NPM1 mutations are more stable than *FLT3* mutations during the course of disease in patients with acute myeloid leukemia

***NPM1* mutations have been reported to be the most frequent mutations in acute myeloid leukemia (AML). They are associated with a wide spectrum of morphologic subtypes of AML, normal karyotype and *FLT3* mutations. The high frequency of *NPM1* mutations might provide a suitable marker for monitoring residual disease of AML.**

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Nucleophosmin (NPM) is a multifunctional phosphoprotein with tumor-suppressor and oncogenic functions. *NPM1* exon 12 mutations were found in 25-35% of adult *de novo* acute myeloid leukemia (AML). These mutations cause a frameshift and the formation of novel C-termini, and generate NPM mutants that localize aberrantly in the cytoplasm.[1-4] Due to their frequency, *NPM1* mutations may become a new tool for monitoring residual disease in AML.

We report on a comparison of the *NPM1* and *FLT3* mutational status during the clinical course of 28 adult AML patients. Bone marrow samples were collected from all patients after informed consent. The patients were diagnosed at our Institute and received induction-chemoterapy including standard dose Ara-C, Idarubicin and Etoposide, and consolidation therapy including Idarubicin and intermediate dose Ara-C. Patients with AML-M3 received All-*trans*-Retinoic Acid in addition to the chemotherapy described above. The presence of *FLT3* mutations (Internal Tandem Duplication and point mutation at D835 residue) and *NPM1* mutations were identified by the high sensitive Denaturing-High Performance Liquid Chromatography (D-HPLC) assay and direct sequencing, using the previously described primers for the *FLT3* analysis,[5] and the forward primer NPM1-F (5'GAAGAATTGCTTCCGGATGATC3') and the reverse primer NPM1-R (5'CCTGGACAACATTTATCAAACACGGTA3') for the amplification of *NPM1* gene.

Mutations of *NPM1* gene were present in 11/28 (39%) AML cases: type A mutation (960_963dupTCTG) occurred in 9/11 (82%) samples, type B (960_963ins CATG) (patient #2) and type D mutation (960_963ins CCTG) (patient #11) were each present in 1 case (9%).[1] Overall, 12/28 patients (43%) carried a mutation of *FLT3* at diagnosis (6 single *ITD*-mutations and 6 D835-mutations) (Table 1).

We analyzed *NPM1* and *FLT3* mutations during the progression of disease, in a median follow-up of 11 months (range 3-31). The first relapse occurred at a median of 9.5 months (range 3-31) after diagnosis. Each patient had matched diagnostic and first relapse samples available for analysis. One of them also had a second relapse sample. Samples from 12 patients were also available at the time of first complete remission (CR1), and samples from 2 patients were obtained in second complete remission (CR2). CR and relapse were defined by classical morphologic criteria (i.e. less and more than 5% blasts in the bone marrow respectively). For the *NPM1* mutation, we observed that the same mutation as that detected at diagnosis was identified again at first relapse in all *NPM1*-mutated patients. Furthermore, patient #1 also showed the same mutation at second relapse. No mutation in *NPM1* was detected in relapses of patients

Table 1. Summary of patient characteristics and comparison of mutations during the clinical course.

Patient no.	*Status*	*Interval (mo)**	*FAB*	*Karyotype*	*NPM1 mutation*	*FLT3 mutation*
			Patients harboring both mutations at diagnosis			
1.	Diagnosis		M4	N	+TCTG	ITD
	CR1	3			-	-
	Relapse 1	4	M4	N	+TCTG	ITD
	Relapse 2	5	M4	N	+TCTG	ITD
2.	**Diagnosis**		**M2**	**N**	**+CATG**	**ITD**
	Relapse	**4**	**M2**	**N**	**+CATG**	**ITD**
3.	Diagnosis		M5	N	+TCTG	D835
	Relapse	9	M5	N	+TCTG	D835
4.	**Diagnosis**		**M3**	**t(15;17)**	**+TCTG**	**D835**
	CR	**7**			**–**	**–**
	Relapse	**24**	**M3**	**t(15;17)**	**+TCTG**	**D835**
5.	Diagnosis		M5	N	+TCTG	D835
	Relapse	5	M5	N	+TCTG	ITD
			Patients harboring NPM1 mutation at diagnosis			
6.	**Diagnosis**		**M2**	**N**	**+TCTG**	**–**
	CR	**2**			**–**	**–**
	Relapse	**9**	**M2**	**N**	**+TCTG**	**–**
7.	Diagnosis		M5	complex	+TCTG	–
	Relapse	4	M5	complex	+TCTG	–
8.	**Diagnosis**		**M4eo**	**inv(16)**	**+TCTG**	**–**
	Relapse	**9**	**M4eo**	**inv(16)**	**+TCTG**	**–**
9.	Diagnosis		M1	N	+TCTG	–
	Relapse 1	17	M1	N	+TCTG	ITD
	CR2	5			–	–
10.	**Diagnosis**		**M0**	**N**	**+TCTG**	**–**
	Relapse 1	**9**	**M1**	**N**	**+TCTG**	**D835**
	CR2	**5**			**–**	**–**
11.	Diagnosis		M5	N	+CCTG	–
	Relapse	7	M5	N	+CCTG	D835
			Patients harboring FLT3 mutation at diagnosis			
12.	**Diagnosis**		**M4eo**	**inv(16)**	**–**	**ITD**
	Relapse	**3**	**M4eo**	**inv(16)**	**–**	**ITD**
13.	Diagnosis		M4eo	inv(16)	–	ITD
	CR	3			–	–
	Relapse	2	M4eo	inv(16)	–	ITD
14.	**Diagnosis**		**M3**	**t(15;17)**	**–**	**ITD**
	CR	**3**			**–**	**–**
	Relapse	**9**	**M3**	**t(15;17)**	**–**	**ITD**
15.	Diagnosis		M0	+X,+Y	–	D835
	Relapse	11	M0	+X,+Y	–	D835
16.	**Diagnosis**		**M4**	**N**	**–**	**D835**
	Relapse	**3**	**M4**	**N**	**–**	**D835**
17.	Diagnosis		M5	+8	–	ITD
	Relapse	17	M5	+8, t(9;19)	–	–
18.	**Diagnosis**		**M1**	**t(9;22)**	**–**	**D835**
	Relapse	**4**	**M1**	**N**	**–**	**–**
			Patients without mutations at diagnosis			
19.	Diagnosis		M2	t(8;21)	–	–
	CR	2			–	–
	Relapse	16	M2	t(8;21)	–	ITD
20.	**Diagnosis**		**M3**	**t(15;17)**	**–**	**–**
	CR	**8**			**–**	**–**
	Relapse	**4**	**M3**	**t(15;17)**	**–**	**–**
21.	Diagnosis		M3	t(15;17)	–	–
	CR	3			–	–
	Relapse	13	M3	t(15;17)	–	–
22.	**Diagnosis**		**M5**	**N**	**–**	**–**
	CR	**5**			**–**	**–**
	Relapse	**3**	**M5**	**N**	**–**	**–**
23.	Diagnosis		M4eo	inv(16)	–	–
	CR	12			–	–
	Relapse	6	M4eo	inv(16)	–	–
24.	**Diagnosis**		**M4eo**	**t(16;16)**	**–**	**–**
	CR	**5**			**–**	**–**
	Relapse	**13**	**M4eo**	**t(16;16)**	**–**	**–**
25.	Diagnosis		M4eo	inv(16)	–	–
	Relapse	6	M4eo	inv(16)	–	–
26.	**Diagnosis**		**M5**	**N**	**–**	**–**
	Relapse	**10**	**M5**	**N**	**–**	**–**
27.	Diagnosis		M2	t(8;21)	–	–
	CR	6			–	–
	Relapse	17	M2	t(8;21)	–	–
28.	**Diagnosis**		**M2**	**N**	**–**	**–**
	Relapse	**18**	**M2**	**N**	**–**	**–**

*Mutational status of NPM1 and FLT3 mutations was analyzed in 28 patient samples during the follow-up. Italics in data field indicate a change in mutational status. no.: number; FAB: French-American-British; NPM1: nucleophosmin; FLT3: fms-like tyrosine kinase 3; N: normal; ITD: internal tandem duplication; CR: complete remission. *Interval (months) between two consecutive studies.*

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that revealed wild-type *NPM1* at diagnosis (17/28), as reported by others,[2,4,6] although at relapse two of them showed a different karyotype from that at diagnosis (patients #17 and 18). In the samples obtained at the time of CR from patients harbouring *NPM1* mutated at diagnosis, the mutation became undetectable. This shows that these were somatic mutations related only to the leukemic clone. Thus, in our experience, the *NPM1* gene status was stable during disease. By contrast, we found that *FLT3* mutational status changed between diagnosis and relapse in 7/28 patients (25%), 4 of them (patients #5, 9, 10, 11) also carried a *NPM1* mutation. Two patients (#17 and 18) lost the mutation at relapse, 4 patients (#9, 10, 11, 19) acquired the mutation at relapse and patient 5 modified the mutation from D835 to *ITD*. In three patients, the change of *FLT3* status was correlated to a modification of FAB or karyotype. In the patient 10, who also carried a *NPM1* mutation, the FAB at diagnosis was M0, at relapse it evolved to M1 and acquired the *FLT3-D835* mutations. Patient #17 acquired the t(9;19) and lost the *FLT3-ITD* at relapse. Patient #18, who harbored the t(9;22) and the *FLT3-D835* mutation at diagnosis, lost both these alterations at relapse. Patients #1-2 and 12-14 relapsed with the identical *FLT3-ITD* length mutation types and patients #3-4 and 15-16 exhibited the same D835-mutation types at both stages. All samples obtained at the time of CR were negative for the presence of *FLT3* mutations (Table 1).

In conclusion, *NPM1* gene status was stable during disease evolution, in contrast to *FLT3*.[6-8] The results reported suggest that *NPM1* mutation, not *FLT3* mutation may be considered as a potential marker for monitoring minimal residual disease. It could be useful to monitor residual disease in patients with normal karyotype, in which no alternative molecular markers are available. If the stability of *NPM1* mutations at relapse is confirmed, these mutations may be useful to monitor residual disease in a large subgroup of AML patients.

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Key words: AML, FLT3 mutation, NPM1 mutation, minimal residual disease.

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