Potential Prognostic Significance of Patterns of Deletion (13q) in Plasma Cell Myelomas—Reappraisal of a Perennial Bone of Contention

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Abstract

Deletion 13q is recommended in the initial cytogenetic workup of myeloma patients. The patterns of this abnormality have been shown to have differential prognostic value. The presence of monosomy 13 is associated with a significantly poor progression-free survival, while interstitial deletion 13q is associated with significant improvement in the overall survival. We analyzed the patterns of 13q abnormalities on fluorescent in situ hybridization (FISH) assay results in myeloma patients. Deletion 13q abnormalities were observed in 38% (55 of 138) of the myeloma patients. Ten (18%) and 44 (80%) patients showed interstitial deletion and terminal deletion, respectively. One had a mosaic of both the patterns. Nine of the ten patients with interstitial deletions were males. For terminal deletion 13q, there appeared to be a slight female predilection, with a male to female ratio of 0.83:1. Half of the patients with deletion 13q had coexistent cytogenetic abnormalities. We suggest a baseline FISH for deletion 13q and specification of the type of abnormality (terminal vs. interstitial) in patients with myeloma. Based on our observation in conjunction with the available literature, further studies in a large cohort of patients with survival data are warranted to clearly delineate the role of deletion 13q in myeloma.

Keywords
- myeloma
- deletion 13q
- prognosis
- interstitial
- terminal

Plasma cell myeloma (PCM) is a clonal disorder of the bone marrow plasma cells. The initial cytogenetic workup includes detection of genomic abnormalities by fluorescent in situ hybridization (FISH) assay. Although the metaphase cytogenetics provide a global picture of the chromosomal aberrations, mitotic quiescence in the plasma cells compounded by low proliferation potential and complexity of the karyotype limits the use of this technique. Moreover, cryptic abnormalities and interstitial deletions cannot be detected by conventional cytogenetics.[1] The National Comprehensive Cancer Network (NCCN) guidelines recommend an interphase FISH panel on the bone marrow plasma cells for del (13q), t(4;14), t(14;16), t(11;14), t(14;20), del (17p), 1q amplification, and del (1q) during the initial evaluation of PCM.[1] NCCN as well as the Mayo clinic risk stratification for multiple myeloma (mSMART) lists t(4;14), t(14;16), t(14;20), del (17p), and 1q gain in the high-risk category.[2] Del (13q) and other genetic abnormalities are grouped under the standard risk category.[2] However, based on the previous and recent data, there is ambiguity pending further

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clarification on the role of del (13q) in PCM.[3-7] That being the case, whether 13q abnormalities should at all be assessed in the initial workup of myeloma, and if included what should be the FISH reporting pattern(s) of this aberration, including their possible relevance.

Herein, we attempted to address the above-mentioned issue based on the FISH results from our center. This is a retrospective analysis, following approval from the Ethics Committee Board, CORE Diagnostics, Gurgaon, Haryana, India (CORE_IRB_001) Institutional review board, and did not have any impact on the patient care. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1964, as revised in 2013. The cases of PCM with del (13q) in isolation or in conjunction with other abnormality were retrieved from the electronic database. All the saved FISH images were further analyzed independently by three molecular pathologists (MB, SS, and SKM) and a consensus on the type of deletion was made. The study period was from January 2017 to December 2018. As part of the evaluation of PCM, we typically conduct FISH testing to identify del (13q), t(4;14), t(14;16), t(11;14), t(14;20), and del (17p) following plasma cell enrichment by adding anti-CD138 monoclonal antibody-coated magnetic beads. For del (13q), we use locus specific dual color (LSP) FISH probe to detect the deletions in the 13q14.2 (*RB1*) and 13q34 (*LAMP1*), respectively. Other probes used in our laboratory include [LSP (*TP53/CCP17*) and locus specific dual color and dual fusion probes including *IgH/FGFR1, IgH/CCND1, IgH/MAF, and IgH/MAFB*] for del(17p), and IgH- related translocations (Cytotest Inc., Rockville, Maryland, United States). FISH assay was performed based on the manufacturer's protocol.[8] A total of 200 interphase plasma cell nuclei were evaluated. The cutoff used for positivity with del (13q) and del (17p) is 6%, and the cutoff for the dual fusion translocation probes is 15%.

Del (13q) abnormalities were detected in 55 (38%) of the 145 patients studied. The median age for the positive cases was 62 years (range = 35–90 years), and a three quarter of them were above 55 years. Of these 55 positive cases, 10 (18%) showed interstitial deletion (►**Fig. 1A**), 44 (80%) had terminal deletion/monosomy13 (►**Fig. 1B**), and one was a mosaic of both the patterns. Nine of the ten patients with interstitial deletions were males denoting a male preponderance and could be an incidental finding. For terminal deletion 13, there appeared to be a slight female predilection, with a male to female ratio of 0.83:1. Half of the patients with 13q deletions had coexistent cytogenetic abnormalities. While 85% (23 of 27) of the patients with combined abnormalities [del(17p) (n = 5); t(4;14) (n = 7); del(17p) and t(4;14) (n = 3); t(11;14) (n = 5); t(14;16) (n = 3)] had terminal deletions, only 15% (4 of 10) had interstitial deletions [del(17p) (n = 1); t(4;14) (n = 3)] (►**Table 1**).

In the natural history of PCM, trisomies and immunoglobulin heavy-chain translocations are the initial events or the first hit in tumorigenesis.[2] In the transition from a monoclonal gammopathy of unknown significance (MGUS) to PCM, secondary molecular events or second hit are accrued in the neoplastic plasma cells.[2] In spite of the presence of del (13q) in a large proportion of PCM patients and its relative rarity in MGUS, its role in the pathogenesis is unclear.[3,9] It is possibly a part of the second hit in the oncogenesis of myeloma cells. Most deletions are localized to the 13q14 locus that harbors the retinoblastoma (*RB*) gene.[10-12] *RB* belongs to the family of tumor suppressor genes. Heterozygous deletion of the *RB* gene is a common event in the myeloma cells with inconsequential effect on the downstream gene transcription and protein expression. It has been observed that many components of the *RB* pathway such as cyclin D, p16, and E2F are dysregulated in neoplastic plasma cells.[10-12] Mutations in the "disrupted in B-cell malignancy" tumor suppressor gene, located 530kb telomeric to the *RB* gene locus, may render

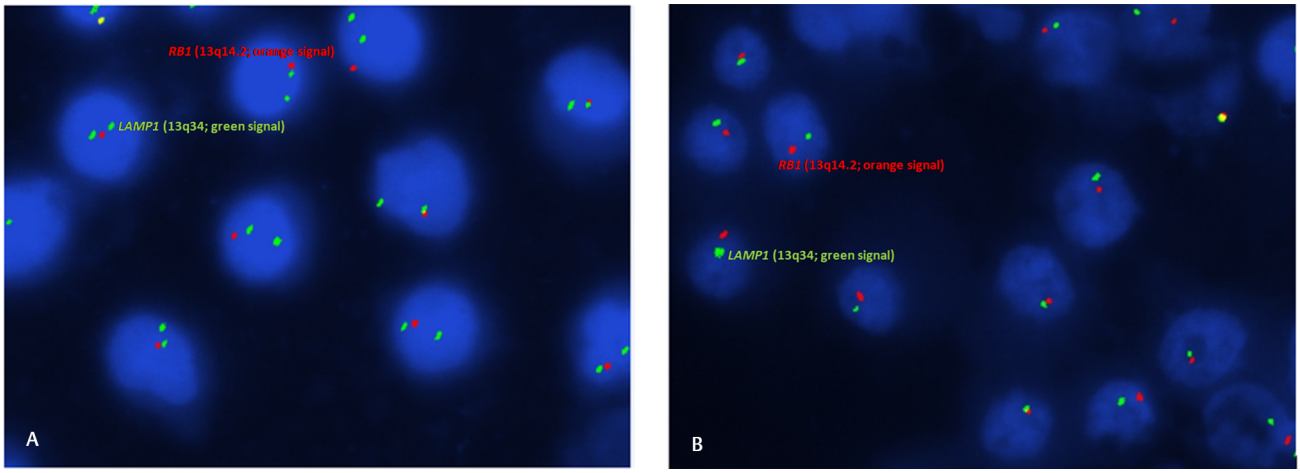

Fig. 1 (**A**) Locus specific probe (LSP) for *RB1* (13q14.2; orange signal) and LSP for *LAMP1* (13q34; green signal) on the interphase cells showing interstitial del(13q)/loss of *RB*1 gene as indicated by one orange and two green signals (fluorescent in situ hybridization [FISH] image at 60x magnification; Olympus 61 microscope using Bioview software, Israel). (**B**) LSP for*RB1* (13q14.2; orange signal) and LSP for *LAMP1* (13q34; green signal) on the interphase cells showing terminal del(13q)/loss of *RB1* and *LAMP1*genes as indicated by one orange and one green signals (FISH image at 60x magnification; Olympus 61 microscope using Bioview software).

Table 1 Patterns of del (13q) in plasma cell myeloma cases

Types of del(13q)	Mean age	Gender (M:F)	Other abnormalities	≥ 55 years	< 55 years
Interstitial deletion 10 (18.18%)	61.3	9:1	4 (14.8%)	7 (70%)	3 (30%)
Terminal deletion 44 (80%)	60.77	0.83:1	23 (85.18%)	34 (77.27%)	10 (22.72%)

plasma cells resistant to apoptosis. Since the *RB* suppresses interleukin 6 (IL-6) production and secretion, its deletion may result in the dysregulation of IL-6 expression and hence expansion of IL-6 dependent myeloma clones.[3]

Except for one study, which reported del (13q) in 86% of their FISH samples, others have reported this abnormality in 30 to 50% of the patients.[13,14] There was an abstract from India with limited number of cases that showed 15% incidence of del (13q).[15] Deletions in the long arm of chromosome 13 can be either interstitial or terminal. Monosomy 13 is the commonest 13q abnormality in myeloma and accounts for 85 to 90% of all 13q abnormalities across multiple studies.[3-7] Interstitial deletions were reported in 6 to 15% patients and were usually large deletions spanning across the 13q14 till the telomeric end of the q arm.[3,13]

The sensitivity of conventional metaphase cytogenetics in detecting del (13q) ranges between 30 and 50% and hence 50 to 70% of these deletions can be missed.[4,6] This results from the low proliferative index of the neoplastic plasma cells and can be resolved with interphase FISH studies. There appears to be a high degree of concordance between del (13q) detected on conventional cytogenetics and FISH.

Though not considered as a high-risk abnormality, there is ample controversy regarding the prognostic implication of del (13q) in PCM. Based on studies conducted in the 90s, it was reported that partial or total loss of 13q was an independent and one of the most important parameters associated with poor prognosis in patients with myeloma.[16] Subsequently, it was observed that del (13q) was associated with poor prognosis, only if detected by conventional cytogenetics and not by FISH.[17] Majority of these del(13q) were terminal deletions and hence there appeared to be no prognostic value in triaging sample for FISH to detect interstitial del(13q). In other studies, del (13q) was an independent marker for poor prognosis on univariate analysis, but not so on multivariate regression analysis.[18,19] These observations led to the conclusion that del (13q) as a standalone marker was not associated with adverse outcomes, rather the poor prognosis was due to cosegregation of other high-risk cytogenetic abnormalities such as del(4;14) and del(17p).[5]

In this context, the analysis by Binder et al sheds some light on the prognostic implications of del (13q) in patients with myeloma.[7] Of the 1181 patients analyzed by FISH and conventional cytogenetics, a del (13q) abnormality was seen in 42% patients. There were 411 (35%) patients with monosomy 13, 73 (6%) with interstitial deletions, and 9 (1%) with a mosaic pattern. The presence of monosomy 13 (compared with its absence) was associated with a significantly poor progression-free survival (median PFS: 1.75 vs. 2.00 years, $p = 0.013$) as well as overall survival (median OS: 5 vs. 8.3 years, $p < 0.001$). In contrast, the presence of interstitial del (13q) (in comparison to its absence) had a salutary effect on the prognosis with significant improvement in OS (median not reached vs. 6.4 years, $p = 0.006$) without an impact on the PFS (median: 1.93 vs. 1.66 years, $p = 0.314$). Binder at al also highlighted that segregation of the 13q abnormality into interstitial or terminal deletions had impact on the prognosis of del (13q) in myelomas.[7] Interestingly, the prognosis associated with these abnormalities was retained even after adjusting for age, gender, international staging system stage, first-line therapy, and the presence of the established high-risk cytogenetic abnormalities.[7] This is the first study demonstrating the contrasting effects of patterns of del (13q) on the outcome of patients treated for myeloma. So, there appears to be a prognostic dichotomy regarding the differential effect of patterns of del (13q) in the myeloma patients as observed in the above[7] and prior studies. First of all, chromosome 13 abnormalities have been analyzed as a single entity throughout and not as two different patterns, that is, monosomy and del (13q) as detected on FISH. Second, considering the small number of patients with interstitial deletions, most studies lacked sufficient power to detect an impact on survival. Lastly, almost all of these studies predated the introduction of novel antimyeloma drugs and may not have been able to detect their differential effect on specific cytogenetic abnormalities.[20]

In conclusion, we recommend a baseline FISH for del (13q) including the pattern of abnormality (terminal vs. interstitial) in addition to karyotyping in patients with PCM. Based on our observation in conjunction with the available literature, further studies in a large cohort of patients are warranted to clearly delineate the prognostic value of del (13q) in PCMs.

Note
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Conflict of Interest
The authors have stated that they have no conflicts of interest.

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