

***NPM1* gene deletions in myelodysplastic syndromes with 5q- and complex karyotype**

The deletion of 5q (5q-) is a frequent clonal chromosomal abnormality detected in patients with myelodysplastic syndromes (MDS). MDS with 5q- as a sole chromosome alteration is characterized by a more favorable prognosis when compared to other forms of MDS.¹ By contrast, when detected in association with other chromosome abnormalities in the context of a complex karyotype, the 5q- aberration is associated in both MDS and acute myeloid leukemia (AML) with adverse prognostic outcome.^{2,3}

The nucleophosmin (*NPM1*) gene located on chromosome 5q35 encodes a multifunctional nucleolar protein that plays multiple roles in cell growth and proliferation. Grisendi *et al.* reported that *NPM1* is essential to maintain genomic stability in the mouse model. In fact, these authors showed that *NPM1* is haploinsufficient for regulating centrosome duplication as *NPM1* heterozygous (*NPM1* +/-) cells show aberrant centrosome numbers, genomic instability and aneuploidy.⁴ The 5q35 region where *NPM1* maps is deleted in a number of MDS and loss of chromosome 5 is a frequent finding in MDS.⁵⁻⁷ To investigate haploinsufficiency of *NPM1* in human myeloid malignancies, we analyzed *NPM1* status in terms of deletion, methylation and mutations in 53 patients with MDS or secondary AML (sAML) carrying the 5q- abnormality as a sole chromosomal alteration or associated with additional chromosome defects.

Cytogenetic methanol/acetic acid fixed cell suspensions and DNA samples were available from 53 unselected patients with a diagnosis of MDS (48 cases) or MDS-sAML (5 cases) with 5q abnormalities. Patients were diagnosed at the Genetic Department of the University of Navarra, Spain and at the Department of Biopathology of the University Tor Vergata of Rome, Italy. MDS were classified according to the French-American-British (FAB) system which was in use at the time of the diagnosis. Bone marrow samples were collected for the study in all cases at the time of presentation.

Cytogenetic and FISH studies were performed according to standard methods. *NPM1* FISH analysis was carried out with bacterial artificial chromosome (BAC) (RP11-117L6 chr: 170,746,923 – 170,922,033), kindly provided by Prof M. Rocchi (Department of Genetics and Microbiology, University of Bari, Italy; <http://www.biologia.uniba.it/rmc/2-YAC-BAC/BAC-Chromosome/BAC-05.html>), and directly labeled with spectrum red dUTPs with a Nick Translation Kit (Vysis

inc, IL, USA). To investigate whether the *NPM1* gene was deleted in cases with karyotypically present 5q-, we also analyzed both interphase nuclei and metaphases with the LSI 5ptel48 spectrum green probe (Cytocell, Cambridge, UK). Detection of *NPM1* mutations was carried out by RT-PCR followed by capillary gel electrophoresis as previously described.⁸ The methylation status of the *NPM1* gene promoter was investigated by methylation-specific PCR.⁹ Bone marrow DNA from healthy donors was used as a negative control and human male genomic DNA universally methylated for all genes was used as a positive control for methylation-specific assays.

Twenty-three patients presented the 5q- as a sole chromosome abnormality (group 1) while 30 patients presented the 5q- in conjunction with other chromosome changes (group 2). Detailed FAB classification and karyotypic groups are reported in Table 1. Interestingly, in group 1 only 43% (10 of 23) of patients had more than 5% blast infiltration whereas in group 2, the blast count was greater than 5% in 80% (24 of 30) of cases ($P=0.009$). Methylation analysis of the *NPM1* gene promoter and mutational studies of *NPM1* exon 12 disclosed unmethylated CpG islands and wild-type *NPM1* exon 12 in all patients. FISH analysis of the *NPM1* locus, revealed deletion of one copy of the gene in 7 of 30 cases with complex karyotype and in none of 23 of those with isolated 5q- ($P=0.01$). Six out of these 7 cases with deleted *NPM1*, 5q- and complex karyotype showed more than 10% marrow blast infiltration at diagnosis (Table 2). A representative case with FISH analysis showing 5q- and *NPM1* gene deletion is shown in Figure 1. Neither *NPM1* hypermethylation, nor mutations were found in patients with 5q-MDS. After the submission of this study, La Starza *et al.* reported the occurrence of *NPM1* deletion in 40% of patients with complex karyotype MDS/AML with 5q-.¹⁰

Table 1. FAB classification and partial karyotypic details of 53 patients with 5q- abnormality.

| FAB | N. of cases | Group 1 5q- as sole chromosome abnormality | Group 2 5q deletion in conjunction with other chromosome changes |
|--------------|-------------|---|---|
| RA | 17 | 12 | 5 |
| RARS | 2 | 1 | 1 |
| RAEB | 10 | 3 | 7 |
| RAEB-T | 19 | 6 | 13 |
| AML | 5 | 1 | 4 |
| TOTAL | 53 | 23 | 30 |

Table 2. Karyotype analysis of 7 patients with *NPM1* deletion.

| UPN | SEX | Blasts % | FAB | KARYOTYPE |
|-----|-----|----------|--------|--|
| 1 | F | 24 | RAEB-T | 48,XX,der(1)t(7;1)(?;p)-,3,del(5)(q13q32),-6,-7,add(11)(q23),-12,-19,-21,+8mar[30] |
| 2 | M | 16 | RAEB | 45,X,-Y,del(3)(q23),del(5)(q13q33),-7,der(12)t(7;12)(q11;q13),-21,+2mar[29]/46,XY[1] |
| 3 | M | 15 | RAEB | 44,XX,del(5)(q?),-6,-7,-16,-18,+2mar[14]/46,XX[16] |
| 4 | F | 13 | RAEB | 45,XX,del(5)(q13q34),der(6)t(7;6)(?;q),-7[29]/46,XX[1] |
| 5 | M | 3 | RARS | 46,XY,del(5)(q13q33),t(7;13)(q32;q13),del(17)(q?) [20]/46,XY[10] |
| 6 | F | 54 | sAML | 45,XX,del(5)(q13q32),der(6)t(7;6)(?;p),-7,del(9)(q?) [30] |
| 7 | F | 15 | RAEB-T | 45,XX,t(3;6)(q21;p21),del(5)(q?),-7,-17,-18,+2mar[28]/46,XX[2] |

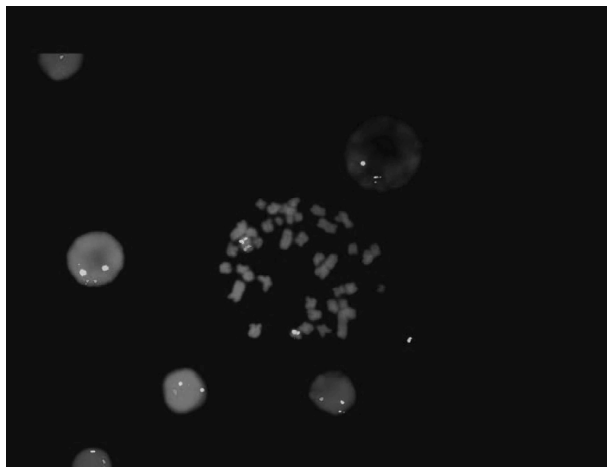


Figure 1. Metaphase and interphase FISH analysis of a representative MDS case with 5q- and deletion of *NPM1* gene. Spectrum red: *NPM1* specific BAC (RP11-117L6). Spectrum green: 5ptel48 probe.

In our series, none of the 7 cases with *NPM1* deletions showed at karyotypic examination a deletion that included the *NPM1* gene locus at 5q35. However, it should be noted that the inaccuracy of the deleted region assignment by chromosome banding has been reported for 5q deletions in both MDS and AML.¹¹

Our findings suggest that *NPM1* haploinsufficiency may have a role in myeloid malignancies associated with large 5q- deletions contributing to MDS development likely through genetic instability. This would be in line with the finding that *NPM1* is haploinsufficient in the control of centrosome duplication in the mouse model, as well as with evidence that *NPM1* hypomorphic mouse embryonic fibroblasts reveal high levels of tetraploidy and aneuploidy.⁴ Alternatively, *NPM1* deletions may represent secondary events linked to the progression of the disease in MDS.

Given the low number of cases and heterogeneity of treatments, we were unable to determine here the clinical significance of *NPM1* deletion in MDS with 5q-. Hence a larger series of cases including homogeneously treated patients is needed to investigate the prognostic impact of these abnormalities.

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Prognostic value of the International Scoring System and response in patients with advanced Waldenström macroglobulinemia

Waldenström macroglobulinemia (WM) is a lymphoproliferative disorder characterized by the production of serum monoclonal immunoglobulin (Ig) M and lymphoplasmacytic bone marrow infiltration.¹ Median survival ranged from 60 to 77 months^{1,2} and the International Scoring System for WM (ISSWM) identified 3 risk subgroups with significantly different survival after starting first treatment.²

Despite this difference in outcome, almost all patients progressed, even low-risk patients (15% of cases), although their median survival was estimated to be 12 years and few events occurred during their first four years of follow up.² Therefore, it is unlikely that improving prognostic assessment at the initiation of first-line therapy will identify a large subset of patients requiring new treatment approaches. In order to identify later during the follow up the patients who may need reinforced therapy or an innovative approach, we assessed the role of ISSWM in patients who required second or a subsequent line of therapy.

Eighty-two WM patients have been included in one of 3 trials evaluating the following combination regimens: fludarabine and cyclophosphamide³ (n=47), fludarabine, cyclophosphamide and rituximab⁴ (n=29), and fludara-