The discovery of NPM1 mutations in acute myeloid leukemia

Paolo Sportoletti

Institute of Hematology and CREO, University and Hospital Santa Maria della Misericordia of Perugia, Perugia, Italy
E-mail: paolo.sportoletti@unipg.it

https://doi.org/10.3324/haematol.2024.286026

TITLE	Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype.
AUTHORS	Falini B, Mecucci C, Tiacci E, *et al.*
JOURNAL	The New England Journal of Medicine. 2005;352(3):254-266. doi: 10.1056/NEJMoa041974.

In 2005 a paper appeared in *The New England Journal of Medicine* describing cytoplasmic nucleophosmin (NPM1) and *NPM1* gene mutations in acute myeloid leukemia (AML) with a normal karyotype.[1] These findings were going to change the way we manage patients with AML. Falini and his colleagues discovered *NPM1* mutations in the era before whole sequencing, stemming from immunohistochemical studies with an anti-NPM1 monoclonal antibody on anaplastic large cell lymphoma with t(2;5) that revealed the NPM1-ALK fusion protein to be aberrantly dislocated in the cytoplasm of lymphoma cells.[2] Further immunohistochemical studies of fixed, paraffin-embedded biopsies from other human tumors showed the expected nuclear expression of NPM1, with the exception of bone marrow biopsies from AML patients who aberrantly expressed NPM1 (a nucleolar located protein) in the cytoplasm of leukemic cells.[1] The authors named these cases NPMc$^+$ (cytoplasmic positive) to distinguish them from the NPMc$^-$ (cytoplasmic negative) ones.[1]

Notably, ectopic cytoplasmic expression of NPM1 was mainly associated with a *de novo* origin of the leukemia, normal karyotype (85% of cases), negativity for CD34 and high sensitivity to chemotherapy.[1] Moreover, it was mutually exclusive with the known recurrent translocations defining AML entities, such as *PML-RARA*, inv(16), t8(;21) and *MLL* rearrangements.[1] The authors also found that about 30% of NPMc$^+$ AML cases carried *FLT3*-internal tandem duplication, establishing, for the first time, a mechanistic link between the two mutations in promoting AML[1] that has been further confirmed in mouse models.[3] These immunohistochemical findings prompted sequencing of the *NPM1* gene which led to mutations being found in exon 12. Finally, the authors used transfection techniques to demonstrate a causal relationship between *NPM1* mutations, which were predicted to alter the protein at its C-terminal, and the cytoplasmic dislocation of NPM1.[1]

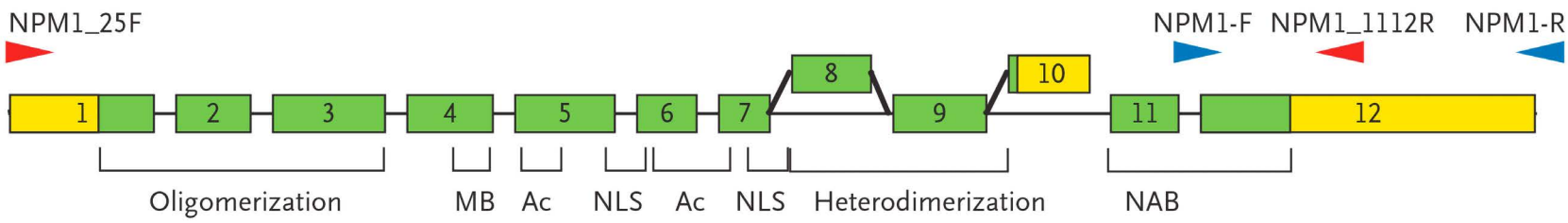
This landmark study contributed to unravel the molecular abnormalities underlying AML with a normal karyotype, since *NPM1* mutations accounted for 60% of cases with these characteristics. Moreover, the authors predicted that *NPM1*-mutated AML may represent a new leukemia entity and suggested that "*Immunohistochemistry plus mutational analysis of NPM may assist in the monitoring of minimal residual disease in a setting (normal karyotype and CD34 negativity) in which no molecular or immunophenotypic markers are available*" and that "*Understanding the mechanisms in NPMc+ AML may lead to more specific anti-leukemic therapies*".[1]

Remarkably, all the authors' predictions came true.[4] Because of its distinctive clinico-pathological features, *NPM1*-mutated AML is now recognized as a distinct leukemia entity both in the 2022 International Consensus Classification and in the fifth edition of the World Health Organization's classification of myeloid neoplasms. A search for *NPM1* mutations (in combination with other mutations) represents a critical step in the genetic-based risk stratification of AML patients according to the European LeukemiaNet. Evaluation of measurable residual disease by quantitative polymerase chain reaction or next-generation sequencing can serve as a tool to guide therapeutic decisions. Finally, better knowledge of the molecular basis of aberrant transport of mutant NPM1, association of NPMc$^+$ with a unique gene expression profile characterized by upregulation of HOX genes, and the activity of NPM1 mutant at the chromatin level have resulted in the development of new promising therapies for *NPM1*-mutated AML based on XPO1 (CRM1) and menin inhibitors.

Disclosures

No conflicts of interest to disclose.

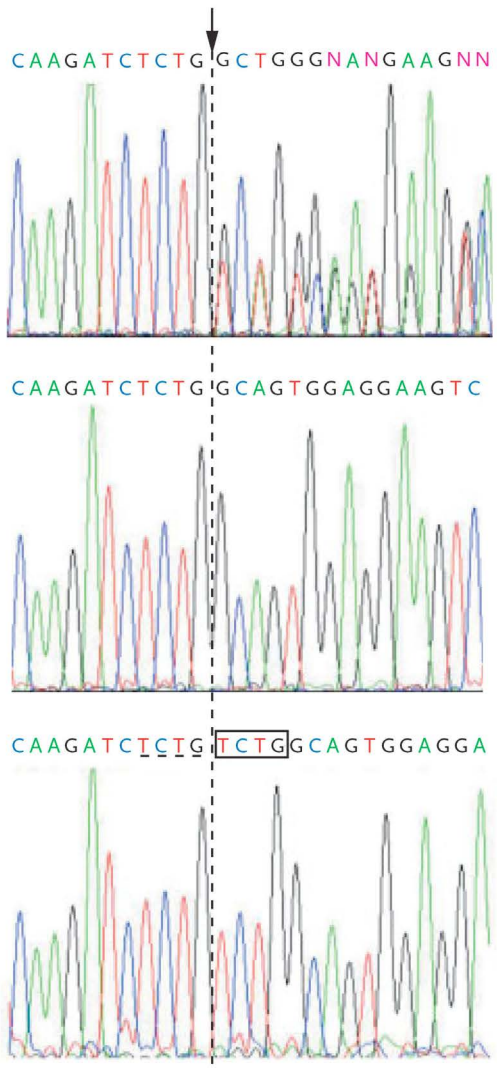
A



B

Type of Mutation	GenBank Accession No.	Sequence	Predicted Protein	No./No. of Cases (%)
None (wild type)	NM_002520	GATCTCTG....GCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLWQWRKSL	40/52 (77)
Mutation A	AY740634	GATCTCTGTCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCLAVEEVSLRK	7/52 (13)
Mutation B	AY740635	GATCTCTGCATGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCMAVEEVSLRK	1/52 (2)
Mutation C	AY740636	GATCTCTGCGTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCVAVEEVSLRK	1/52 (2)
Mutation D	AY740637	GATCTCTGCCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCLAVEEVSLRK	1/52 (2)
Mutation E	AY740638	GATCTCTG....GCAGTCTCTTGCCCAAGTCTCTTTAAGAAAATAG	-DLWQSLAQVSLRK	1/52 (2)
Mutation F	AY740639	GATCTCTG....GCAGTCCCTGGAGAAAGTCTCTTTAAGAAAATAG	-DLWQSLEKVSLRK	1/52 (2)

C



D

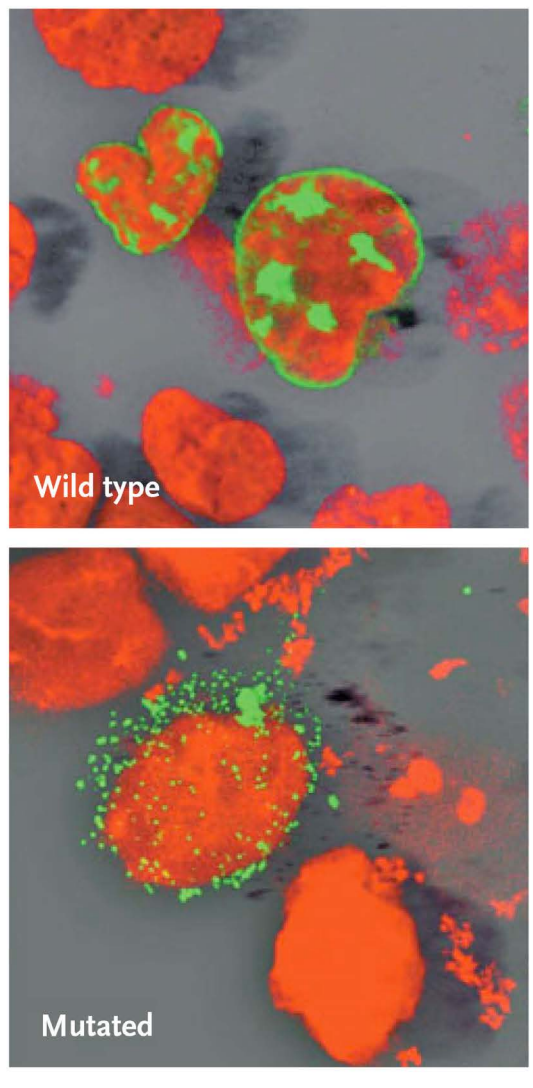


Figure 1. Mutations in exon 12 of the nucleophosmin (*NPM*) gene and in the encoded protein. (A) A schematic representation of the *NPM* gene as deduced from GenBank sequences NM_002520, NM_199185, and AB042278. Green indicates coding sequences, and yellow 3' and 5' untranslated regions. MB: metal-binding domain; Ac: acidic domain; NLS: nuclear localization signal; NAB: nucleic acid-binding domain. Primers for amplification of genomic DNA (NPM1-F and NPM1-R [blue arrowheads]) and complementary DNA (NPM1_25F and NPM1_1112R [red arrowheads]) are shown in their approximate positions above the map. (B) The wild-type NPM sequence (nucleotides 952 through 989) is aligned with six mutant variants, called A to F. Red type indicates

Continued on following page.

nucleotide insertions. The predicted protein is also shown, with boxed areas indicating the positions of the two C-terminal tryptophan (W) residues; the wild-type tryptophan residue is shown in yellow, and the mutated residues are shown in gray. The new amino-acid sequence common to all the mutated proteins is shown in green. For each variant, the number and percentage of affected cases, of the 52 total NPMc$^+$ cases, are given. (C) Sequencing results from one patient bearing mutation A, as obtained by direct sequencing (top diagram) and after cloning and sequencing of the two individual alleles (middle [wild-type] and bottom [mutated allele] diagrams). The arrow and the dashed line indicate the position where the two alleles diverge, and the box indicates the most frequently mutated nucleotides. (D) As shown in the images, the mutated NPM protein is dislocated in the cytoplasm. The images are tridimensional reconstructions of confocal micrographs of NIH-3T3 cells transfected with plasmids encoding wild-type and mutant *NPM* alleles tagged with enhanced green fluorescent protein; the nuclei were counterstained with propidium iodide (which appears as red). The wild-type protein is located in the nucleoli and nuclear membrane, whereas the mutated NPM shows aberrant cytoplasmic localization. Reproduced, with permission, from the paper by Falini *et al*. in *The New England Journal of Medicine*.[1]

References

1. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352(3):254-266.
2. Falini B, Pulford K, Pucciarini A, et al. Lymphomas expressing ALK fusion protein(s) other than NPM-ALK. Blood. 1999;94(10):3509-3515.
3. Sportoletti P, Varasano E, Rossi R, et al. Mouse models of NPM1-mutated acute myeloid leukemia: biological and clinical implications. Leukemia. 2015;29(2):269-278.
4. Falini B, Brunetti L, Sportoletti P, Martelli MP. NPM1-mutated acute myeloid leukemia: from bench to bedside. Blood. 2020;136(15):1707-1721.