Rare coincident *NPM1* and *RUNX1* mutations in intermediate risk acute myeloid leukemia display similar patterns to single mutated cases

Recently, Mendler *et al*. reported a low incidence of 4 of 472 (0.85%) acute myeloid leukemia (AML) cases that carried concurrent *NPM1* and *RUNX1* mutations.[20] Interestingly, they found that *RUNX1* mutations in these rare cases with concurrent *NPM1* mutations were structurally unusual when compared to *RUNX1* mutations observed in *NPM1* wild-type cases. All these 4 cases had *RUNX1* mutations that were in-frame, located outside the Runx homology (RH) domain and were also present in the germline.[20]

To further investigate these findings in an independent cohort, we screened 2722 adult *de novo* AML cases with intermediate-risk cytogenetics (1171 females, 1551 males; median age 68.4, range 15.7-100.4 years) for *NPM1* and *RUNX1* mutations. Patients provided written informed consent and study protocols were in accordance with the Declaration of Helsinki. We found co-existent *NPM1* and *RUNX1* mutations in a similar rare subset of 0.44% of all cases (11 of 2722 cases) as described by Mendler *et al*.[1] Clinical and molecular characteristics of these patients are shown in Table 1. Three patients were female, 8 patients were male. Median age was 67.7 years (range 42.0-82.0 years). Regarding *NPM1* mutations, 9 patients had subtype A, one patient subtype I, and one patient harbored an unusual mutation in *NPM1* consisting of a missense mutation (p.Trp290Leu) and a complex frameshift mutation in the 3'-UTR.

In the *NPM1* mutated cases, we confirmed a high percentage of *RUNX1* missense mutations: n=6 (54.5%) as compared to 37.0% in an independent cohort of *RUNX1* mutated/*NPM1* wild-type cases.[2] However, in 5 cases, other various *RUNX1* mutations were detected: n=2 frame-shift; n=2 nonsense; n=1 splice-site mutation. Regarding the localization of the *RUNX1* mutations, 4 mutations were localized in the RH domain, 4 mutations in the TAD domain, and only 1 *RUNX1* mutation was located downstream the RHD domain. This is in contrast to the report of Mendler *et al*. who report that all their 4 mutations detected in *RUNX1* were located outside the TAD or RHD domain.

In 5 of our cases, follow-up material was available. In 3 cases (Patient ns. 4, 5 and 10), *RUNX1* mutations were clearly somatic as they were non-detectable in complete remission material. In 2 cases (Patient ns. 2 and 7), a germline mutation could not be excluded as *NPM1* and *RUNX1* mutation loads did not decrease during follow up even though complete remission had been achieved. In 6 patients, no follow up or germline material was available.

Regarding cytogenetics, the patients did not differ from single *NPM1* - or single *RUNX1* mutated cases.[2,3] In detail, 8 patients were cytogenetically normal, one patient had trisomy 8, one case showed loss of a sex chromosome, and one patient had a translocation t(5;12)(q33;p13).

RUNX1 mutation loads ranged between 3% and 48%. Interestingly, all 3 assured somatic mutations had a very low mutation load of less than 10%. In contrast, the *NPM1* mutation load ranged between 30% and 50%. To analyze the disease-causing potential of *RUNX1* muta-

Table 1. Clinical and molecular characteristics of primary AML patients with co-existing *NPM1* and *RUNX1* mutations[#].

Patient N.	Gender	Age	Karyotype	*NPM1* mutation subtype	*NPM1* allele change	*RUNX1* amino acid change	*RUNX1* allele change[#]	*RUNX1* mutation load (%)	Type of mutation	Mutation taster	COSMIC
1	M	62	46,XY	A	c.860_863dupTCTG	p.Arg293*	c.877C>T	41	no follow up	disease causing	mutation
2	M	63	46,XY,t(5;12)	A	c.860_863dupTCTG	p.Tyr328*	c.984C>G	5	unknown**	disease causing	no entry
3	M	76	47,XY,+8	A	c.860_863dupTCTG	p.Phe194Leufs*7	c.579dupC	48	no follow up	disease causing	no entry
4	F	51	45,X,-X	A	c.860_863dupTCTG	p.Lys83Arg	c.248A>G	3	somatic	disease causing	mutation
5	M	42	46,XY	I	c.863_864insCTTG	p.Ala297Val	c.890C>T	5	somatic	disease causing	no entry
6	M	68	46,XY	A	c.860_863dupTCTG	p.Phe326Ser	c.977T>C	2	no follow up	disease causing	no entry
7	F	76	46,XX	A	c.860_863dupTCTG	p.Lys144Asn	c.432A>T	47	unknown**	disease causing	no entry
8	F	73	46,XX	A	c.860_863dupTCTG	p.Phe326Ser	c.977T>C	3	no follow up	disease causing	no entry
9	M	66	46,XY	Trp290Leu	c.964G>T	p.Tyr113Leufs*4	c.337dupT	23	no follow up	disease causing	no entry
10	M	82	46,XY	A	c.860_863dupTCTG	Splice site mutation	c.886+1G>A	5	somatic		no entry
11	M	72	46,XY	A	c.860_863dupTCTG	p.Thr65Ala	c.193A>G	42	no follow-up	disease causing	no entry

***Patient did not reach CR. [#]RUNX1 mutations are numbered according to Ensemble cDNA sequence ENSG00000159216 transcript RUNX1-001(ENST00000344691).*

Table 2. Clinical and molecular characteristics of relapsed AML patients with co-existing *NPM1* and *RUNX1* mutations.[#]

Patient N.	Gender	Age	Karyotype	*NPM1* mutation subtype	*NPM1* allele change	*RUNX1* amino acid change	*RUNX1* allele change[#]	*RUNX1* mutation load (%)	Type of mutation	Mutation taster	COSMIC
12	M	47	46,XY	B	c.959insCATG	Splice site mutation	c.886+2_886+5delTAAG	4	somatic		no entry
13	M	65	46,XY,+8	D	c.959insCCTG	p.Glu429Phefs*144	c.1284_1285ins17	30	somatic**		no entry

[#]RUNX1 mutations are numbered according to Ensemble cDNA sequence ENSG00000159216 transcript RUNX1-001(ENST00000344691).

tions, all mutations were analyzed by PolyPhen prediction (*genetics.bwh.harvard.edu/pph2/*) and Mutation Taster (*mutationtaster.org*) algorithms and were identified as probably damaging to the protein function. We also subjected the detected *RUNX1* mutations to the catalog of somatic mutations in cancer (*COSMIC; cancer.sanger.ac.uk/cancergenome/projects/cosmic/*), an online tool for storage and display of somatic mutation information and related details, also containing information relating to human cancers. Two mutations had an entry in COSMIC (p.Lys83Arg and p.Arg293*), the others had not yet been described. However, as all *RUNX1* mutations except one involved functional domains of *RUNX1*, we suspect them to be disease-associated rather than polymorphisms.

Besides the 11 patients described above, our cohort also contained 2 patients with *NPM1* mutated *de novo* AML who gained a *RUNX1* mutation at relapse (Table 2), indicating that *RUNX1* mutations can be acquired during disease progression

Taken together, we were able to confirm the rare concomitance of *NPM1* and *RUNX1* mutations in *de novo* intermediate risk karyotype AML. However, we could not confirm that *RUNX1* mutations are always structurally unusual or germline in *NPM1* mutated cases. In fact, in our cohort, most of them were not structurally unusual as had been postulated by Mendler *et al.*[1] In our cohort, the majority of detected *RUNX1* mutations in NPM1 mutated cases were located in functional domains of *RUNX1*, the remaining cases had one mutation located downstream the RHD domain and two splice-site mutations. This pattern does not differ from mutation patterns reported for *RUNX1* mutations in *NPM1* wild-type cases.

Annette Fasan, Claudia Haferlach, Alexander Kohlmann, Frank Dicker, Christiane Eder, Wolfgang Kern, Torsten Haferlach, and Susanne Schnittger

MLL Munich Leukemia Laboratory, Munich, Germany

Correspondence: susanne.schnittger@mll.com

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References

1. Mendler JH, Maharry K, Becker H, Eisfeld AK, Senter L, Mrozek K, et al. In rare acute myeloid leukemia patients harboring both RUNX1 and NPM1 mutations, RUNX1 mutations are unusual in structure and present in the germline. Haematologica. 2013;98(8):e92-e94.
2. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood. 2011;117(8):2348-57.
3. Haferlach C, Mecucci C, Schnittger S, Kohlmann A, Mancini M, Cuneo A, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood. 2009;114(14):3024-32.