Commentary

Nucleophosmin (*NPM1*) Mutations in Acute Myeloid Leukemia: An Ongoing (Cytoplasmic) Tale of Dueling Mutations and Duality of Molecular Genetic Testing Methodologies

Gerald Wertheim and Adam Bagg

*From the Department of Pathology and Laboratory Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania*

Contemporary laboratory evaluation and classification of acute myeloid leukemia (AML) is multimodal, using a diverse array of tools such as morphology, cytochemistry, flow cytometric immunophenotyping, and genetic testing. Genetic testing incorporates both conventional cytogenetic karyotyping and molecular analysis, with the latter including fluorescence *in situ* hybridization- and polymerase chain reaction (PCR)-based approaches. Of all of these parameters, genetic features are the most pertinent with regard to the biology, classification, prognosis and, ultimately, therapy of AML.^{1–4} The importance of genetic changes was, for the first time, codified in the 2001 World Health Organization classification of AML and will assume an even greater role in the update, slated for publication later this year.

In the 2001 classification, four recurrent cytogenetic abnormalities have been selected to define specific diagnostic entities. Those AMLs that harbor gene fusions resulting from t(8;21), t(15;17), or inv(16) have a favorable prognosis, whereas tumors with 11q23 translocations tend to be more aggressive, with a poor or occasionally intermediate prognosis, depending on the chromosomal partner. Not only does the karyotype define prognosis, but it determines specific treatment options as well.⁵ Thus, the decision to use bone marrow transplant as a first line treatment following induction of remission is guided, in large part, by the karyotype of the AML, and the use of targeted therapies, such as all-trans retinoic acid, is essentially restricted to those AMLs with t(15;17). Together, the aforementioned karyotypically detectable lesions are seen in approximately 30% of adult AMLs.² Approximately 30% of AMLs may contain other extremely diverse, but far less frequent, non-random chromosomal abnormalities.² Indeed, over 150 recurrent, and sometimes prognostically relevant, lesions have been described in AML, yet most have not currently been formally incorporated into classification schemes.⁶ However,

three additional genetically defined AML categories to be included in the new (2008) World Health Organization classification are AMLs with t(6;9), inv(3) and t(1;22).

Although these numerous recurrent karyotypic aberrations are both biologically and clinically significant, approximately 40% of AMLs in adults (and ~25% of pediatric cases) do not harbor genetic alterations that can be detected by conventional cytogenetics.² Variability in the frequency of these so-called cytogenetically normal (CN) AMLs as reported in the literature is likely due to a number of factors. These include age (more CN AMLs are evident with increasing age), methodology (direct preparation versus 24- or 48-hour culture, with the latter sometimes providing a higher yield of clonal abnormalities), and specimen source (in ~5% of cases, bone marrow may be superior to peripheral blood in detecting clonal abnormalities). A fourth factor to consider is biological, in that a minor proportion of cases harboring one of the four common translocations/genetic fusions alluded to above may be karyotypically cryptic. Either reverse transcription (RT)-PCR or fluorescence *in situ* hybridization can be used to uncover these infrequent events, and given the clinical importance of proper diagnosis, they should not be misclassified as CN-AML.

In large clinical outcome analyses, CN-AMLs, collectively the most common group of AMLs, are assigned to an intermediate prognosis category.² However, CN-AMLs are clearly not homogeneous, and numerous insights into the diversity present in this group have been unraveled recently.⁷ Indeed, distinct subsets of CN-AMLs have been recognized to possess genetic or epigenetic alterations in specific genes, as well as (some-

Supported, in part, by a SCOR grant from the Leukemia and Lymphoma Society of America (to A.B.).

Accepted for publication February 29, 2008.

Address reprint requests to Adam Bagg, MD, Director, Hematology, Department of Pathology and Laboratory Medicine, University of Pennsylvania, 7.103 Founders Pavilion, 3400 Spruce Street, Philadelphia, PA 19104-4283. E-mail: adambagg@mail.med.upenn.edu.

times related) expression profiles, dictating their biological and clinical characteristics. While the entire spectrum of genetic alterations has certainly not been defined, many recurrent genetic abnormalities have been identified and have been demonstrated to be important in both tumorigenesis and the clinical outcome of CN-AMLs. For example, internal tandem duplication (ITD) within *FLT3*, which leads to ligand-independent activation of this receptor tyrosine kinase, has a significantly adverse effect on clinical outcome.⁸ Similarly, partial tandem duplications of *MLL* (also disrupted in 11q23 translocations, noted above) are associated with a poor prognosis. Conversely, mutations in *CEBPA* correlate with favorable prognosis. Yet other biologically and clinically relevant genetic lesions are being uncovered, including mutations of *RUNX1*, *KIT*, *N-RAS*, and *K-RAS*, as well as overexpression of *BAALC*, *ERG*, *MN1*, and *EV11*.

Although enriched in the group of CN-AMLs, the above lesions are not found exclusively in these cases, and a number of genetic aberrations have been noted to occur simultaneously, with intriguing biological and clinical associations. In brief, most of the aforementioned translocations disrupt transcription factors (such as *RUNX1* and *RARA* in the t(8;21) and t(15;17), respectively), in a dominant-negative fashion, and lead to a block in normal myeloid differentiation (so-called class 2 mutations).⁹ Experimentally, and also likely clinically, such defects are necessary but not sufficient for the AML phenotype. Only when combined with an additional mutation, typically one having a positive effect on proliferation (a so-called class 1 mutation), does the AML manifest, at least experimentally. In patients, the combination of the two types of mutations typically has prognostically adverse connotations. Thus, prognosis for patients with t(8;21) and an associated *KIT* mutation, or with t(15;17) and a *FLT3*-ITD mutation, is typically worse than for patients with the translocations alone.^{10,11}

To date, the most frequently identified mutated gene in CN-AML is nucleophosmin (*NPM1*).¹² Mutations in this gene, which are typically small insertions (usually of 4 bp, sometimes up to 11 bp) in the coding region of the terminal exon (exon 12), occur in ~50 to 60% of CN-AMLs, equivalent to ~20 to 25% of all AMLs.¹² The nucleophosmin gene product is an ~37-kDa protein that actively shuttles between the nucleolus, nucleoplasm, and cytoplasm; however, as its name suggests, it is predominantly found in the nucleolus. The nucleophosmin protein was initially suspected to be involved in promoting cell growth, in part through its mediation of ribosomal biogenesis. Consistent with this hypothesis, *NPM1* is overexpressed in a variety of human tumors. Studies have demonstrated that *NPM1* might also promote growth inhibition by its functional interactions with the tumor suppressors p14ARF and p53.¹³ Mutations in *NPM1* found in CN-AML alter tryptophan residues required for proper nucleolar localization and create a putative nuclear export signal at the C terminus of the protein. Consequently, the mutant nucleophosmin protein is predominantly localized to the cytoplasm and, through dimerization, causes the mislocalization of the wild-type protein as well.¹⁴ This leads to the mislocalization and

destabilization of p14ARF and to the inhibition of p53 activity.¹³ Thus cytoplasmic nucleophosmin (NPMc) may be oncogenic, a hypothesis that is supported by tissue culture studies.¹³ In addition to this interesting pathology, the protein mislocalization may be exploited diagnostically since cytoplasmic nucleophosmin can be detected immunohistochemically, and some reports suggest an essentially 100% correlation between immunohistochemical (NPMc) and molecular (*NPM1* mutations) findings. Despite this concordance, the terms NPMc and *NPM1* mutation signify fundamentally distinct phenomena—with distinct methods of detection—and should probably not, therefore, be used interchangeably.

Given the high frequency of *NPM1* mutations in CN-AMLs, multiple studies assessing the relationship between these mutations and survival have been performed. One study showed a weak, but statistically significant increase in disease-free and overall survival in patients with an AML that possessed an *NPM1* mutation.¹⁵ This benefit, however, is affected by the *FLT3* status. *FLT3* ITDs are enriched in AMLs with *NPM1* mutations, in that they are seen twice as often in this group as compared with AMLs with wild-type *NPM1*.¹⁶ Numerous studies have demonstrated that patients who lack an *NPM1* mutation and possess an *FLT3*-ITD have a much worse prognosis, as gauged by both disease-free survival and overall survival, than do patients who possess a *NPM1* mutation without a concomitant *FLT3*-ITD.^{15,17,18} Overall survival of these latter patients approaches that of patients with AMLs that harbor karyotypes correlated with a favorable prognosis, such as t(8;21), t(15;17), or inv(16) and for whom bone marrow transplant may not have survival benefit. In fact, CN-AML patients with mutant *NPM1* and wild-type *FLT3* may not benefit from bone marrow transplant. It has been proposed that combining the status of these two “dueling” mutations allows for stratification into three prognostic groups.^{18,19} Accordingly, patients may be assigned to good (*FLT3*-ITD⁻/*NPM1*⁺), intermediate (*FLT3*-ITD⁻/*NPM1*⁻ or *FLT3*-ITD⁺/*NPM1*⁺), and poor (*FLT3*-ITD⁺/*NPM1*⁻) categories.

Detection of a mutant *NPM1* allele may also be of clinical benefit in following the treatment course of patients with CN-AML. Longitudinal studies have demonstrated that *NPM1* mutations are stable throughout the course of the disease, presumably because they often occur as an early or initiating event in the genesis of the AML and are required for tumor maintenance.^{15,20} Thus, an *NPM1* mutation, if present, represents a marker that can be used to monitor for minimal residual disease (MRD). It has further been demonstrated that quantitative assessment of these mutations correlates well with responsiveness to initial induction chemotherapy.²¹ Monitoring patients by quantitative assessment of *NPM1* mutations can predict overt clinical relapse. Accordingly, these data suggest that *NPM1* mutation detection may have clinical and treatment ramifications not only at initial diagnosis but also subsequently for monitoring remission and predicting relapse.

Nascent molecular discoveries and their demonstrated associated clinical relevance are characteristically and predictably followed by a flurry of publications detailing

Table 1. Reported Methodologies for Detecting *NPM1* Mutations

Technology	Substrate	Diagnostic sensitivity (%)	Analytic sensitivity (%)	References
PCR and capillary electrophoresis*	DNA	~100	~5	22
ASO-Nested PCR and capillary electrophoresis	RNA	~75–80	~0.001	23
PCR and melting curve analysis/FRET	DNA	~90	0.01	24
PCR/Sequencing	RNA	~98–100	NR	12,25
PCR/Sequencing	DNA	~100	NR	15,17
PCR/dHPLC	DNA	~100	NR	25
LNA-mediated PCR clamping	DNA	~100	0.1–1	26
Quantitative PCR	DNA	>95	0.01–0.1	27
Quantitative RT-PCR	RNA	~90	0.01	27
Immunohistochemistry*	Protein	~100	NR	14

FRET, fluorescence resonance energy transfer; dHPLC, denaturing high-performance liquid chromatography; LNA, locked nucleic acid; NR, not reported (for sequencing of other mutations, this is typically 15 to 25%).

* Assay may be performed on paraffin-embedded tissue.

methodologies that might be used in a molecular diagnostic laboratory. These laboratories are then charged with determining the most appropriate assay to adopt. Such is the case for *NPM1*, and in this regard, two papers in this issue of *The Journal of Molecular Diagnostics* are of note. The study by Szankasi and colleagues²² details a non-quantitative, genomic DNA-based PCR assay for detecting *NPM1* mutations. This assay can be used on paraffin-embedded tissue and can detect mutations when the leukemic cells represent 5% of the population. Since the assay is performed using genomic DNA as a template, intronic primers are used to avoid the amplification of the known pseudogenes. Moreover, since small-length nucleotide insertions distinguish wild-type and mutant alleles, a polymerase with editing capabilities is used. By contrast, the methodology described by Ottone et al²³ begins with an RNA template, employs an RT-PCR protocol with and without semi-nesting, and uses allele-specific oligonucleotide (ASO) primers. The assay only detects the most common *NPM1* A mutation, which represents 75 to 80% of mutated cases and consists of a tetranucleotide TCTG insertion in exon 12. Although the latter protocol does not detect the full range of *NPM1* mutations, it can detect mutant clones that represent as little as 0.001% of the population. The greater lability of the RNA template in this assay is a potential shortcoming; however, since the molecular assays to detect translocations in AML require RNA, this ought not to be a major issue for molecular diagnostic laboratories.

Although both articles in this issue present viable methods for detection of *NPM1* mutations, a comparison of the two methodologies highlights recurring issues surrounding both mutational testing in general and *NPM1* in particular. The genomic DNA-based method detects all known *NPM1* mutations, yet the diagnostic utility of the test is limited to cases in which leukemic cell proportion represents at least 5% of the assayed population. While this relatively low analytic sensitivity is not a limitation at diagnosis, since by definition there will be >20% blasts, it diminishes the utility of the assay for MRD detection. The RNA-ASO-based method has superior analytic sensitivity. However, this advantage comes at a price, in that diagnostic sensitivity is restricted to detection of one specific mutation and bypasses other *NPM1* mutations that are seen in 20 to 25% of positive cases. The trade-off

evident in these two different methodologies is thus quite striking and is practically inherent in the detection of multiple mutant alleles: a gain in the range of mutation detection is associated with a sacrifice in the sensitivity of detecting a specific allele. Such factors are clearly germane to deciding which assay to institute in a molecular diagnostic laboratory, which in turn is dictated by the specific clinical questions that are being asked. If the primary goal of *NPM1* testing is to stratify patients at diagnosis, a qualitative PCR of *NPM1* exon 12 from genomic DNA followed by separation of products by capillary electrophoresis (similar to the assay described by Szankasi et al²²) may be instituted. Alternatively, if the major goal of testing is MRD detection in a subset of *NPM1*-mutated AMLs, an ASO-PCR or RT-PCR assay (similar to that described by Ottone et al²³), is preferred. If both allele detection at diagnosis and MRD detection are desirable, a combination of methods is required. In this scenario, both assays could be performed at diagnosis to classify patients, and MRD testing would only be performed if the allele-specific assay were positive. Alternatively, individual patient/mutation-specific ASO assays could be developed, although this may be prohibitively expensive. The methods described in the reports in this issue of the *JMD*, and highlighted in this commentary, are among numerous other assays that have been reported for *NPM1* mutation detection. Table 1 outlines some of the key features of various different assays, which may help determine which assay is most applicable to a particular laboratory. Ultimately, the decision as to which assay to use is likely predicated on a number of factors, including i) what is the clinical question and ii) what material is available.

Real-time quantitative PCR provides a highly sensitive approach that is well suited for MRD detection, evaluation of response to therapy, and prediction of overt clinical relapse.²¹ Unfortunately, real-time quantitative PCR approaches may be limited by their requirement for allele-specific primers or probes, with an attendant increase in cost. Furthermore, given the remarkable sensitivity of real-time quantitative PCR, and the need to compare product levels from discrete time points (and possibly different laboratories), extensive standardization and quality control protocols will need to be established, analogous to the recent efforts to harmonize testing for *BCR*-

ABL1 levels in patients with chronic myelogenous leukemia.²⁸

As noted, the clinical impact of *NPM1* mutations is affected by the mutational status of the *FLT3* gene. Indeed, *NPM1* mutation analysis is of little or no value in the absence of *FLT3* testing and the two should always be performed together. Thus, clinical laboratories establishing an *NPM1* assay may wish to attempt to multiplex this assay with an *FLT3*-ITD assay. Detection by capillary electrophoreses of *FLT3*-ITD mutations and *NPM1* mutations in a single assay has been reported.²⁹ We have previously used a multiplex approach, with gel-based detection, to identify recurrent translocations in acute leukemia.³⁰ However, multiplex mutational testing may be particularly well suited to the use of a Luminex-based systems using X-MAP technology, as has been adopted at our institution to identify these recurrent translocations. Theoretically, if PCR and hybridization conditions were similar to those used for detection of recurrent translocations, identification of both recurrent karyotypic abnormalities and these intragenic mutations could be distilled into a single (RNA-based) assay. Additional clinically relevant mutations, such as those seen in *CEBPA*, *KIT*, and others, may also be included to increase significantly and comprehensively the prognostic yield. Indeed, using this bead-based technology, the number of discrete mutations that can be detected in a single assay is vast. Thus, the ultimate goal of detecting all clinically relevant mutations for AML—the “mutationome”—in a single reaction is theoretically feasible.

Although the two articles in this issue of *JMD*, as well as multiple other studies, focusing on *NPM1* have used molecular techniques to identify mutations, the cytoplasmic mislocalization of the mutant protein allows for the use of immunohistochemistry to determine *NPM1* status.¹² Using immunohistochemistry to detect underlying mutations or dysregulated gene expression is certainly not without precedent in hematopathology, with the detection of *BCL2* in follicular lymphoma (in contrast to follicular hyperplasia), *CyclinD1* in most mantle cell lymphoma, and *ALK* in a subset of anaplastic large cell lymphoma being central to diagnosis of these lymphomas. Accordingly, in laboratories that lack molecular diagnostic capabilities but can perform adequate immunohistochemistry, this method may be a viable alternative to direct analysis of the *NPM1* gene. However, interpretation of *NPM1* localization may be difficult, especially in blasts that lack abundant cytoplasm. Moreover, *NPM1* immunohistochemistry is unlikely to be useful for MRD detection, and *NPM1* localization is only relevant with known *FLT3* mutational status, which is only obtained through molecular characterization. Thus, it appears currently appropriate to recommend nucleic acid-based assays rather than immunohistochemical approaches for the detection of *NPM1* mutations.

Despite *NPM1* being only one of many genes that are frequently mutated in AML, the high occurrence of mutation in CN-AML, the correlation with prognosis, the temporal stability of the mutation, and the possible impact the mutation may have on treatment options strongly argue that testing for *NPM1* mutations should be routinely per-

formed in molecular laboratories. However, the existence of multiple *NPM1* mutant alleles complicates testing strategies, which currently ensures that a single testing methodology is not ideal for all clinical situations. As such, to institute the appropriate test or tests, laboratory directors must not only be familiar with the benefits and limitations of the various approaches (the duality of methodologies), but more importantly, must also be cognizant of specific and contextual (dueling mutations) clinical questions that are being asked, and what the clinical consequences of the answers are going to be.

References

- Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD: Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood* 2002, 100:4325–4336
- Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A: The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998, 92:2322–2333
- Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, Paietta E, Willman CL, Head DR, Rowe JM, Forman SJ, Appelbaum FR: Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood* 2000, 96:4075–4083
- Wheatley K, Burnett AK, Goldstone AH, Gray RG, Hann IM, Harrison CJ, Rees JK, Stevens RF, Walker H: A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. *Br J Haematol* 1999, 107:69–79
- Estey E, Dohner H: Acute myeloid leukaemia. *Lancet* 2006, 368:1894–1907
- Kelly L, Clark J, Gilliland DG: Comprehensive genotypic analysis of leukemia: clinical and therapeutic implications. *Curr Opin Oncol* 2002, 14:10–18
- Mrozek K, Bloomfield CD: Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program* 2006, 169–177
- Small D: *FLT3* mutations: biology and treatment. *Hematology Am Soc Hematol Educ Program*. 2006, 178–184
- Kelly LM, Gilliland DG: Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet* 2002, 3:179–198
- Callens C, Chevret S, Cayuela JM, Cassinat B, Raffoux E, de Botton S, Thomas X, Guerci A, Fegueux N, Pigneux A, Stoppa AM, Lamy T, Rigal-Huguet F, Vekhoff A, Meyer-Monard S, Ferrand A, Sanz M, Chomienne C, Fenaux P, Dombret H: Prognostic implication of *FLT3* and *Ras* gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. *Leukemia* 2005, 19:1153–1160
- Lasa A, Carricondo MT, Carnicer MJ, Perea G, Aventin A, Nomdedeu JF: A new D816 c-KIT gene mutation in refractory AML1-ETO leukemia. *Haematologica* 2006, 91:1283–1284
- Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettrossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF: Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med* 2005, 352:254–266
- Cheng K, Grisendi S, Clohessy JG, Majid S, Bernardi R, Sportoletti P, Pandolfi PP: The leukemia-associated cytoplasmic nucleophosmin mu-

- tant is an oncogene with paradoxical functions: arf inactivation and induction of cellular senescence. *Oncogene* 2007, 26:7391–7400
14. Falini B, Martelli MP, Bolli N, Bonasso R, Ghia E, Pallotta MT, Diverio D, Nicoletti I, Pacini R, Tabarrini A, Galletti BV, Mannucci R, Roti G, Rosati R, Specchia G, Liso A, Tiacci E, Alcalay M, Luzi L, Volorio S, Bernard L, Guarini A, Amadori S, Mandelli F, Pane F, Lo-Coco F, Saglio G, Pelicci PG, Martelli MF, Mecucci C: Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. *Blood* 2006, 108:1999–2005
 15. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G: Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood* 2006, 107:4011–4020
 16. Falini B, Nicoletti I, Martelli MF, Mecucci C: Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. *Blood* 2007, 109:874–885
 17. Dohner K, Schlenk RF, Habdank M, Scholl C, Rucker FG, Corbacioglu A, Bullinger L, Frohling S, Dohner H: Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood* 2005, 106:3740–3746
 18. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, Linch DC; Medical Research Council Adult Leukaemia Working Party: The impact of FLT3 internal tandem duplication mutant level, number, size and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood* 2008, 111:2776–2784
 19. Gallagher RE: Dueling mutations in normal karyotype AML. *Blood* 2005, 106:3681–3682
 20. Palmisano M, Grafone T, Ottaviani E, Testoni N, Baccarani M, Martinelli G: NPM1 mutations are more stable than FLT3 mutations during the course of disease in patients with acute myeloid leukemia. *Haematologica* 2007, 92:1268–1269
 21. Chou WC, Tang JL, Wu SJ, Tsay W, Yao M, Huang SY, Huang KC, Chen CY, Huang CF, Tien HF: Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. *Leukemia* 2007, 21:998–1004
 22. 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, 10:236–243
 23. Ottone T, Ammatuna E, Lavorgna S, Noguera NI, Buccisano F, Venditti A, Gianni L, Postorino M, Federici G, Amadori S, Lo Coco F: An allele-specific RT-PCR assay to detect type A mutation of the nucleophosmin-1 gene in acute myeloid leukemia. *J Mol Diagn* 2008, 10:212–216
 24. Scholl S, Mugge LO, Landt O, Loncarevic IF, Kunert C, Clement JH, Hoffken K: Rapid screening and sensitive detection of NPM1 (nucleophosmin) exon 12 mutations in acute myeloid leukaemia. *Leuk Res* 2007, 31:1205–1211
 25. Roti G, Rosati R, Bonasso R, Gorello P, Diverio D, Martelli MF, Falini B, Mecucci C: Denaturing high-performance liquid chromatography: a valid approach for identifying NPM1 mutations in acute myeloid leukemia. *J Mol Diagn* 2006, 8:254–259
 26. Thiede C, Creutzig E, Illmer T, Schaich M, Heise V, Ehninger G, Landt O: Rapid and sensitive typing of NPM1 mutations using LNA-mediated PCR clamping. *Leukemia* 2006, 20:1897–1899
 27. Gorello P, Cazzaniga G, Alberti F, Dell'Oro MG, Gottardi E, Specchia G, Roti G, Rosati R, Martelli MF, Diverio D, Lo Coco F, Biondi A, Saglio G, Mecucci C, Falini B: Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. *Leukemia* 2006, 20:1103–1108
 28. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, Baccarani M, Cortes J, Cross NC, Druker BJ, Gabert J, Grimwade D, Hehlmann R, Kamel-Reid S, Lipton JH, Longtine J, Martinelli G, Saglio G, Soverini S, Stock W, Goldman JM: Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood* 2006, 108:28–37
 29. Noguera NI, Ammatuna E, Zangrilli D, Lavorgna S, Divona M, Buccisano F, Amadori S, Mecucci C, Falini B, Lo-Coco F: Simultaneous detection of NPM1 and FLT3-ITD mutations by capillary electrophoresis in acute myeloid leukemia. *Leukemia* 2005, 19:1479–1482
 30. Salto-Tellez M, Shelat SG, Benoit B, Rennert H, Carroll M, Leonard DG, Nowell P, Bagg A: Multiplex RT-PCR for the detection of leukemia-associated translocations: validation and application to routine molecular diagnostic practice. *J Mol Diagn* 2003, 5:231–236