Leukemia Research 76 (2019) 29–32

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Letter to the Editor

Impact of *NPM1* mutation subtypes on treatment outcome in AML: The Lyon-University Hospital experience

The nucleophosmin member 1 (NPM1) is an abundant multifunctional nucleolar protein involved in the maintenance of genome stability and ribosome biogenesis [1]. Mutations in the *NPM1*gene are detected in approximately one-third of all patients with acute myeloid leukemia (AML) and up to 60% of those with normal cytogenetics [2]. The gene encoding *NPM1* is located on 5q35.1 and contains 12 exons. *NPM1* is involved in epigenetic control, ribosomal protein assembly, and regulation of p53 tumor suppressor pathway [3]. *NPM1* mutations are generally considered of favorable prognosis in the absence of additional internal tandem duplication in the fms-related tyrosine 3 gene (*FLT3-ITD*) [4], and *NPM1* mutations represent an attractive target for minimal residual disease (MRD) monitoring [5,6]. There are more than 50 various types of *NPM1* mutations resulting in a frameshift due to an insertion of bases, which cluster in exon 12 [7]. These frameshift mutations generally lead to the modifications of two tryptophanes. The most common is type A mutations (TCTG) found in approximately 80% of cases, followed by type B (CATG) and type D (CGTG) mutations in about 10%, and a spectrum of other mutations accounting for 10% [6]. Little is known about the prognostic relevance of *NPM1* non-A mutations. A few published studies have addressed this topic revealing controversial results. A tendency was shown for patients with non-A mutations to have an adverse impact on survival [8], while other studies showed the opposite [9,10] and others did not demonstrate any differences regarding mutation types [11,12]. More recently, Alpermann et al. reported an adverse effect of *FLT3-ITD* mutation in type A patients and of *DNMT3A* mutations in types A and D [13].

The purpose of the present study was to evaluate the relevance of A type and non-A type *NPM1* mutations with regard to clinical outcome. Our analyses were based on 175 *NPM1*-mutated patients (median age: 61 years; range: 20–93) with newly diagnosed AML seen in our institution over a 4-year period. The *NPM1* mutations were detected by high resolution melting, confirmed by next-generation sequencing. Detailed information regarding initial clinical, cytomorphological, cytogenetic and molecular characteristics of patients are presented in Table 1. Regarding *NPM1* mutation types, type A was found in 128 cases (73%) and non-A types in 47 cases (27%) including B type mutation (17 cases), D type mutation (14 cases), and other rare mutations (16 cases). The karyotype (defined according to the European LeukemiaNet classification [2]) was intermediate-risk in 85% of cases, unfavorable-risk in 5%, and failed in 19 patients, a profile in line with prior reports [14]. An *FLT3-ITD* mutation was detected in 38% of cases (Table 1). Although involving few patients, *FLT3-TKD* mutation appeared more frequent in type non-A *NPM1* mutations than in type A.

Out of 175 patients, 134 (77%) were treated with anthracycline- and cytarabine-based intensive chemotherapy, while 41 (23%) received low-intensity therapy (hypomethylating agent or low-dose cytarabine courses) (25 patients) or only hydroxyurea or 6-mercaptopurine and best supportive care (16 patients). Median follow-up for overall survival (OS) was 5.5 years (95% CI: 4.7–6.8). Complete remission (CR) was achieved in 124 patients (92%) treated with intensive chemotherapy and in 7 patients (17%) treated less intensively. Forty patients underwent allogeneic stem cell transplantation (ASCT) in first CR. MRD-negativity (RT-qPCR; sensitivity: 10^{-6}) was obtained significantly more frequently in the group with type A mutation after intensive induction chemotherapy ($P = 0.02$). Achievement of MRD-negativity after induction chemotherapy was of prognostic value both in terms of disease-free survival (DFS) (median: not reached versus 7.1 months; $P < 0.0001$) and OS (median: not reached versus 13.4 months; $P < 0.0001$). OS curves according to MRD status and type of *NPM1* mutation are shown in Fig. 1. Median OS of the whole cohort was 24.7 months (95% CI: 18.2–44) with a 3-year OS at 44%. Median disease-free survival (DFS) was 22.9 months with a 3-year DFS at 48%. Outcomes according to *NPM1* mutation types and treatment received are summarized in Table 2. In univariate analysis, we did not find any statistical difference with regard to DFS and OS among patients with A type and non-A type *NPM1* mutations. However, a better median OS was observed in non-A type mutation patients treated with intensive chemotherapy (median: not reached versus 63.7 months) (Fig. 2A) and an advantage in terms of OS was found in non-A type mutation patients who received only low-intensity therapy (median: 9.4 versus 2.9 months) (Fig. 2B). Those results should nevertheless be taken with caution because of the limited number of patients in each group. In multivariable analysis, younger age [Hazard ratio (HR): 3.24; 95% confidence interval (CI): 1.66–6.23; $P = 0.004$], MRD-negativity [HR: 5.86; 95% CI: 2.88–11.70; $P < 0.0001$], and non-A type mutations [HR: 2.84; 95% CI: 1.29–6.17; $P = 0.006$] were significantly associated with a better OS in patients treated with intensive chemotherapy. Younger age [HR: 3.44; 95% CI: 1.84–6.35; $P = 0.001$], MRD-negativity [HR: 7.79; 95% CI: 3.70–16.28; $P < 0.0001$], and non-A type mutations [HR: 3.54; 95% CI: 1.61–7.69; $P = 0.001$] also appeared as independent prognostic factors in terms of DFS. When introduced into the model, *FLT3-ITD* mutation status was not a variable independently associated with survival. Age, MRD status, and the type of *NPM1* mutation were still of prognostic value when only considering patients with *FLT3-ITD* mutation. Only younger age remained of prognostic value when only considering patients without *FLT3-ITD* mutation (Table 3).

The incidences of various *NPM1* variants were similar to those previously reported [11–13]. The data from the literature showed similar clinical and biological features among patients with A type and non-A type mutations [8,11,13]. This was confirmed by our study. In the literature, evaluation of clinical outcome based on *NPM1* genotypes showed no differences in terms of CR rates after intensive chemotherapy [8,10,11,13]. Reversely, results were very heterogeneous regarding their impact on treatment outcome in terms of survival. Larger studies reported a trend for a better leukemia-free survival (LFS)

https://doi.org/10.1016/j.leukres.2018.11.016
Received 13 October 2018
Available online 29 November 2018

Table 1
Initial clinical and biological features in the cohort of A type and non-A type *NPM1*-mutated patients.

Characteristics	Type A (128 patients)	Type non-A (47 patients)	Total cohort (175 patients)
Clinical features			
Sex ratio (M/F)	0.70	0.88	0.75
Age (years)	60 (20–93)*	62 (34–-80)	61 (20–93)
WHO PS			
0–2	107 (84%)**	36 (77%)	143 (82%)
3–4	21 (16%)	10 (21%)	31 (17%)
ND	0	1	1
Blood Features			
WBC (x10^9/L)	22.5 (0.9–300)	14.8 (1.32–193)	21.5 (0.9–300)
Hb (g/L)	96 (54–146)	94 (10–138)	95 (10–146)
Platelets (x10^9/L)	71 (8–415)	69 (15–698)	70 (8–698)
PMN (x10^9/L)	1.8 (0.1–20.7)	1.5 (0.1–11)	1.75 (0.1–20.7)
PB blasts (%)	56 (0–100)	45 (0–97)	55 (0–100)
BM blasts (%)	80 (20–98)	80 (20–95)	80 (20–98)
LDH (UI/L)	639 (119–6200)	552 (172–3165)	609 (119–6200)
FAB classification			
M0	2	0	2
M1	42 (33%)	11 (23%)	53 (30%)
M2	20 (16%)	9 (19%)	29 (17%)
M4	17 (13%)	4 (9%)	21 (12%)
M5	43 (34%)	17 (36%)	60 (34%)
M6	1	1	2
M7	1	0	1
ND	2	5	7
Cytogenetics			
Intermediate-risk	108 (84%)	40 (85%)	148 (85%)
Unfavorable-risk	5 (4%)	3 (6%)	8 (5%)
Failure	15	4	19
Molecular mutations			
FLT3-ITD	50 (39%)	17 (36%)	67 (38%)
FLT3-TKD	3 (2%)***	6 (13%)***	9 (5%)

Abbreviations: BM, bone marrow; F, female; FAB, French-American-British; Hb, hemoglobin; LDH, lacticodehydrogenase; M, male; ND, not determined; PMN, polymorphonuclar cells; PS, performance status; WBC, white blood cells; WHO, World Health Organization.
* Median (range); ** Number of patients (percentage); *** $P = 0.01$.

and OS in patients with non-A type *NPM1* mutations, despite a higher rate of additional cytogenetic abnormalities [10,13]. In our study, while no differences were also noted in terms of CR rates, MRD levels after induction seemed to be influenced by the type of *NPM1* mutation with early MRD-negativity achieved in type A cases. Despite the prognostic value of MRD-negativity, the deeper response in type A *NPM1* mutation patients did not impact on OS. Our results could certainly be influenced by the limited number of patients. In the other hand,

Table 2
Outcomes according to *NPM1* mutation types and treatment received.

Outcomes	Type A (128 patients)	Type non-A (47 patients)	Total cohort (175 patients)
CR			
All patients	93 (73%)	38 (81%)	131 (75%)
Intensive chemo	88 (92%)	36 (92%)	124 (92%)
Low intensity/BSC	5 (15%)	2 (25%)	7 (17%)
MRD negativity**			
All patients	57 (75%)***	16 (53%)***	73 (69%)
Intensive chemo	57 (77%)****	15 (54%)****	72 (71%)
Low intensity/BSC	NA	NA	NA
DFS			
All patients	21.5 months 3-y: 48%	32.3 months 3-y: 48%	22.9 months 3-y: 48%
Intensive chemo	31.7 months 3-y: 49%	32.3 months 3-y: 48%	31.7 months 3-y: 49%
Low intensity/BSC	NA	NA	NA
OS			
All patients	24.7 months 3-y: 44%	20.3 months 3-y: 44%	24.7 months 3-y: 44%
Intensive chemo	63.7 months 3-y: 56%	NR 3-y: 51%	63.7 months 3-y: 55%
Low intensity/BSC	2.9 months 1-y: 23%*	9.4 months 1-y: 50%*	3.8 months 3-y: 6%

Abbreviations: BSC, best supportive care; chemo, chemotherapy; CR, complete remission; MRD1, minimal residual disease evaluated after induction therapy; NA, not applicable; NR, not reached; y, year.
*$P = 0.03$ (Breslow); ** Evaluated after induction in 106 patients achieving CR; ***$P = 0.03$ (evaluated in 76 type A patients and 30 type non-A patients); ****$P = 0.02$ (evaluated in 74 type A patients and 28 type non-A patients).

discrepancies between early MRD achievement and OS could perhaps be explained by a less aggressive but more resistant leukemic clone in patients with non-A type mutations. Nearly all *NPM1*-mutated AML patients showed concurrent mutations in genes involved in regulation of DNA methylation, in RNA splicing, in the cohesion complex, or in cell signaling pathways [15]. In a prior report, Alpermann et al. suggested that the prognosis of patients with type B mutation was unaffected by *FLT3-ITD* and *DNMT3A* mutations [13]. However, *FLT3-ITD* mutation did not appear as an independent prognostic factor when introduced into our multivariate analysis model. Surprisingly, factors of prognostic impact on survival varied according to *FLT3-ITD* status, suggesting a relationship between *FLT3-ITD* mutation and the type of *NPM1* mutation and/or MRD status after induction chemotherapy although no correlation was found in univariate analysis. No correlations were described between *FLT3-TKD* mutation and *NPM1* mutation subtypes. However, co-occurrence of *FLT3-TKD* and *NPM1* mutations has

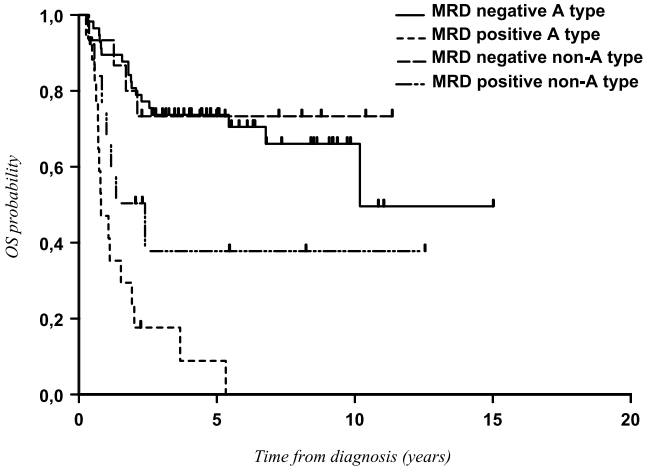

Fig. 1. OS according to type of *NPM1* mutation and MRD status after induction with intensive chemotherapy (MRD negative A type: 57 patients; MRD positive A type: 17 patients; MRD negative non-A type: 15 patients; MRD positive non-A type: 13 patients).

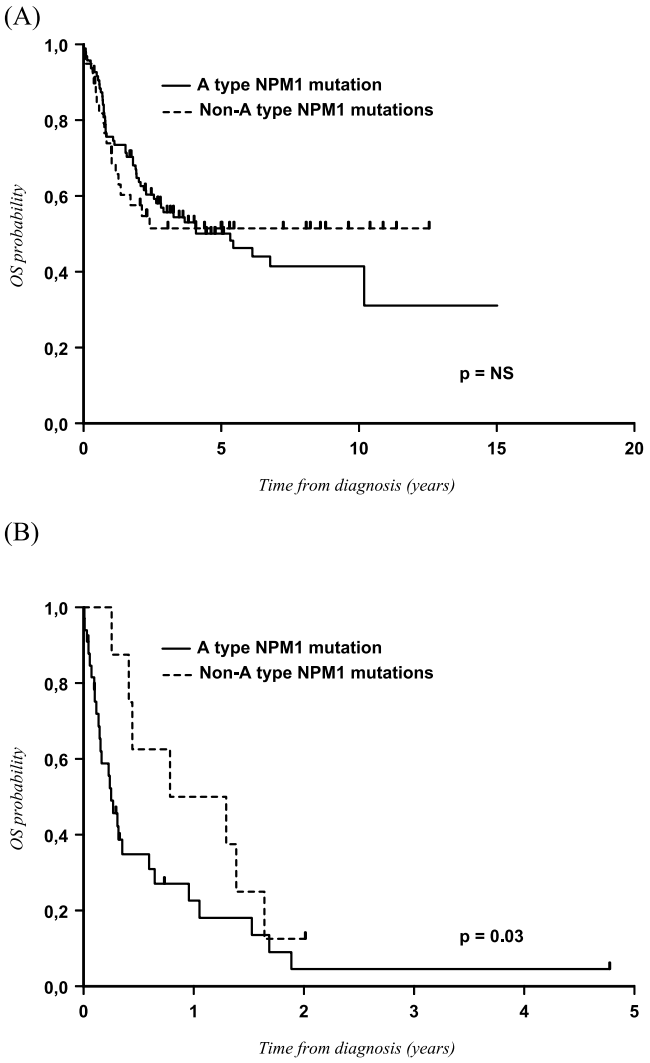

Fig. 2. OS according to *NPM1* mutation subtypes: (A) Patients receiving intensive chemotherapy (A type: 95 patients; non-A type: 39 patients), (B) patients receiving only low-intensity therapy or best supportive care (A type: 33 patients; non-A type: 8 patients).

Table 3
Multivariate analyses in patients receiving intensive chemotherapy.

Features	HR	95% CI	*P* value
DFS (all patients)*			
A type *vs* non-A type mutations	3.54	1.61–7.69	0.001
MRD-negative *vs* MRD-positive	7.79	3.70–16.28	< 0.0001
Age ≥ 60 *vs* Age < 60 years	3.44	1.84–6.35	0.001
OS (all patients)*			
A type *vs* non-A type mutations	2.84	1.29–6.17	0.006
MRD-negative *vs* MRD-positive	5.86	2.88–11.70	< 0.0001
Age ≥ 60 *vs* Age < 60 years	3.24	1.66–6.23	0.004
OS (patients *FLT3-ITD* positive)**			
A type *vs* non-A type mutations	4.93	1.32–18.17	0.013
MRD-negative *vs* MRD-positive	34.55	6.29–188.67	< 0.0001
Age ≥ 60 *vs* Age < 60 years	4.65	1.32–16.11	0.011
OS (patients *FLT3-ITD* negative)***			
Age ≥ 60 *vs* Age < 60 years	2.58	1.10–5.92	0.031

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; MRD, minimal residual disease after induction chemotherapy; OS, overall survival.

* 95 patients were involved in the multivariate analysis. Factors included into the model were: cytogenetic-risk group (intermediate *vs* unfavorable), age (≥60 years *vs* < 60 years), MRD status after induction (negative *vs* positive), *FLT3-ITD* status (mutated *vs* non-mutated).

** 38 patients were involved in the multivariate analysis.

*** 59 patients were involved in the multivariate analysis.

recently been defined as a highly favorable prognostic AML subgroup, which could explain the advantage of non-A type mutations in terms of survival [16]. While OS of type A and non-type A *NPM1* mutations did not differ significantly in univariate analysis among patients receiving intensive therapy, the difference appeared surprisingly highly significant in the multivariate analysis. Although not clearly understandable, patients were not censored at the time of transplant in the univariate analysis and ASCT was not considered as a variable in the multivariate analysis, which could influence the outcomes.

NPM1 gene mutations are important as molecular markers for diagnosis, prognosis and monitoring of MRD in AML patients. Several groups have reported the prognostic impact of *NPM1* mutation MRD response, especially when early assessed [5,17–19]. Ivey et al. prospectively showed that *NPM1* MRD performed after the first cycle of consolidation was highly predictive of relapse-free survival (RFS) and OS, independently of *FLT3-ITD* and *DNMT3A* mutational status [6]. These results were recently confirmed with postinduction MRD, which showed that patients harboring at least a 4-log reduction did not benefit from ASCT, independently of *FLT3-ITD* status [5]. The prognostic impact of MRD-negativity after induction therapy was confirmed in our study, in which it appeared as the main prognostic factor in patients with *NPM1* mutation.

Because of their low frequency and their diversity, it is very challenging to assess non-A *NPM1* mutation clinical relevance. Larger

studies need to be conducted to evaluate the prognostic value of *NPM1* mutation subtypes and their relationship with the other molecular markers in order to define whether they should be taken into account for treatment decision-making, especially regarding consolidation by ASCT.

Conflict of interest

The authors declare no conflicts of interest.

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