Roland Jäger, M.Sc., is currently in his final year of training within the PhD Program of the Center for Molecular Medicine in Vienna, Austria. Dr. Robert Kralovics is an independent Principal Investigator at the Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.

The work was supported by grants from the Austrian Science Fund (FWF) and the MPD Foundation.

No potential conflicts of interests relevant to this article were reported.

References

1. Stegelmann F, Bullinger L, Griesshammer M, Holzmann K, Habdank M, Kuhn S, et al. High-resolution single-nucleotide polymorphism array-profiling in myeloproliferative neoplasms identifies novel genomic aberrations. Haematologica. 2010;95(4)666-9.
2. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144-8.
3. Kralovics R, Passamonti F, Buser A, Teo S, Tiedt R, Passweg J, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779-90.
4. Levine R, Wadleigh M, Cools J, Ebert B, Wernig G, Huntly B, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7(4):387-97.
5. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365(9464):1054-61.
6. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356(5):459-68.
7. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood. 2008;111(3):1686-9.
8. Kralovics R. Genetic complexity of myeloproliferative neoplasms. Leukemia. 2008;22(10):1841-8.
9. Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J, et al. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. Blood. 2008;111(8):3931-40.
10. Kralovics R, Skoda RC. Molecular pathogenesis of Philadelphia chromosome negative myeloproliferative disorders. Blood Rev. 2005;19(1):1-13.
11. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289-301.
12. Grand F, Hidalgo-Curtis C, Ernst T, Zoi K, Zoi C, McGuire C, et al. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood. 2009;113(24):6182-92.
13. Tefferi A, Pardanani A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia. 2009;23(5):905-11.
14. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289-301.
15. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930-5.
16. Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. Nature. 2009;460(7257):904-8.
17. Schmidt MH, Dikic I. The Cbl interactome and its functions. Nat Rev Mol Cell Biol. 2005;6(12):907-18.
18. Reynolds RM, Browning GG, Nawroz I, Campbell IW. Von Recklinghausen's neurofibromatosis: neurofibromatosis type 1. Lancet. 2003;361(9368):1552-4.
19. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet. 1996;12(2):144-8.
20. Birnbaum RA, O'Marcaigh A, Wardak Z, Zhang YY, Dranoff G, Jacks T, et al. Nf1 and Gmcsf interact in myeloid leukemogenesis. Mol Cell. 2000;5(1):189-95.
21. Balgobind BV, Van Vlierberghe P, van den Ouweland AM, Beverloo HB, Terlouw-Kromosoeto JN, van Wering ER, et al. Leukemia-associated NF1 inactivation in patients with pediatric T-ALL and AML lacking evidence for neurofibromatosis. Blood. 2008;111(8):4322-8.
22. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41(4):450-4.
23. Schaub FX, Jager R, Looser R, Hao-Shen H, Hermouet S, Girodon F, et al. Clonal analysis of deletions on chromosome 20q and JAK2-V617F in MPD suggests that del20q acts independently and is not one of the predisposing mutations for JAK2-V617F. Blood. 2009;113(9):2022-7.
24. Buxhofer-Ausch V, Gisslinger H, Berg T, Gisslinger B, Kralovics R. Acquired resistance to interferon alpha therapy associated with homozygous MPL-W515L mutation and chromosome 20q deletion in primary myelofibrosis. Eur J Haematol. 2009;82(2):161-3.
25. Beer PA, Jones AV, Bench AJ, Goday-Fernandez A, Boyd EM, Vaghela KJ, et al. Clonal diversity in the myeloproliferative neoplasms: independent origins of genetically distinct clones. Br J Haematol. 2009;144(6):904-8.
26. Saint-Martin C, Leroy G, Delhommeau F, Panelatti G, Dupont S, James C, et al. Analysis of the ten-eleven translocation 2 (TET2) gene in familial myeloproliferative neoplasms. Blood. 2009;114(8):1628-32.
27. Beer PA, Delhommeau F, Lecouedic JP, Dawson MA, Chen E, Bareford D, et al. Two routes to leukemic transformation following a JAK2 mutation-positive myeloproliferative neoplasm. Blood. 2009. Epub ahead of print.
28. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C, et al. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood. 2009;113(24):6182-92.
29. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. Nat Genet. 2009;41(4):446-9.
30. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. Nat Genet. 2009;41(4):455-9.
31. Olcaydu D, Skoda RC, Looser R, Li S, Cazzola M, Pietra D, et al. The 'GGCC' haplotype of JAK2 confers susceptibility to JAK2 exon 12 mutation-positive polycythemia vera. Leukemia 2009;23(10):1924-6.

Molecular and alternative methods for diagnosis of acute myeloid leukemia with mutated *NPM1*: flexibility may help

Brunangelo Falini,[1] Maria Paola Martelli,[1] Stefano A. Pileri,[2] and Cristina Mecucci[1]

[1]Institute of Hematology, University of Perugia, Perugia, Italy; [2]Institute of Hematology, Chair of Hematopathology, University of Bologna, Bologna, Italy. E-mail: faliniem@unipg.it doi:10.3324/haematol.2009.017822

(Related Original Article on page 670)

Acute myeloid leukemia (AML) is a molecularly and clinically heterogeneous disease.[1] Nucleophosmin (*NPM1*) gene mutations resulting in cytoplasmic delocalization of nucleophosmin (NPMc+)[2] are the most common genetic alteration in AML, being detected in about one-third of cases. Because of its unique molecular, genotypic, immunophenotypic and prognostic features[3-6] (Table 1), AML with mutated *NPM1* was included as a

separate provisional entity in the 2008 World Health Organization (WHO) classification of myeloid neoplasms, under the heading of "AML with recurrent genetic abnormalities".[7] In the 2001 WHO classification, this category included only AML with t(15;17), t(8;21), inv(16), and *MLL* rearrangements and in 2008 it was expanded to include AMLs carrying t(6;9), inv(3) or t(3;3), AML (megakaryoblastic) with t(1;22) and two provisional entities, i.e. AML with mutated *NPM1* and AML with mutated *CEBPA*. Thus, in the 2008 WHO classification, the category of "AML with recurrent genetic abnormalities" covers about 60% of AML.

The identification of the specific genetic alteration underlying each of the AML subtypes listed in the category of "AML with recurrent genetic abnormalities" is of critical importance since it helps to assign patients to different prognostic groups, thus influencing therapy. For example, in AML patients under 60 years of age, *NPM1* mutations consistently predict favorable prognosis,[6] when no concomitant *FLT3*-ITD mutation is present, whilst in AML patients 70 years old or over *NPM1* mutations appear to be the only factor influencing prognosis in multivariate analysis.[8]

The search for genetic alterations using molecular methods would ideally be the gold standard for diagnosis. Unfortunately, not all centers, especially in developing countries, are equipped for molecular studies and this may hamper the worldwide use of the WHO classification. These problems could potentially be solved by developing simple surrogates for molecular studies. Here, we briefly review the molecular methods and their alternatives that are currently available for diagnosing AML with mutated *NPM1*.

Detection of NPM1 mutations by molecular techniques

The normal *NPM1* gene configuration and the first six *NPM1* mutations we identified in AML,[2] which lead to common structural changes of the NPM1 protein C-terminus, are shown in Figure 1. Over the past five years, several qualitative and quantitative molecular assays for identifying *NPM1* mutations have been developed and tested in a large number of AML patients.

(i) Qualitative assays for NPM1 mutations: these highly sensitive and specific assays for detecting *NPM1* mutations[9-15] are best applied to RNA or DNA extracted from fresh bone marrow or peripheral blood leukemic cells[13] but plasma[16] and paraffin-embedded samples[17] are also suitable. More than 50 molecular variants of *NPM1* mutations have been identified to date,[18] mostly involving exon-12, and occasionally other exons.[19] *NPM1* mutations occur in about 30% of adult AML[4] (50-60% of AML with normal karyotype). *NPM1* mutation A (a duplication of TCTG at position 956 to 959 of the reference sequence) accounts for 75-80% of cases.[2] Mutation B and D account for about 10% and 5% of cases, respectively; other mutations are very rare. *NPM1* mutations are less frequent in childhood (about 8% of pediatric AML)[20,21] and have been never found in children under three years of age.[21] Pediatric and adult AML with mutated *NPM1* appear to differ not only in frequency but also in the type of mutation as, unlike adults, the majority of children carry non-type A mutations.[22] Identifying the specific type of mutation by molecular techniques is essential when PCR (Polymerase Chain Reaction)-based quantitative studies are planned (*see below*).

(ii) Monitoring of minimal residual disease. Since *NPM1* mutations are frequent and very stable over the course of disease,[23] they are an optimal marker for monitoring minimal residual disease (MDR)[24] in approximately 30% of adult AML. Indeed, the clinical value of PCR-based quantitative assessment of *NPM1* mutant copies in predicting relapse and prognosis of AML with mutated *NPM1* was demonstrated in several studies.[25-28] Notably, Schnittger *et al.*[28] reported the best clinical outcome was associated with the greatest reduction in the number of *NPM1* mutant copies (<0.01 *NPM1/ABL* ratio). Monitoring of NPM1 mutant copies every 4-6 months is advisable.[29]

Detection of NPM1 mutated proteins by Western blot

The Western blot assay uses antibodies that recognize

Table 1. Main features of acute myeloid leukemia with mutated NPM1 (NPMc+ AML).

- *NPM1* mutations leading to cytoplasmic dislocation of nucleophosmin
- *NPM1* mutations/cytoplasmic NPM are specific for AML*, usually *de novo*
- One-third of all adult AML (about 8% in children)
- Higher incidence in female (in most trials)
- Close association with normal karyotype (about 85% of cases)
- Mutually exclusive with other AML carrying recurrent genetic abnormalities
- About 15% cases carry secondary chromosome aberrations (+8, del9(q), +4)
- Wide morphological spectrum (more often M4 and M5)
- Frequent multilineage involvement
- Negativity for CD34 (>95% of cases)
- Unique GEP molecular signature (↓ *CD34* gene; ↑*HOX* genes)
- Distinct microRNA profile
- High frequency of *FLT3*-ITD (about 40% of cases)
- Good response to induction therapy (80-85% of cases)
- Relatively good prognosis (in the absence of *FLT3*-ITD)

** Not detectable in other human neoplasms by immunohistochemistry (more than 5,000 samples tested) and/or mutational analysis. GEP: gene expression profiling.*

NPM1 mutants but not wild-type NPM1 protein in lysates from AML samples.[30] These antibodies identify the specific band (37kDa) of mutated NPM1 protein only in *NPM1*-mutated AML cases and recognize over 95% of *NPM1* mutations.[30]

Morphology and immunophenotype of NPM1-mutated acute myeloid leukemia

Advanced molecular or biochemical techniques are not always available or easy to apply and, therefore, a "realistic classification" based on morphological appearance was, in the past, suggested as a compromise solution for some AML subtypes.[31] Since some typical FAB categories such as M3, M2 with increased eosinophils (M2eo), or M4 with increased eosinophils (M4eo), closely correlated with the presence of t(15;17), t(8;21), and inv(16), respectively, recognition of these morphological features was proposed as a surrogate for cytogenetic studies. The morphology-based approach is, however, limited by imperfect morphological-genetic correlations[32] since inv(16) or t(16;16) correlate with M4eo morphology in only some cases, whilst AML with t(8;21) sometimes shows an M1 or M4 morphology. Morphology is also a poor predictor of *NPM1* mutations because, although often associated with M4 and M5 morphology,[2,33] AML with mutated *NPM1* encompasses all other FAB categories except M3, M4eo and M7.[2] Interestingly, a similar broad morphological spectrum is also seen in AML with *MLL* rearrangements.[34]

Immunophenotype combined with morphology may further increase the ability to identify specific AML genetic entities. Examples include positivity for CD19 and PAX5 in AML with t(8;21),[35] low expression of HLA/DR and CD34 in acute promyelocytic leukemia (APL),[7] expression of the condroitin sulfate molecule NG2 omolog (encoded by *CSGP4*)[36] in AML with *MLL* rearrangements, and the consistent CD34 negativity in *NPM1*-mutated AML,[2,4] but again the correlation immunophenotype/genotype is not complete.

Thus, in most AML cases, morphological/immunophenotypic studies alone cannot reliably predict genetic lesions, and other surrogates for molecular investigations need to be found.

Immunohistochemical detection of cytoplasmic nucleophosmin

In the 2008 WHO classification, AML with mutated *NPM1* is also indicated with the synonym of NPMc+ AML (NPM cytoplasmic positive AML).[7] In fact, one simple, low cost, highly-specific alternative approach to diagnosis is immunohistochemical detection of cytoplasmic nucleophosmin.[37,38] This assay is fully predictive of *NPM1* mutations since all molecular variants of *NPM1* mutations (including those affecting exons other than 12) result in aberrant export of NPM1 mutant from the nucleus to the cytoplasm of leukemic cells.[37,38] Immunohistochemistry is usually performed with monoclonal antibodies that recognize wild-type and mutated NPM1 proteins.[38] Cytoplasmic nucleophosmin is optimally detected in paraffin sections from B5-fixed/EDTA-decalcified bone marrow trephines[2,38,39] (Figure 2). Partial concordance between sub-cellular expression of nucleophosmin and *NPM1* gene status was seen in one study[40] where samples were fixed in formalin and decalcified in formic acid. It is at present unclear whether the technical problem is due to formalin fixation and/or formic acid decalcification.

Searching for cytoplasmic nucleophosmin is reminiscent

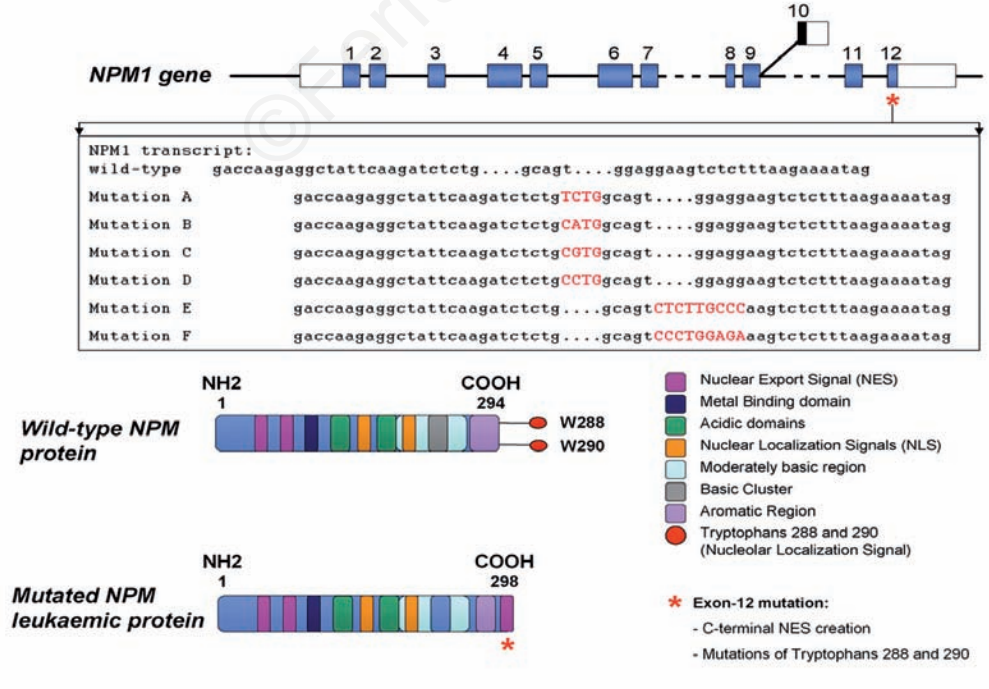

Figure 1. Configuration of the normal *NPM1* gene. *NPM1* mutations in AML occur almost exclusively at exon-12 (asterisk). (A – F) The first 6 discovered mutations.[2] Mutation A is the most frequent (75-80% of cases). All *NPM1* mutations (about 50 so far identified) result in common changes at the C-terminus end (asterisk) of the NPM1 protein, i.e. changes of tryptophans (288 and 290) and insertion of a new nuclear export signal (NES) motif. These changes cause aberrant cytoplasmic accumulation of NPM1 mutants which is easily detectable by immunohistochemistry (see Figure 2). This figure is provided by the Author (BF) and was originally reported in the 2008 WHO classification.[7]

of identifying APL with t(15,17) by means of the PG-M3 (anti-PML) monoclonal antibody.[41] In fact, the rationale for both tests is similar. The *NPM1* mutation and the *PML/RAR*-alpha fusion gene both cause ectopic subcellular relocalization of the respective proteins: nucleophosmin is found in cytoplasm (instead of nucleolus) and PML is observed as nuclear microspeckles (instead of nuclear bodies). In a recent study, Rego *et al.*[42] combined morphology with the PML immunofluorescence test (PG-M3 monoclonal antibody) to investigate 102 APL patients from developing countries (Brazil, Mexico and Uruguay). Notably, this approach resulted in a more accurate and rapid diagnosis which led in turn to the immediate start of chemotherapy plus ATRA. Consequently, overall survival markedly increased from the unsatisfactory 50% in historical controls to about 80%. This study provides an example model of the big impact of using a simple test as surrogate for molecular investigations in the therapy of AML.

Immunohistochemical detection of cytoplasmic nucleophosmin may also be useful in developed countries as simple front-line screening for *NPM1* mutations.[37,38] The expected 30% of adults with aberrant cytoplasmic nucleophosmin could then be referred to more specialized centers for confirmation and identification of mutation type by molecular techniques. Moreover, immunohistochemistry is critical for diagnosis of AML cases presenting with "dry tap" or as myeloid sarcoma.[17]

As bone marrow trephines are not routinely performed in AML patients in all hematologic centers, the ability to predict *NPM1* mutations from cytoplasmic expression of nucleophosmin in smears or cytospins would be particularly useful. In the present issue of Haematologica, Mattsson *et al.*[43] found no significant correlation between sub-cellular expression of nucleophosmin and *NPM1* gene status in their immunocytochemical study of smears and cytospins from 60 AMLs (31 *NPM1*-mutated; 29 *NPM1* wild-type). Why cytoplasmic nucleophosmin is detected in fixed paraffin-embedded material but not cytological samples remains unclear. A possible explanation is that preparation and/or fixation of smear and cytospins leads to nucleophosmin diffusion across cell compartments and even out of the cells, thus preventing accurate tracking of the protein. In contrast, fixation and paraffin-embedding may optimally stabilize cell membranes (especially nuclear membrane) thus allowing accurate visualization of sub-cellular distribution of nucleophosmin.

Mattsson *et al.*'s[43] claim that cytoplasmic nucleophosmin in paraffin sections may represent non-specific staining appears unfounded because there is much strong evidence indicating that in *NPM1*-mutated AML immunohistochemistry on paraffin sections depicts the real status of nucleophosmin sub-cellular distribution. In fact, aberrant cytoplasmic expression of nucleophosmin in B5 fixed/EDTA decalcified bone marrow trephines is fully predictive of *NPM1* mutations.[37,39] Aberrant nuclear export of nucleophosmin is consistent with the molecular alterations at the C-terminus of all NPM1 mutant proteins (Figure 1) including those generated by *NPM1* mutations involving exons other than exon 12.[38] Moreover, antibodies that specifically recognize mutant but not wild-type NPM1 protein, consistently label the cytoplasm of *NPM1*-mutated AML cells at both immunohistochemistry[44] and

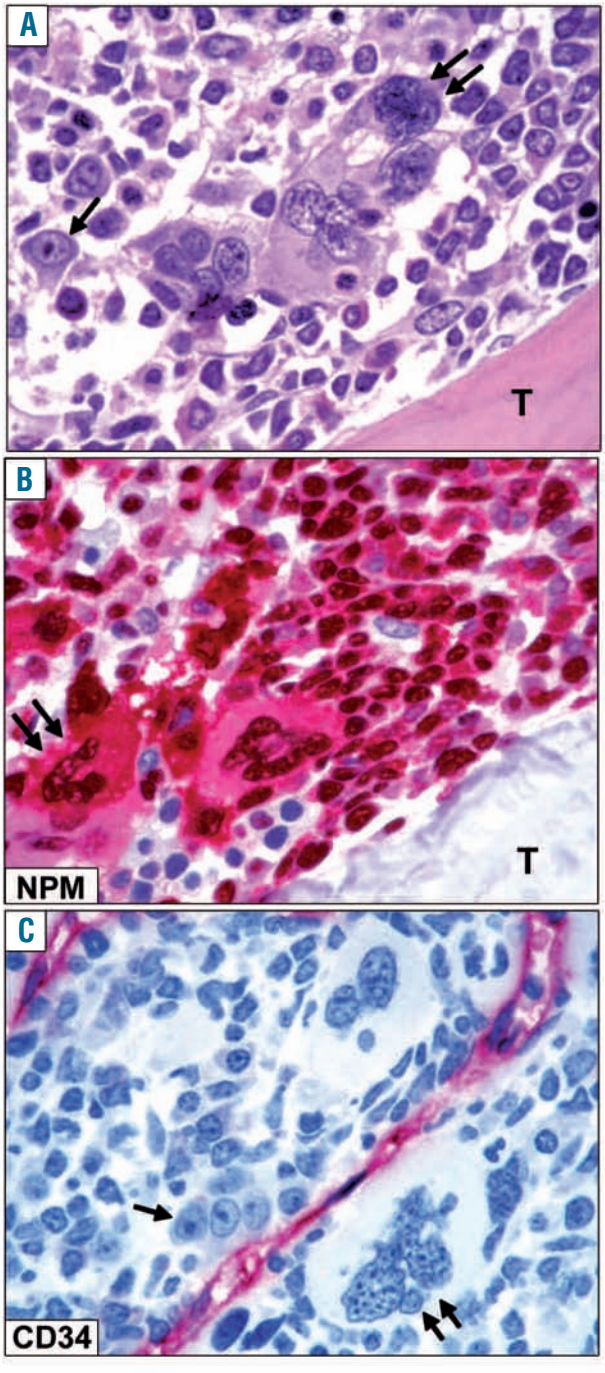

Figure 2. Detection of cytoplasmic nucleophosmin by immunohistochemistry (bone marrow trephines, paraffin sections). (A) AML with mutated *NPM1*. Marrow infiltration by myeloid blasts admixed with megakaryocytes (double arrows) and erythroid precursors (single arrow). T indicates a bone trabecula (hematoxylin-eosin; x1,000). (B) Myeloid blasts, megakaryocytes (double arrows) and erythroid precursors show aberrant cytoplasmic expression of nucleophosmin (multilineage involvement) (APAAP technique; x1,000). T indicates a bone trabecula. (C) Myeloid blasts, as well as megakaryocytes (double arrows) and erythroid precursors (single arrow) belonging to the leukemic clone, are CD34-negative (APAAP technique; x1,000). (B and C) Hematoxylin counterstaining.

Western blotting.[30] Finally, laser confocal microscopy of transfected cells clearly showed that fluorescent-tagged wild-type and mutated NPM1 proteins localized respectively in the nucleolar and cytoplasmic cell compartments.[4,38]

Whatever the reason for the failure to detect cytoplasmic nucleophosmin in smears or cytospins, the comprehensive study by Mattsson *et al.*[43] clearly indicates this method cannot be at present recommended as surrogate marker for *NPM1* mutations. One promising approach based on the use of intracellular flow cytometry for rapid, specific detection of nucleophosmin in the cytoplasm of leukemic cells has been recently proposed as an alternative to bone marrow trephines in the diagnosis of *NPM1*-mutated AML.[45] Because of its simplicity, this test may emerge in the future as a valuable surrogate to molecular studies for initial screening of AML samples. Another method that can be used in alternative to bone marrow trephines for detection of cytoplasmic nucleophosmin is immunostaining of sections cut from paraffin-embedded pellets of peripheral blood leukemic cells (B. Falini, unpublished observation, 2009).

Surrogates for molecular studies are expected to be particularly useful in older patients where *NPM1* mutations appear to play a prognostic role independently of the *FLT3* gene status.[8] For younger patients, whose favorable prognosis is associated with the *NPM1*-mutated/*FLT3*-ITD negative genotype,[4,6] we must search for rapid, inexpensive assays which can also allow us to assess the *FLT3* gene status, which is still only evaluable by molecular methods.

Conclusions

Because of its distinctive features, AML with mutated *NPM1* (NPMc+ AML) was included as a provisional entity in the 2008 WHO classification of myeloid neoplasms. Over the past five years several methods have been developed to diagnose this new entity. *NPM1* mutations can be detected by molecular techniques or surrogates such as immunohistochemistry, Western blotting and possibly flow cytometry. These methods are complementary rather than competitive and offer a flexible approach to diagnosis which is essential if the WHO Classification is to be implemented, as intended, worldwide.

Brunangelo Falini is Professor of Hematology at the University of Perugia, Perugia, Italy. Maria Paola Martelli is a Researcher in Hematology at the University of Perugia, Perugia, Italy. Stefano A Pileri is Professor of Pathology and Director of the Hematopathology Unit at Bologna University, Bologna, Italy. Cristina Mecucci is Associated Professor of Hematology at the University of Perugia, Perugia, Italy.

Supported by the Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.) and Fondazione Cassa di Risparmio di Perugia (Grants n. 2007.0099.020 and 2008.020.058). The authors would like to thank Claudia Tibido` for her excellent secretarial assistance and Dr. Geraldine Anne Boyd for her help in editing this paper. The authors apologize to those whose papers could not be cited owing to space limitations.

Drs. Falini and Mecucci applied for a patent on the clinical use of NPM1 mutants. No other potential conflicts of interests relevant to this article were reported.

References

1. Lowenberg B. Acute myeloid leukemia: the challenge of capturing disease variety. Hematology Am Soc Hematol Educ Program 2008;1-11.
2. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352(3):254-66.
3. Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, et al. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood. 2005;106(3):899-902.
4. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007;109(3):874-85.
5. Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, et al. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci USA. 2008;105(10):3945-50.
6. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909-18.
7. Arber D, Brunning RD, Le Beau MM, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Swerdlow SH et al., Eds, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. International Agency for Research on Cancer (IARC), Lyon, 2008, p.110-23.
8. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrozek K, Margeson D, et al. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a cancer and leukemia group B study. J Clin Oncol. 2010;28(4):596-604.
9. Roti G, Rosati R, Bonasso R, Gorello P, Diverio D, Martelli MF, et al. Denaturing high-performance liquid chromatography: a valid approach for identifying NPM1 mutations in acute myeloid leukemia. J Mol Diagn. 2006;8(2):254-9.
10. Laughlin TS, Becker MW, Liesveld JL, Mulford DA, Abboud CN, Brown P, et al. Rapid method for detection of mutations in the nucleophosmin gene in acute myeloid leukemia. J Mol Diagn. 2008;10(4):338-45.
11. Ottone T, Ammatuna E, Lavorgna S, Noguera NI, Buccisano F, Venditti A, et al. An allele-specific rt-PCR assay to detect type A mutation of the nucleophosmin-1 gene in acute myeloid leukemia. J Mol Diagn. 2008;10(3):212-6.
12. Szankasi P, Jama M, Bahler DW. A new DNA-based test for detection of nucleophosmin exon 12 mutations by capillary electrophoresis. J Mol Diagn. 2008;10(3):236-41.
13. Wertheim G, Bagg A. Nucleophosmin (NPM1) mutations in acute myeloid leukemia: an ongoing (cytoplasmic) tale of dueling mutations and duality of molecular genetic testing methodologies. J Mol Diagn. 2008;10(3):198-202.
14. Huang Q, Chen W, Gaal KK, Slovak ML, Stein A, Weiss LM. A rapid, one step assay for simultaneous detection of FLT3/ITD and NPM1 mutations in AML with normal cytogenetics. Br J Haematol. 2008;142(3):489-92.
15. Dvorakova D, Lengerova M, Pospisilova J, Palasek I, Mayer J. A novel quantitative assessment of minimal residual disease in patients with acute myeloid leukemia carrying NPM1 (nucleophosmin) exon 12 mutations. Leukemia. 2009;23(4):793-6.
16. Ma W, Kantarjian H, Zhang X, Jilani I, Sheikholeslami MR, Donahue AC, et al. Detection of nucleophosmin gene mutations in plasma from patients with acute myeloid leukemia: clinical significance and implications. Cancer Biomark. 2009;5(1):51-8.
17. Falini B, Lenze D, Hasserjian R, Coupland S, Jaehne D, Soupir C, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. Leukemia. 2007;21(7):1566-70.
18. Rau R, Brown P. Nucleophosmin (NPM1) mutations in adult and childhood acute myeloid leukaemia: towards definition of a new leukaemia entity. Hematol Oncol. 2009;27(4):171-81.
19. Albiero E, Madeo D, Bolli N, Giaretta I, Bona ED, Martelli MF, et al. Identification and functional characterization of a cytoplasmic nucleophosmin leukaemic mutant generated by a novel exon-11 NPM1 mutation. Leukemia. 2007;21(5):1099-103.
20. Cazzaniga G, Dell'Oro MG, Mecucci C, Giarin E, Masetti R, Rossi V, et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. Blood. 2005;106(4):1419-22.
21. Brown P, McIntyre E, Rau R, Meshinchi S, Lacayo N, Dahl G, et al.

The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood. 2007;110(3):979-85.
22. Thiede C, Creutzig E, Reinhardt D, Ehninger G, Creutzig U. Different types of NPM1 mutations in children and adults: evidence for an effect of patient age on the prevalence of the TCTG-tandem duplication in NPM1-exon 12. Leukemia. 2007;21(2):366-7.
23. Falini B, Martelli MP, Mecucci C, Liso A, Bolli N, Bigerna B, et al. Cytoplasmic mutated nucleophosmin is stable in primary leukemic cells and in a xenotransplant model of NPMc+ acute myeloid leukemia in SCID mice. Haematologica 2008;93(5):775-9.
24. Gorello P, Cazzaniga G, Alberti F, Dell'Oro MG, Gottardi E, Specchia G, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia. 2006;20(6):1103-8.
25. Chou WC, Tang JL, Wu SJ, Tsay W, Yao M, Huang SY, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. Leukemia. 2007;21(5):998-1004.
26. Barragan E, Pajuelo JC, Ballester S, Fuster O, Cervera J, Moscardo F, et al. Minimal residual disease detection in acute myeloid leukemia by mutant nucleophosmin (NPM1): comparison with WT1 gene expression. Clin Chim Acta. 2008;395(1-2):120-3.
27. Bacher U, Badbaran A, Fehse B, Zabelina T, Zander AR, Kroger N. Quantitative monitoring of NPM1 mutations provides a valid minimal residual disease parameter following allogeneic stem cell transplantation. Exp Hematol. 2009;37(1):135-42.
28. Schnittger S, Kern W, Tschulik C, Weiss T, Dicker F, Falini B, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. Blood. 2009;114(11):2220-31.
29. Ommen HB, Schnittger S, Jovanovic JV, Ommen IB, Hasle H, Ostergaard M, et al. Strikingly different molecular relapse kinetics in NPM1c, PML-RARA, RUNX1-RUNX1T1 and CBFB-MYH11 acute myeloid leukemias. Blood. 2010;115(2):198-205.
30. Martelli MP, Manes N, Liso A, Pettirossi V, Verducci Galletti B, Bigerna B, et al. A western blot assay for detecting mutant nucleophosmin (NPM1) proteins in acute myeloid leukaemia. Leukemia. 2008;22(12):2285-8.
31. Arber DA. Realistic pathologic classification of acute myeloid leukemias. Am J Clin Pathol. 2001;115(4):552-60.
32. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292-302.
33. Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005;106(12):3733-9.
34. Schoch C, Schnittger S, Kern W, Dugas M, Hiddemann W, Haferlach T. Acute myeloid leukemia with recurring chromosome abnormalities as defined by the WHO-classification: incidence of subgroups, additional genetic abnormalities, FAB subtypes and age distribution in an unselected series of 1,897 patients with acute myeloid leukemia. Haematologica. 2003;88(3):351-2.
35. Tiacci E, Pileri S, Orleth A, Pacini R, Tabarrini A, Frenguelli F, et al. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. Cancer Res. 2004;64(20):7399-404.
36. Wuchter C, Harbott J, Schoch C, Schnittger S, Borkhardt A, Karawajew L, et al. Detection of acute leukemia cells with mixed lineage leukemia (MLL) gene rearrangements by flow cytometry using monoclonal antibody 7.1. Leukemia. 2000;14(7):1232-8.
37. Falini B, Martelli MP, Bolli N, Bonasso R, Ghia E, Pallotta MT, et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. Blood. 2006;108(6):1999-2005.
38. Falini B, Bolli N, Liso A, Martelli MP, Mannucci R, Pileri S, et al. Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. Leukemia. 2009;23(10):1731-43.
39. Luo J, Qi C, Xu W, Kamel-Reid S, Brandwein J, Chang H. Cytoplasmic expression of nucleophosmin accurately predicts mutation in the nucleophosmin gene in patients with acute myeloid leukemia and normal karyotype. Am J Clin Pathol. 2010;133(1):34-40.
40. Konoplev S, Huang X, Drabkin HA, Koeppen H, Jones D, Kantarjian HM, et al. Cytoplasmic localization of nucleophosmin in bone marrow blasts of acute myeloid leukemia patients is not completely concordant with NPM1 mutation and is not predictive of prognosis. Cancer. 2009;115(20):4737-44.
41. Falini B, Flenghi L, Fagioli M, Lo Coco F, Cordone I, Diverio D, et al. Immunocytochemical diagnosis of acute promyelocytic leukemia (M3) with the monoclonal antibody PG-M3 (anti-PML). Blood. 1997;90(10):4046-53.
42. Rego EM, Kim HT, Ruiz-Arguelles GJ, Uriarte R, Jacomo RH, Gutierrez-Aguirre H, et al. Improving the treatment outcome of acute promyelocytic leukemia in developing countries through International Cooperative Network. Report on the International Consortium on Acute Promyelocytic Leukemia Study Group. Blood. (ASH Annual Meeting Abstracts) 2009;114, 6.
43. Mattsson G, Turner SH, Cordell J, Ferguson DJ, Schuh A, Grimwade LF, et al. Can cytoplasmic nucleophosmin be detected by immunocytochemical staining of cell smears in acute myeloid leukemia? Haematologica. 2010;95(4):670-3.
44. Pasqualucci L, Liso A, Martelli MP, Bolli N, Pacini R, Tabarrini A, et al. Mutated nucleophosmin detects clonal multilineage involvement in acute myeloid leukemia: Impact on WHO classification. Blood. 2006;108(13):4146-55.
45. Oelschlaegel U KS, Schaich M, Kroschinsky F, Parmentier S, Ehninger G, Thiede C. A rapid flow cytometric method for the detection of NPM1 mutated patients with acute myeloid leukemia (AML). Blood. (ASH Annual Meeting Abstracts) 2008;112:1490.

Splenic marginal zone lymphoma: a hydra with many heads?

Luca Arcaini,[1] Marco Paulli[2]

[1]Division of Hematology, Department of Oncohematology and [2]Department of Pathology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Italy. E-mail: luca.arcainiunipv.it doi:10.3324/haematol.2009.021576

(Related Original Article on page 604)

In this issue of the Journal, Baseggio *et al.*[1] report on a series of 24 patients with CD5-positive, t(11;14)-negative splenic marginal zone lymphoma (SMZL) diagnosed by means of cytology and flow cytometry of peripheral blood. All the patients were splenectomized at diagnosis or during follow-up and, consequently, spleen specimens were available for histological examination in all cases. The biological features of the CD5-positive SMZL cases did not appear to be different from those of a comparative series of 42 CD5-negative SMZL cases with the exception of a tendency to a more mutated immunoglobulin variable heavy chain genes (IGHV) status. Clinically, CD5-positive SMZL were characterized only by more marked lymphocytosis at diagnosis and more frequent diffuse bone marrow infiltration. No significant differences were found in outcome.

Marginal zone lymphomas

The marginal zone is an anatomically distinct B-cell compartment that surrounds the lymphocytic corona of the mantle. In the past, cases of lymphoma considered to be derived from monocytoid/marginal zone B cells have been described,[2] including cases primarily involving extranodal sites. The 1992 updated Kiel classification[3] first