2%	82%	41
2	10%	10%	88%	9
3	1%	1%	57%	57
4	4%	1%	74%	74
5	8%	7%	85%	12
6	3%	12%	73%	6
7	<1%	8%	28%	4
8	50%	14%	63%	5
9	1%	10%	43%	14
10	4%	6%	72%	12
11	43%	28%	96%	3
12	35%	19%	93%	5
13	27%	47%	96%	4
14	1%	12%	56%	5
15	3%	13%	49%	4
16	10%	10%	75%	8
17	<1%	7%	89%	13
18	3%	3%	89%	30
19	2%	8%	64%	8
20	<1%	7%	51%	7
21	63%	18%	93%	5
22	2%	7%	62%	9
23	4%	6%	52%	9
24	2%	5%	56%	11
25	6%	11%	88%	8
26	6%	8%	77%	10
27	2%	5%	59%	12
28	1%	2%	63%	32

Abbreviations: PC, plasma cell; non-PCE, non-enriched for plasma cells; PCE, enriched for plasma cells; EF, enrichment factor

Table 3
Results of FISH Analysis in Positive Routine, Non-enriched, and Enriched Samples from 14 FISH-positive cases*

Case	IgH			del(13q/ RBB1)	del(17p/ TP53)	1q21 gain
	IgH/ CCND1-XT	IgH/MAF	uIgH			
1	-	-	-	-	N/N/43	-
2	N/N/57	-	-	-	-	-
3	-	-	-	-	N/N/50	-
4	-	-	-	-	N/N/64	-
5	-	N/N/23	-	N/85/93	-	-
6	N/N/76	-	-	-	-	-
7	-	-	-	-	N/N/56	NT/N/49
8	-	-	19/32/68	24/42/89	23/33/92	-
9	-	-	N/N/34	-	-	NT/N/59
10	-	-	-	N/N/56	-	-
11	-	-	N/32/78	-	-	-
12	23/32/83	-	-	-	-	-
13	-	-	-	-	N/34/84	-
14	-	-	-	N/N/76	-	-

*The first, second, and the third number in the table represents the percentage of interphases positive for the corresponding individual probe in routine, non-enriched, and enriched sample, respectively. Percentage of FISH-positive cells lower than cutoff value in all the 3 types of sample was shown as “-”

Abbreviations: FISH, fluorescence in situ hybridization; N, negative; NT, not tested

Note – cases 1, 4, 8, and 11 were newly diagnosed when plasma cell enrichment was performed

Table 4

Summary of Cytogenomic Abnormalities identified by FISH analysis

Sample type	IgH			Del(13q/ RBI)	Del(17p/ TP53)	1q21 gain	Total # positive Test (p-Value)	Total # positive case (p=Value)	Total # HR positive treated case (p=Value)
	CCND1-XT	MAF	uIgH						
Routine	1	0	1	1	1	NT	4 (0.0018)	2 (<0.0001)	0 (0.0089)
	3	1	3	4	6	2	19	14	7
Study	1	0	2	2	2	0	7 (0.0143)	5 (0.0111)	2 (0.1324)

* The number of cases with high-risk cytogenomic abnormalities detected by FISH from the 22 treated cases

Abbreviations: FISH, fluorescence in situ hybridization; PCE, plasma cell enriched; non-PCE, non-enriched for plasma cells; HR, high-risk