

## The Value of FLT3, NPM1 and DNMT3A Gene Mutation Analysis in Acute Myeloid Leukemia Diagnosis

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Myeloid neoplasms, especially acute myeloid leukemia (AML), are characterized by abnormal proliferation of hematopoietic cells and by genomic instability with a high diversity of chromosomal and molecular abnormalities (1,2). The 2016 revised World Health Organization classification of AML and myeloid neoplasm emphasized the value of molecular genetics testing in AML risk stratification and prognosis, especially for patients with a normal karyotype (3, 4). The gene mutation data are adding an important contribution to the immunophenotype and the clinical features in defining clinicopathological relevant disease entities (3,5). The investigation of the genome profile in AML has fundamentally changed the approach of AML patients. AML patients are routinely investigated for the presence of mutations in FMS-related tyrosine kinase 3 (*FLT3*) and *NPM1* genes. Recently some studies recommended also the DNA methyltransferase 3A (*DNMT3A*) gene mutation analysis at the AML diagnosis time (6-8). Roloff et al. sug-

gested that AML cases have on average three acquired mutations at the diagnostic although the European LeukemiaNet (ELN) 2017 prognostic model considers only one gene mutation in the most of AML cases and two in a few cases (9).

### ***FLT3* gene mutation**

*FLT3* mutations are common molecular abnormalities in AML (10) and have therefore proven to be a target for *FLT3* tyrosine kinase inhibitors (*FLT3* TKIs) enabling the personalized treatment of AML. *FLT3* internal tandem duplications (ITD) were reported in 30% of AML patients and the tyrosine kinase domain (TKD) point mutations detected in about 5% of AML cases. The prognostic significance of *FLT3* ITD is influenced by allelic ratio (*FLT3* ITD to *FLT3* wild-type). According to the 2017 ELN recommendations, a high allelic ratio is considered to be greater than 0.5 (*FLT3* ITD high; >0.5) while a low allelic ratio is considered less than

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0.5 (*FLT3* ITD low; <0.5) (11). Mutation within *FLT3* gene are common in AML cases with normal karyotype but they are also associated with cytogenetic aberrations, such as t(15;17)/*PML-RARA*, core binding factor-AML (*CBF-AML*) t(6;9) and DEK-NUP214 abnormalities (12). *FLT3* mutation testing is recommended in all AML patients in parallel with cytogenetic analyses as finding a *FLT3* gene mutation is a negative prognostic marker and Midostaurin, a *FLT3* TKI targeted therapy, is available (12). In addition, *FLT3* gene mutation may be used as a predictor for relapse in AML, had a negative effect on survival time (13), and is frequent in older patients (14). Considering the fact that *FLT3*-ITD is acquired late in leukemogenesis and that *FLT3*-ITD mutation may be lost at the time of relapse, it is not recommended to use *FLT3* mutation as a marker for minimal residual disease (MRD) monitoring (9).

### ***NPM1* gene mutation**

*NPM1* mutations are the commonest AML mutations identified up to now in adult patients, occurring in about 30% of cases, including half of cytogenetically normal AML patients and it is associated with a good prognosis in the absence of *FLT3* ITD mutation (14, 15). *NPM1* mutations were almost always found in association with co-occurrence mutations and reported usually as late driver secondary events (8). Papaemmanuil et al. reported that the most frequently observed co-mutations in AML patients with a *NPM1* mutation were *DNMT3A* (54%), *FLT3* ITD (39%), *NRAS* (19%), *TET2* (16%) and *PTPN11* (15%) (8). The same study observed that co-occurrence of mutations in *NPM1*, *FLT3* ITD, and *DNMT3A* predicted an especially adverse prognosis of the young AML patients (8). Recently, the study performed by Patel et al indicated that a high *NPM1* mutant allele at diagnosis in *de novo* AML is an independent predictor

of unfavorable clinical outcomes, particularly in patients treated with stem-cell transplant and in patients with *DNMT3A* co-mutation (16). The prognostic effect of *NPM1* mutation in *de novo* AML may be influenced by the mutated allele burden (16). Other studies reported also that co-occurrence of *NPM1* mutations with other common gene mutations in AML patients is associated with a poor prognosis (17). *NPM1* mutations are considered to be mutually exclusive with other genomic rearrangements and/or chromosomal aneuploidy in AML patients. It is estimated that 75% of *NPM1*-mutated AML associate mutations in DNA hydroxymethylation genes [such as *DNMT3A*, Ten-Eleven Translocation 2 (*TET2*), isocitrate dehydrogenase genes *IDH1* and *IDH2*)] (18).

Furthermore, *NPM1* gene mutation has been validated as a good marker for assessment of minimal residual disease and as a predictor for AML relapse (9).

### ***DNMT3A* gene mutation**

*DNMT3A* gene mutation are found in about 25%-30% of all AML cases, the most of them are localized in R882 domain and are more frequent in older AML cases. Mutations in *DNMT3A* gene are associated with poor prognosis and with a higher relapse rate (4). Recently it was reported that in the absence of high-risk cytogenetics, *DNMT3A* mutation status has a negative impact on prognostic outcome in the presence or absence of *FLT3* and/or *NPM1* mutation (6, 14). *DNMT3A* mutation is an important predictor of shorter overall survival (OS) in patients diagnosed with AML, especially in those with normal karyotype or intermediate-risk cytogenetics. The OS of the AML cases with *DNMT3A* mutation was reported by Kumar et al. to be poorer than that of AML patients with *NPM1* mutation but was better than that of the AML cases with *FLT3* ITD mutation (6). About 80% of AML

patients harboring *DNMT3A* mutation had also *NPM1* mutation (19).

A recent study revealed that R882 mutations in *DNMT3A* gene were associated with an increased number of white blood cells (WBC) and a higher percentage of blasts at the moment of diagnosis, with M4-M5 AML subtype according to French-American-British (FAB) classification and with normal karyotype (20). The same study observed that the mutation burden decreased after allogeneic hematopoietic stem cell transplantation (alloHSCT) and suggested it as the optimal therapy choice for the eradication of *DNMT3A* R882 mutation in AML cases (20). Recently it was observed that *DNMT3A* is one of the most frequently commutated gene with isocitrate dehydrogenase-2 (*IDH2*) in relapsed or refractory AML cases (21).

### ***FLT3* ITD mutation in association with *NPM1* mutation**

The relapse rate and overall survival in AML patients with *FLT3* ITD mutation is influenced by the ITD allelic ratio. It was reported that patients with *NPM1* mutation and *FLT3* ITD mutation but with a low allelic ratio (<0.5) have a similar outcome (favorable prognosis) as patients with a *NPM1* mutation but without *FLT3* ITD; therefore, both groups are now considered favorable (and added to favorable risk group according to ELN 2017 recommendations) (11). Contrariwise, AML with wild-type *NPM1* and *FLT3* ITD with a high allelic ratio (>0.5) has an adverse prognosis and is included in the adverse-risk group (11).

### ***FLT3* ITD mutation in association with *DNMT3A* R882**

Recently, it was reported by Tang et al. that AML patients with *FLT3* ITD and *DNMT3A* R882 double mutation had a poor prognosis, and that *FLT3* ITD+ and *DNMT3A* R882+ double muta-

tion was an independent factor for poor outcome post-transplantation (7).

Another recent study performed by Ardestani et al. revealed that patients with both *DNMT3A* R882 and *FLT3* ITD mutations had the worst OS and relapse-free survival compared with AML cases with one mutation (22). The OS at 5-year was 0% for AML patients with *FLT3* ITD + *DNMT3A* R882+ double mutation versus those with no *DNMT3A* R882 or *FLT3* ITD mutation (62%). Based on their findings, presence of *FLT3* ITD+ and *DNMT3A* R882+ double mutations represent an unfavorable prognostic factor in AML patients even after allogeneic hematopoietic stem cell transplantation (22). Recently it was reported that *FLT3* ITD+ and *DNMT3A* R882+ double mutation is significantly associated with a lower OS (P=0.016) and it has a significant negative effect on complete remission rates (14).

### ***NPM1*, *DNMT3A* and *FLT3* combined mutations**

*NPM1*, *DNMT3A*, and *FLT3* ITD combined mutated genotype represented the most frequent three-gene co-occurrence in a representative cohort consisting of 1540 AML patients identified in 6% of the investigated cases and that co-occurrence of *NPM1*, *FLT3* ITD, and *DNMT3A* mutations predict a particularly adverse prognosis (8). The same study noticed that deleterious effect of *FLT3* ITD was most clinically important in cases with concomitant *DNMT3A* and *NPM1* mutations (8). The frequency of triple *NPM1*, *DNMT3A* and *FLT3*-ITD combined mutations was lower in AML patients from Egypt (1.6%) (14) than that reported by Papammanuil et al (8) for AML cases included in three multicenter clinical trials of the German–Austrian AML Study Group. Presence of *NPM1*, *DNMT3A* and *FLT3* ITD triple combined mutations was associated with a short survival in

Egyptian AML patents (14).

Taking into account that there are few data regarding the *NPM1*, *DNMT3A*, and *FLT3* combined mutations in AML additional mutation studies are necessary, to determine the appropriate prognosis value of concomitant mutations in AML, especially those focused on investigation of all three genes. Recently, a rare AML case with concomitant four somatic mutations [(namely *FLT3* ITD, *FLT3* D835 (also known as c.2504A>T, D835V), *DNMT3A* R882C, and *NPM1* c.863\_864insTCTG)] was reported to be associated with an adverse prognostic and a very short survival (23).

**In summary**, reviewing the published data, it is not recommended to use *FLT3* mutation as a marker for minimal residual disease (MRD) monitoring. *FLT3* ITD+ and *DNMT3A* R882+ double mutations represent an unfavorable prognostic factor in AML patients even after allogeneic hematopoietic stem cell transplantation while *DNMT3A* and *IDH2* mutations frequently co-occured in relapsed or refractory AML cases. The evaluation of *FLT3*, *NPM1* and *DNMT3A* mutational status and routine quantification of mutational burden at diagnosis is therefore an important factor in predicting AML patient outcome. This comprehensive approach will provide data for diagnosis and prognostic significance and will also offer the potential for personalized treatment.

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### Conflict of interest

None to declare

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