G, Wassmann B, et al. Sustained complete molecular remissions after treatment with imatinib-mesylate in patients with failure after allogeneic stem cell transplantation for chronic myelogenous leukemia: results of a prospective phase II open-label multicenter study. J Clin Oncol 2005;23:7583-93.
8. Dazzi F, Szydlo RM, Craddock C, Cross NC, Kaeda J, Chase A, et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood 2000;95:67-71.
9. Kaeda J, O'Shea D, Szydlo RM, Olavarria E, Dazzi F, Marin D, et al. Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. Blood 2006;107:4171-6.
10. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 1995;86:1261-8.
11. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. Blood 2004;104:2204-5.
12. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood 2007;109:58-60.

Late relapse of acute myeloid leukemia with mutated *NPM1* after eight years: evidence of *NPM1* mutation stability

Late relapse (>5 years) of acute myeloid leukemia (AML) is rare.[1] Detecting at the time of late relapse the same genetic alteration as at diagnosis strongly suggests it may play a critical role in leukemogenesis. We previously reported in this journal that in AML with mutated nucleophosmin (NPM1), NPM1 mutation is very stable at relapse.[2] However, this conclusion was based upon molecular analysis of AML patients in whom the interval between diagnosis and relapse was short (median one year).[2] Here, we describe for the first time the clinico-pathological and molecular features of a patient with *NPM1*-mutated, *FLT3*-ITD positive AML who, after eight years, relapsed with *NPM1*-mutated, *FLT3-ITD* negative AML. These findings provide the most compelling clinical evidence to date that *NPM1* mutation is stable, strongly suggesting it is a founder genetic lesion and further supporting the view that AML with mutated NPM13 is a separate entity with distinct biological and clinical features.[4,5]

In May 2000, a 19-year old woman was diagnosed with acute myelo-monocytic leukemia (*Online Supplementary Figures 1A*) at the Institute of Hematology, "La Sapienza" University, Rome. The leukemic cells' immunophenotype in cell suspension was: $CD33^+$ (95%), myeloperoxidase-positive (55%), $CD13^+$ (23%), $CD14^+$ (25%), $CD15^+$ (46%), $CD117^+$ (1%), $HLA\text{-}DR^+$ (1%), $CD34^+$(0%). Results of immunohistochemistry are shown in *the Online Supplementary Figures 1B and C*. Immunohistochemical staining of bone marrow trephine revealed aberrant nucleophosmin expression in leukemic cell cytoplasm[6] (Figure 1A), predicting *NPM1* mutation. *NPM1* gene sequencing showed a 4 base pair (TCTG) insertion after nucleotide 1018 corresponding to mutation A3. Molecular studies also revealed an internal tandem duplication of the FLT3 gene (FLT3-ITD) (Figure 1B). The number of NPM1 mutant copies assessed by quantitative PCR at diagnosis is shown in Figure 1C. Cytogenetic analysis showed a der(13;14) constitutional Robertsonian recombination associated with trisomy 4 in six metaphases and der(13;14) as the sole finding in five (*data not shown*).

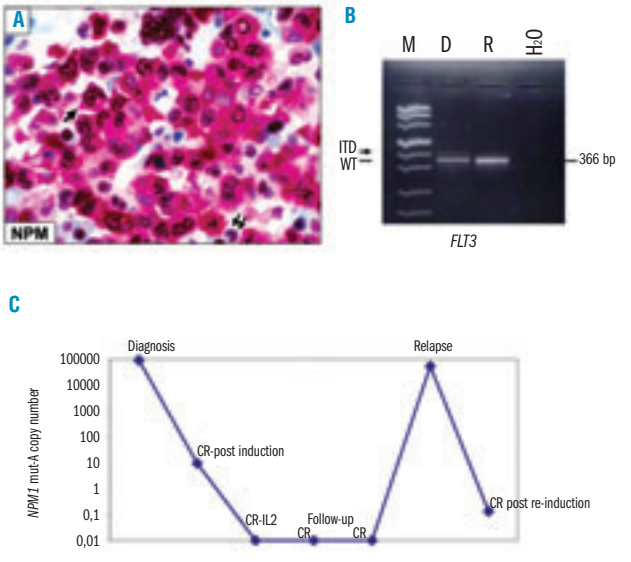

Figure 1. (A) **NPM subcellular expression at diagnosis. Acute myeloid leukemia cells show aberrant cytoplasmic positivity for nucleophosmin (NPM) (arrow) whilst residual hemopoietic cells exhibit a nucleus-restricted positivity for NPM (double arrows). APAAP technique; × 800; hematoxylin counterstaining. (B)** ***FLT3*** **gene status at diagnosis and relapse. The arrow shows the presence of a faint extra-band corresponding to the** ***FLT3-ITD*** **expressed at low level at diagnosis but not at relapse. The black line indicates the 366bp product corresponding to the amplification of FLT3 wild-type. M: marker; D: diagnosis; R: relapse; H2O=negative control, water line. C) Retrospective quantification of** ***NPM1*** **mut-A by real time RT-PCR. Variation in** ***NPM1*** **mut-A transcript level at diagnosis, complete remission (CR) and relapse is shown. The highest transcript copies number was detected at diagnosis and relapse, whereas the transcript copies number decreased following induction and became undetectable during follow-up. After re-induction therapy a reduction of NPM1 mut-A copies number was detected again. IL2 indicates interleukin-2.**

The patient was treated under the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA)/EORTC AML12 protocol (*Online Supplementary Appendix*). Complete hematologic remission, first documented in June 2000, continued until May 2008 when the patient relapsed. Immunohistochemical and molecular studies confirmed nucleophosmin was dislocated into leukemic cell cytoplasm (C23/nucleolin was nucleus-restricted) (Figure 2 A, B) and NPM1 mutation A was present (Figure 2C). FLT3 gene analysis revealed no ITD or D835 mutations (Figure 1B). Cytogenetic investigation again found a 46,XX, der(13;14) (q10;q10), +4 karyotype in 4/9 metaphases (Figure 2D). Rescue therapy was immediately started with ARA-C, idarubicin and mylotarg (*Online Supplementary Appendix*). At present (September 2008), the patient is in complete hematological remission.

Our results raise interesting questions about the significance and stability of NPM1 and FLT3 mutations in

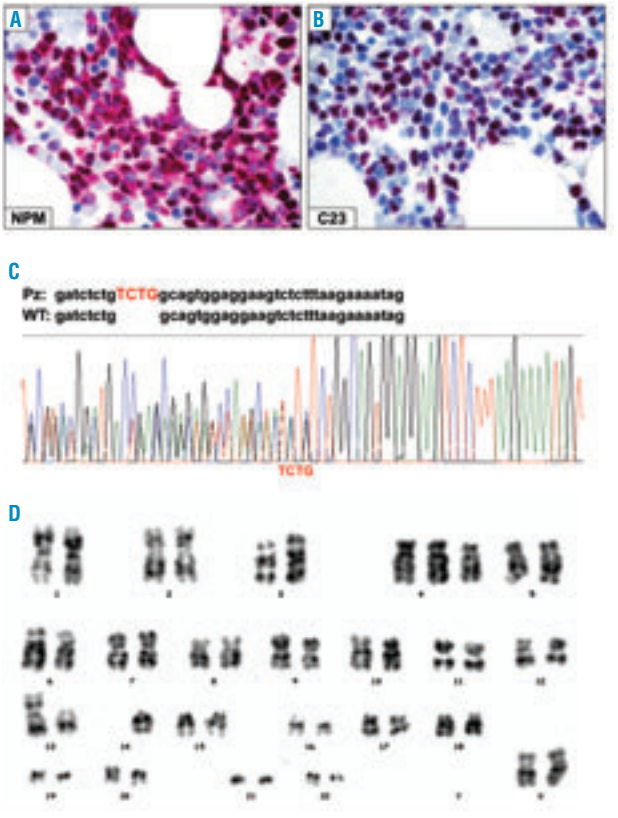

Figure 2. NPM status and cytogenetics at relapse. (A) Leukemic cells retain the aberrant cytoplasmic expression of nucleophosmin (NPM). (B) AML cells show the expected nucleus-restricted positivity for nucleolin/C23. A and B, immuno-alkaline phosphatase anti-alkaline phosphatase (APAAP) technique; × 800; hematoxylin counterstaining. (C) Electropherogram of NPM1 mutation A at relapse. (D) G-banded karyotype at relapse showing 46,XX, der(13;14) (q10;q10), +4.

AML. We previously reported an NPMc$^+$ (cytoplasmic-positive) myeloid sarcoma occurring 20 years after diagnosis of AML[7], but no information was available on cytogenetics or *NPM1* gene status of AML at presentation. In the present report, retention of *NPM1* mutation-A and aberrant nucleophosmin expression in leukemic cell cytoplasm after eight years is a remarkable example of NPM1 mutation stability, which has otherwise been observed to date only in a NOD-SCID mouse model[2], and strongly suggests the *NPM1* mutation is needed for leukemia growth and survival. Interestingly, FLT3-ITD had disappeared at relapse, in keeping with evidence that *FLT3*-ITD is a secondary genetic alteration[8] that is not stable over the course of disease.[9,10]

Clinical findings in this case of NPM1-mutated AML are also quite peculiar. Despite a concomitant FLT3-ITD, which usually antagonizes the favorable prognostic impact of the *NPM1* mutation,[4] the patient achieved a long-lasting complete hematologic remission that was associated with an undetectable number of NPM1 mutant A copies at quantitative PCR (Figure 1C). This may be because a low burden of leukemic cells carried *FLT3*-ITD at diagnosis (Figure 1B). On the contrary increasing *FLT3*-ITD mutant levels appear to associate with a highly significant trend for worsening relapse risk and overall survival.[11] The reasons for the very late relapse are poorly understood but, as *FLT3-ITD* was absent at relapse, other underlying biological factors may have come into play such as, for example, trisomy 4 which was found at diagnosis and relapse.

Finally, the favorable leukemic genotype (NPM1-mutated/*FLT3-ITD* negative) at relapse raises the question of the most appropriate therapy. Should this young woman receive an allogeneic stem cell transplantation or should she be exempted since the AML relapse is very late and the genotype is favorable?[12] In weighing up the pros and cons, one realizes that the outcome of clinical decisions in patients such as this may help improve our understanding of the biology and clinical behavior of AML with mutated *NPM1*.

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Key words: acute myeloid leukemia, nucleophosmin, NPM1, mutation, normal karyotype.

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References

1. Medeiros BC, Minden MD, Schuh AC, Schimmer AD, Yee K, Lipton JH, et al. Characteristics and outcomes of acute myelogenous leukemia patients with very late relapse (>5 years). Leuk Lymphoma 2007;48:65-71.
2. 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;93:775-9.
3. 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;352:254-66.
4. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood 2007;109:874-85.
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; 105:3945-50.
6. 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;108:1999-2005.
7. Bolli N, Galimberti S, Martelli MP, Tabarrini A, Roti G, Mecucci C, et al. Cytoplasmic nucleophosmin in myeloid sarcoma occurring 20 years after diagnosis of acute myeloid leukaemia. Lancet Oncol 2006;7:350-2.
8. Liso A, Castiglione F, Cappuccio A, Stracci F, Schlenk RF, Amadori S, et al. A one-mutation mathematical model can explain the age incidence of acute myeloid leukemia with mutated nucleophosmin (NPM1). Haematologica 2008;93:1219-26.
9. Chou WC, Tang JL, Lin LI, Yao M, Tsay W, Chen CY, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. Cancer Res 2006;66:3310-6.
10. 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;92:1268-9.
11. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, et al. 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;111:2776-84.
12. 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;358:1909-18.

Treatment of light chain deposition disease with bortezomib and dexamethasone

Light chain deposition disease (LCDD) is a rare plasma cell dyscrasia characterized by deposition of immunoglobulin fragments. The kidneys are almost always affected while heart, liver and other tissues are occasionally involved. About 50% of patients with LCDD have concurrent myeloma, however most present with nephrotic range proteinuria and rapidly deteriorating renal function.[1] Renal biopsy shows nodular glomerulosclerosis, resembling diabetic kidney changes, and linear deposits of monoclonal light chains along tubular basement membrane by immunofluorescence. The depositions are granular or amorphous and non-fibrilar, Congo-red negative.[2] The outcome of patients with LCDD is variable. Median time to end-stage renal disease (ESRD) is 2.7 years with 5-year ESRD-free survival of 37%.[2] There is no standard treatment for patients with LCDD. Chemotherapy with alkylating agents and steroids has shown modest results.[3,4] High-dose melphalan (HDM) with autologous stem cell transplantation (ASCT) has been used in some patients and has led to improvement of renal function.[5,6] Bortezomib, a reversible proteasome inhibitor, has shown significant activity in myeloma patients and is safely administered to patients with renal failure, even those under dialysis.[7] Preliminary experience indicates that bortezomib is associated with hematologic and organ responses in patients with light chain amyloidosis.[8] Recent data indicate that this agent may have a protective role of renal parenchyma due to inhibition of NFκB activity.[9] Based on the above data we administered the combination of bortezomib and dexamethasone to 4 consecutive patients with LCDD with typical findings of renal biopsy, serum and urine electrophoresis, and immunofixation and bone marrow biopsy (Table 1). None of the patients had symptomatic myeloma defined by osteolytic bone disease, hypercalcemia, anemia or renal impairment due to myeloma cast nephropathy. Two patients were previously untreated; one was relapsing from prior response to cyclophosphamide with prednisone and one was refractory to vincristine, doxorubicin and dexamethasone (VAD). All patients presented with impaired renal function, non-selective proteinuria and poorly controlled hypertension, defined as blood pressure (BP) > 140/90 mmHg, although they were receiving three or more antihypertensive drugs (Table 1). One patient also had echocardiographic evidence of heart involvement. Serum free light chains (FLCs-FREELITE assay) were elevated in all patients, with abnormal kappa to λ ratio, and were measured at the beginning of each cycle of treatment. Three patients had no measurable monoclonal

Table 1. Patients' characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4
Gender/Age (years)	Female/56	Male/46	Male/52	Male/67
Prior therapy	N/A	VAD (refractory)	N/A	Cyclophosphamide/prednisone (relapse off treatment)
Bone marrow plasma cells	20%	10%	11%	3%
Light chain type	κ	κ	κ	λ
Serum immunofixation	κ	0	IgG-κ	0
Urine immunofixation	κ	κ	κ	λ
Pre/post BD involved free light chain (mg/L)	2670/76.1	523/13.6	297/24.5	232/99.4
Pre/post BD (κ/λ ratio)	252/6.5	54.4/ 0.95	11/0.9	0.2/0.47
Pre/post BD creatinine (mg/dL)	3.6/1.7	2.1/1.8	3.5/2.4	2.4/1.7
Pre/post BD 24h urine protein (mg)	2860/375	6500/416	4920 / < 200	1620 / 680
Pre/post BD serum albumin (gr/dL)	3.2/3.9	3.3/4.2	3.7/4.4	4/4
Pre/post BD antihypertensive treatment	3 drugs/1 drug	3 drug/2 drugs	5 drugs/2 drugs	5 drugs/3 drugs
Response to BD	PR	CR	CR	PR
Time to response (>50% reduction of affected light chain by FREELITE)	21 days (1 cycle of BD)	21 days (1 cycle of BD)	21 days (1 cycle of BD)	84 days (4 cycles)
Time to hematologic CR (Normal free light chains and ratio)	N/A	63 days (3 cycles of BD)	84 days (4 cycles of BD)	N/A
Time to 50% reduction 24h urine protein	42 days (2 cycles of BD)	21 days (1 cycle of BD)	42 days (2 cycles of BD)	63 days (3 cycles of BD)
CD34 collection (*10^6/Kg)	15	63	8	N/A
Time to neutrophil engraftment	Day 8	Day 10	Day 11	N/A
Months without progression	16+	15+	12+	At 2 months relapse of proteinuria without hematologic relapse