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Somatic mutations of isocitrate dehydrogenases 1 and 2 are prognostic and follow-up markers in patients with acute myeloid leukaemia with normal karyotype

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Background. Mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) genes are frequent molecular lesions in acute myeloid leukaemia with normal karyotype (AML-NK). The effects of *IDH* mutations on clinical features and treatment outcome in AML-NK have been widely investigated, but only a few studies monitored these mutations during follow-up.

Patients and methods. In our study samples from 110 adult *de novo* AML-NK were studied for the presence of *IDH1* and *IDH2* mutations, their associations with other prognostic markers and disease outcome. We also analyzed the stability of these mutations during the course of the disease in complete remission (CR) and relapse.

Results. *IDH* mutations were found in 25 (23%) patients. IDH^+ patients tend to have lower CR rate compared to IDH^- patients (44% *vs* 62.2%, p = 0.152), and had slightly lower disease free survival (12 months *vs* 17 months; p = 0.091). On the other hand, the presence of *IDH* mutations had significant impact on overall survival (2 *vs* 7 months; p = 0.039). The stability of *IDH* mutations were studied sequentially in 19 IDH^+ patients. All of them lost the mutation in CR, and the same *IDH* mutations were detected in relapsed samples.

Conclusions. Our study shows that the presence of *IDH* mutations confer an adverse effect in AML-NK patients, which in combination with other molecular markers can lead to an improved risk stratification and better treatment. Also, *IDH* mutations are very stable during the course of the disease and can be potentially used as markers for minimal residual disease detection.

Key words: *IDH1* mutations; *IDH2* mutations; acute myeloid leukaemia; normal karyotype

Introduction

Patients with acute myeloid leukaemia with normal karyotype (AML-NK) comprise 40-50% of all AML patients.[1] They are characterized by high heterogeneity in terms of clinical features, biological characteristics and response to treatment. Nevertheless, all of the AML-NK patients are categorized into intermediate risk group. The need for more precise risk stratification of such cases led to the discovery of numerous new molecular markers. Some of them, such as mutations in fms-related

tyrosine kinase-3 (*FLT3*), nucleophosmin (*NPM1)* and CCAAT/enhancer binding protein alpha (*CEBPA*) genes have made an impact on prognosis of AML-NK patients. Those mutations have been already included in the revised version of World Health Organisation classification of leukaemia.[2] This new classification implies that all AML-NK patients with mutated *NPM1* without *FLT3-* (internal tandem duplication) *ITD* and mutated *CEBPA* have favourable genotype.

Mardis *et al.* have reported the entire genome sequence of leukemic cells from a single *de novo* AML-NK patient and compared it with the genome sequence from normal skin cells of the same patient.[3] After that, from the number of possible somatic mutations, only a handfull of genes were recurrently mutated in multiple AML genomes, including mutations in the genes for isocitrate dehydrogenase 1 (*IDH1*) and isocitrate dehydrogenase 2 (*IDH2*).

The *IDH1* and *IDH2* genes, located at chromosome bands 2q33.3 and 15q26.1 respectively, encode NADPH (reduced nicotinamide adenine dinucleotide phosphate) - dependent isocitrate dehydrogenase 1/2 enzymes, whose main role is to protect cells from oxidative stress.[4]

Heterozygous point mutations in *IDH1* and *IDH2* genes most likely affect the evolutionarily conserved arginine at position R132 in exon 4 of *IDH1 (IDH*^{R132}) and either the homologous position R172 (*IDH*^{R172}) or the second arginine R140 in the *IDH2* gene (*IDH*^{R140}).[5]

IDH1 and *IDH2* mutations occur in approximately 20% of AML-NK cases.[6-11] Clinical characteristics commonly found in these patients compared to those with wild-type *IDH* are older age, higher platelet counts and concurrent presence of *NPM1* mutations.[5,6,8,9,11,18] The relatively high incidence of *IDH* mutations and their association with the most commonly detected mutations in AML patients (*NPM1* mutations) indicates possible mutual interactions in the pathogenesis of the disease.[19,22]

Despite the results of numerous studies investigating the effect of the presence of *IDH* mutations on clinical outcome, the prognostic significance of these mutations remains controversial.[11] A number of studies showed that the presence of these mutations have no effect in response to therapy and survival[5,14-16], while there are others that suggest a negative prognostic effect.[8-10,17-20] Nevertheless, most studies agree with the fact that *IDH* mutations have adverse prognostic impact in the so called low-risk group of patients (*NPM1*$^{+}$/*FLT3-ITD*$^{-}$ AML-NK patients).[8-10,13,20,21]

Some studies investigated the potential of *IDH* mutations as a follow-up markers.[13,16,22-24] *IDH1* and *IDH2* mutations are relatively stable and show direct correlation with disease status. Thus, *IDH* mutations could be useful markers for monitoring disease, including treatment response, minimal residual disease (MRD), and early relapse.

The purpose of our study was to analyze the frequency of mutations in *IDH1/2* genes and their potential associations with other prognostic markers and outcome in 110 adult *de novo* AML-NK patients. We also analyzed the stability of these mutations during the course of the disease in complete remission (CR) and relapse.

Patients and methods

Patients

From 2009-2014, pre-treatment bone marrow (BM) samples from 110 consecutive consenting patients with *de novo* AML-NK were analysed at the Clinic for Hematology. This study was approved by the by the Ethics Committee of the Clinical Centre of Serbia, Belgrade, Serbia. Written informed consent was obtained for all patients. Diagnostic procedures comprised cytomorphology, cytogenetics, molecular genetics and immunophenotyping of BM. Morphologic diagnosis was made according to the French–American–British classification.[25] Conventional G-band karyotyping was employed for cytogenetic analysis.[26] Immunophenotyping by flow cytometry was performed using the direct multicolour immunofluorescent technique applied to whole BM specimens.[2] A WBC count ≥ $30x10^9$/L was considered as leukocytosis. Organ dysfunctions, as well as non-disease mortality risk were estimated by the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI).[27] Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale.[28] All patients < 60 years of age were treated with standard "3+7" induction chemotherapy, consisting of daunorubicin at a daily dose of 60 mg/m^2 on days 1–3, in combination with cytarabine at 200 mg/m^2 daily as a continuous intravenous infusion for 7 days. Patients > 60 years old were treated with reduced doses in the same regimen. Patients who achieved CR after induction chemotherapy received three cycles of consolidation chemotherapy: cytarabine 3 g/m^2 per q12h on days 1, 3 and 5 for those younger than 60 years and cytarabine 0.5-1g/m^{2}per q12h on days 1, 3 and 5 for those older than 60 years. Patients aged ≤55 years under-

went allogeneic stem cell transplantation (SCT), in total 15 (25.42%) patients. Definitions of CR, overall survival (OS), disease free survival (DFS) and early death (ED) were established by proposed criteria.[29]

Molecular analyses

BM samples collected at diagnosis, in CR (after induction therapy and after consolidation) and at relapse were analysed. Mononuclear cells were separated by Ficoll density gradient centrifugation and cryopreserved until mutational analyses. Genomic *DNA* was extracted from the mononuclear cells using a QIAamp Blood Mini Kit (Qiagene, Germany) according to the manufacturer's protocol. *DNA* fragments spanning exons 4 of the *IDH1* and *IDH2* genes were amplified by polymerase chain reaction (PCR) as described before.[24] PCR reaction products were further subjected to direct sequencing, and the resulting sequences compared to wild-type *IDH1* and *IDH2 cDNA* (GenBank Accession numbers NM_005896.2 and NM_002168.2, respectively). Mutational analyses of *FLT3* and *NPM1* gene mutations were performed as previously reported.[30-32] We investigated the impact of *IDH* mutations on OS in AML-NK patients in relation to three different risk groups defined by *FLT3* and *NPM1* mutation status (*favourable* risk-*NPM1*$^+$/*FLT3-ITD*$^-$; *intermediate-NPM1*$^-$/*FLT3*-ITD$^-$; *unfavorable-FLT3-ITD*$^+$), according to the recommendation of European Leukaemia Net.[1]

Statistical analysis

Differences in continuous variables were analysed using the Mann-Whitney *U* test for distribution between two groups. Frequencies were analysed using the Pearson χ^2 test for 2x2 tables or the Fisher exact test for larger tables. Survival probabilities were estimated by the Kaplan-Meier method, and differences in survival distributions were evaluated using the Log rank test. Patients undergoing allogeneic SCT were censored at the time of transplantation. Multivariate logistic regression model was applied to analyse factors related to the probability of CR failure. Cox's regression model was applied to determine the association of *NPM1* mutations with OS and DFS with adjustment for other factors. The statistical analyses were performed using SPSS computer software 15.0 (Chicago, IL, USA). For all analyses, the probability (p) values were 2-tailed and $p < 0.05$ was considered statistically significant.

TABLE 1. Type of *IDH1* and *IDH2* mutations identified in 110 AML-NK patients

Mutation	Nucleotide change	Amino acid change	No. of patients
IDH1			
	c.394C>T	R132C	4
	c.395G>A	R132H	2
	c.394C>G	R132G	1
	c.394C>A	R132S	1
IDH2			
	c.419G>A	R140Q	12
	c.418C>T	R140W	1
	c.419G>T	R140L	1
	c.418C>G	R140G	1
	c.515G>A	R172K	2

C = cysteine; G = glycine; H = histidine; K = lysine; L = leucine; S = serine. Q = glutamine

IDH1 and *IDH2* nucleotide numbering based upon the NCBI sequence NM_005896.2 and NM_002168.2, respectively.

Results

Frequency of IDH1 and IDH2 mutations in AML-NK patients

Among the 110 AML-NK patients, 25 (23%) harboured missense mutations in *IDH* genes. Eight (7%) patients had *IDH1* mutations, all of them *IDH*^{R132}. Seventeen (16%) patients had *IDH2* mutations: fifteen *IDH*^{R140} and two *IDH*^{R172} (Table 1). The wild-type allele was retained in all *IDH* positive samples, and no patient had both *IDH1* and *IDH2* mutations. As *IDH1* and *IDH2* mutations were mutually exclusive and appear to have the same functions, we examined the clinical significance these mutations as a collective group as previously reported.[11]

Association of IDH mutations with clinical characteristics and other molecular markers

Pre-treatment clinical characteristics of the patients are summarized in Table 2. Their mean age was 54 years (range 19–78), while 31 (31.8%) patients were ≥ 60 years of age. There were 62 (56.4%) men and 48 (43.6%) women. Distribution of *IDH*$^+$ patients across FAB groups was uneven, being most frequent in the M2 group - nine (29%) patients, followed by six (27.3%) in the M1 and five (21%) in the M4 group. *IDH*$^+$ patients had higher platelet counts (p = 0.024), as well as a higher percentage of pe-

TABLE 2. Clinical characteristics of patients with *de novo* AML-NK stratified by the presence or absence of *IDH* mutations

Parameter	Total (n = 110)	*IDH*$^+$ (n = 25)	*IDH*$^-$ (n = 85)	*p* value
Sex				**0.617**
Male (%)	62 (56.4)	13 (21)	49 (79)	
Female (%)	48 (43.6)	12 (25)	36 (75)	
Age, years, median (range)	53.5(19-78)	50(23-73)	54(19-78)	0.783
ECOG ≥2				0.081
Yes No	45(40.9) 65(59.1)	14(31.1) 11(16.9)	31 (68.9) 54 (83.1)	
HCT-CI ≥3				**0.300**
Yes No	8(7.3) 102(92.7)	3 (37.5) 22(21.6)	5 (62.5) 80(78.4)	
WBC count, x10^9/l (range)	16.8 (0.5-195)	6.9 (0.5-160)	17.4 (0.8-195)	0.373
Haemoglobin median, range	95.5 (6-178)	100 (57-178)	94 (6-140)	0.810
Platelets (x10^9/L) median, range	68 (1-420)	109(16-193)	56 (1-420)	0.024
LDH (U/L) median, range	917 (273-7180)	901 (315-5105)	922.5 (273-7180)	0.825
Peripheral blood blast (%)	26 (0-96)	60.5 (0-96)	21 (0-96)	0.031
Bone marrow blasts (%)	71 (23-97)	67 (33-97)	73 (23-97)	0.920
FAB (%)				0.139
M0	10	4 (40)	6 (60)	
M1	22	6 (27.3)	16(72.7)	
M2	31	9 (29)	22 (71)	
M4	24	5 (21)	19 (79)	
M5	22	1 (0.05)	21 (95.5)	
M6	1	0 (0.0)	1 (100.0)	
***FLT3*-ITD**				0.626
present (%)	26(23.6)	5 (19.2)	21 (80.8)	
absent (%)	84(76.4)	20 (23.8)	64 (76.2)	
***FLT3*-D835**				0.428
present (%)	9	3 (33.3)	6 (66.7)	
absent (%)	101	22 (21.8)	79 (78.2)	
NPM1				0.496
present (%)	42(38.2)	11(26.2)	31(73.8)	
absent (%)	68(61.8)	14(20.6)	54(79.4)	

ECOG = performance status of the Eastern Cooperative Oncology Group; FAB = French-American-British classification; *FLT3-ITD* = *FLT3* internal tandem duplication; HCT-CI = hematopoietic cell transplantation-comorbidity index; IDH = isocitrate dehydrogenase; LDH = lactate dehydrogenase; *NPM1* = nucleophosmin; WBC = white blood cell count

ripheral blood (PB) blasts (p = 0.031) compared to *IDH*$^-$ patients. There were no differences between *IDH*$^+$ and *IDH*$^-$ patients regarding age, sex, WBC count, BM blast percentage, haemoglobin and serum LDH level.

IDH mutations occurred evenly in *NPM1*$^+$ and *NPM1*$^-$ patients (26.2% *vs* 20.6%, p = 0.496). Moreover, *IDH* mutations were not associated with *FLT3-ITD* mutations: 19.2% *vs* 23.8% (p = 0.626).

Response to induction therapy and prognostic relevance of IDH mutations

Out of the 85 *IDH*$^-$ patients, 51 (62.2%) achieved CR, while 11/25 (44%) of the *IDH*$^+$ patients achieved CR. The difference was not statistically significant (p = 0.152). The presence of *IDH* mutations was not associated with ED (*IDH*$^+$-36% *vs* *IDH*$^-$- 24.7%; p = 0.310), too. Overall 36/110 (32.7%) participants

exhibited disease relapse, 6 (24%) *IDH*$^+$ and 30 (35.3%) *IDH*$^-$patients. The impact of *IDH* mutations on DFS failed to reach statistical significance (*IDH*$^+$- 12 months *vs* *IDH*$^-$- 17 months; p = 0.266). In contrast, OS was significantly impaired in the presence of *IDH* mutations (*IDH*$^+$-2 months vs *IDH*$^-$-7 months; p = 0.039) (Figure 1).

In the univariate analysis, leukocytosis (p = 0.016) was found to be significantly correlated with a poor rate of CR. The most important factor associated with poor CR rate in the multivariate analysis was leukocytosis (p = 0.015, RR 0.34, 95% CI 0,143-0,809). Univariate analysis showed that significant factors for poor DFS were *FLT3-ITD* positivity (p = 0.03) and *NPM1* positivity (p = 0.032). The most significant risk factor for DFS using the multivariate method was *FLT3-ITD* positivity (p = 0.030, RR = 2.465, 95% CI 1.089-5.579). Univariate COX proportional regression analysis indicated that the following tested features were significant predictors of poor OS: age ≥ 55 years (p = 0.023), leukocytosis (p = 0.001) and *IDH* positivity (p = 0.039). The multivariate COX proportional regression method pointed to leukocytosis (p = 0.001, RR = 1.768, 95% CI 1.084–2.883) as the most significant predictor of poor OS.

In our study, patients aged 55 years or less received conventional or reduced intensity allogeneic SCT. OS rate in *IDH*$^+$ patients not given allogeneic SCT was markedly lower than that in *IDH*$^+$ patients who received it (2 *vs* 15 months; p = 0.006) (Figure 2). Conversely, among patients who did receive allogeneic SCT, the difference in OS rates between those with or without *IDH* mutations was not significant (p = 0.07).

We found that the presence of *IDH*$^+$ had a negative impact on OS in the *intermediate* risk subgroup (5 *vs* 12 months; p = 0.050) (Figure 3). However, *IDH* mutations did not affect OS in the *favourable* and *unfavourable* subgroups (1 *vs* 3 months, p = 0.668; 1 *vs* 7 months, p = 0.114, respectively).

Sequential studies of IDH mutation

The *IDH* mutational status was serially studied in relapsed samples of *IDH*$^-$ patients and in follow-up and/or relapsed samples in *IDH*$^+$ patients. None of the available relapsed samples of *IDH*$^-$ patients acquired *IDH* mutations. Among the nineteen *IDH*$^+$ cases who were alive after induction, eleven (44%) achieved CR. Nine of them lost *IDH* mutations after induction therapy but two patients retained it. One of them achieved CR after the first induction therapy. He lost *FLT3-D835* and *NPM1* positive status, but remained *IDH2*$^+$ positive and died

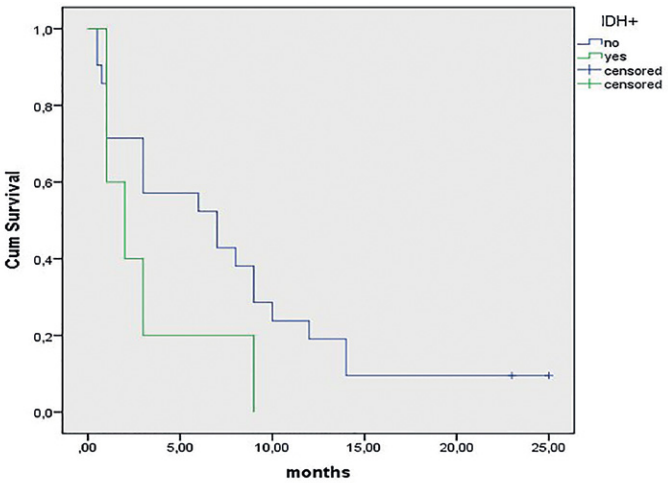

FIGURE 1. Impact of *IDH* mutation on overall survival (p = 0.039 by Kaplan-Meier method).

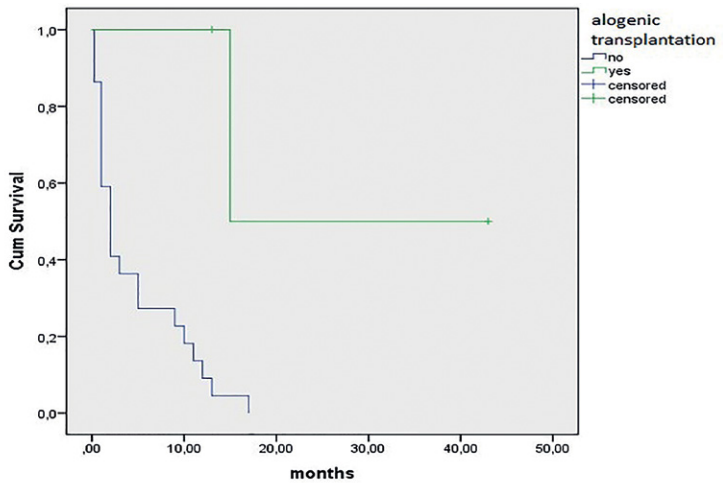

FIGURE 2. Overall survival associated with *IDH* mutations and allogeneic stem cell transplantation in AML-NK patients (p = 0.006 by Kaplan-Meier method).

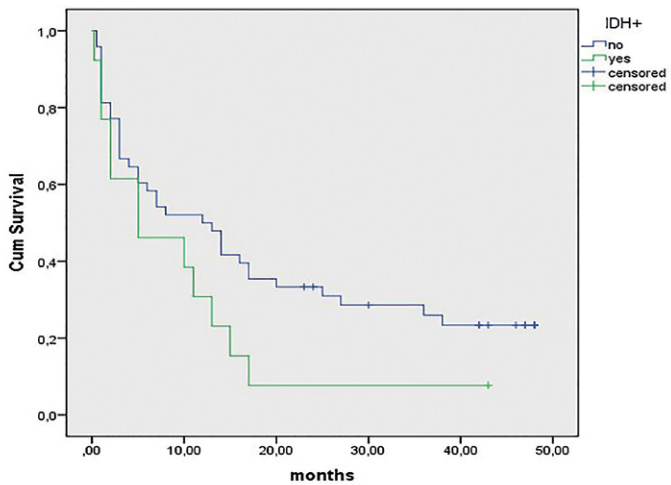

FIGURE 3. Comparison of the overall survival in intermediate group (*NPM1*$^-$/*FLT3*$^-$) between *IDH*$^+$ and *IDH*$^-$ patients (p = 0.050 by Kaplan–Meier method).

TABLE 3. Results of sequential studies of *IDH*+ patients

Patient ID	Age/ sex	*IDH/FLT3/NPM* status on diagnosis	Disease status after induction	*IDH/FLT3/ NPM* status after induction	Disease status after consolidation therapy	*IDH/FLT3/NPM* status after consolidation	Relapse	*IDH/FLT3/NPM* status in relapse
245	53/F	*IDH2*^{R140Q}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	/
275	50/F	*IDH2*^{R172K}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	*IDH2*^{R172K}*/ FLT3-D835/wt*
280	61/F	*IDH1*^{R132H}*/ FLT3-ITD/Type A*	RD	/	*RD*	/	/	/
291	38/F	*IDH2*^{R140Q} */FLT3-ITD/Type A*	RD	/	RD	/	/	/
305	47/M	*IDH2*^{R140Q}*wt/wt*	CR	*wt /wt/wt*	CR	/	yes	/
320	40/M	*IDH2*^{R140W}*wt/wt*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
349	39/M	*IDH2*^{R140L}*/wt/wt*	CR	*IDH2*^{R140L} */ wt/wt*	*CR*	*wt /wt/wt*	/	/
378	66/M	*IDH1*^{R132H} */wt/Type A*	ED	/	/	/	/	/
380	44/F	*IDH1*^{R132C}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	*IDH1*^{R132C}*/wt/wt*
393	54/M	*IDH2*^{R132C}*/ FLT3-D835/wt*	CR	*wt /wt/wt*	CR	/	yes	/
399	23/F	*IDH2*^{R140Q}*/wt/Type A*	ED	/	/	/	/	/
401	69/M	*IDH2*^{R140Q}*/ FLT3-ITD/wt*	RD	/	/	/	/	/
403	73/F	*IDH1*^{R132C}*/wt/wt*	RD	/	/	/	/	/
412	46/M	*IDH2*^{R140Q}*/wt/Type A*	RD	/	/	/	/	/
418	62/F	*IDH1*^{R132G}*/FLT3-ITD/Type A*	CR	*wt /wt/wt*	CR	/	/	/
423	43/M	*IDH2*^{R140Q}*/wt/wt*	ED	/	/	/	/	/
426	56/M	*IDH2*^{R172K}*/wt/wt*	ED	/	/	/	/	/
469	63/M	*IDH1*^{R132C}*/FLT3-D835/ Type A*	CR	*IDH1*^{R132C}*/ wt/wt*	CR	/	yes	/
487	73/M	*IDH2*^{R140Q}*/wt/wt*	RD	*IDH2*^{R140Q}*/ wt/wt*	RD	/	/	/
556	50/M	*IDH2*^{R140Q}*/wt/Type A*	ED	/	/	/	/	/
612	30/M	*IDH2*^{R140Q}*/wt/wt*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
615	40/F	*IDH2*^{R140Q}*/wt/Type A*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
645	43/F	*IDH2*^{R140G}*/ FLT3-ITD/Type A*	RD	/	/	/	/	/
672	33/F	*IDH1*^{R132S}*/wt/Type A*	RD	/	/	/	/	/
680	67/M	*IDH2*^{R140Q}*/FLT3-D835/wt*	ED	/	/	/	/	/

CR = complete remission; ED = early death; RF = refractory disease; wt = wild type

in relapse of disease (patient ID 469). The second one (patient ID 349) achieved CR after of induction therapy but retained *IDH* mutation. The mutation was lost in sequential follow-up sample, but patient died during the consolidation therapy in cyto-morphological remission with bone marrow aplasia from the septic shock (Table 3). Patient with refractory disease (patient ID 487) two months after the beginning of therapy remained *IDH2* positive. Two patients, who lost their *IDH* mutation in CR, regained it in relapse. Two of the nine patients who achieved molecular remission were treated with allogeneic SCT and are still alive. Remaining 7 patients died during therapy and after disease relapse. These results indicate stability of *IDH* mutations during the course of AML.

Discussion

The frequency of *IDH* mutations in patients with AML is 6-19%, but 12-33% in those with AML-

NK.[5,8,17,18,20,33] In our study on adult AML-NK patients, *IDH* mutations were detected in 23% of them. The prevalence of *IDH2* over *IDH1* mutations observed here (15.5% *vs* 7%) was similar to other published results.[5,6,8,22]

Our patients with *IDH* mutations had higher platelet counts and a higher percentage of PB blasts than those without such mutations, which confirms previous findings.[5,6,8,11] We detected *IDH* mutations most frequently in M2 type cases, followed by M1 (36% and 24%, respectively) and M4 type, which is in according with other results.[13,15,19]

Examining correlations between *IDH* mutations and other common genetic alterations in AML, such as *NPM1* and *FLT3* mutations, we found a slight but non-significant prevalence of *NPM1*$^+$ among *IDH*$^+$ patients (*NPM1*$^+$- 26.2% *vs* *NPM1*$^-$- 20.6%; p = 0.496). This is not in line with previous reports.[6, 8 ,9,11,18] The *FLT3* mutations were almost equally distributed between *IDH*$^+$ and *IDH*$^-$ groups of patients, which is in concordance with other studies.[5,7,18]

The prognostic impact of *IDH* mutation is controversial. Most studies have shown that both *IDH1* and *IDH2* mutations confer an unfavourable prognosis in AML-NK, *i.e*. a higher risk of disease relapse and shorter OS.[3,6-8,11,16,17,20] In our study, CR rate was 62.2% in *IDH*$^-$ patients, while in *IDH*$^+$ patients it was somewhat lower (45.8%), but without statistical significance. A similar finding was reported by Nomdedeu *et al*.[17], where the CR rate of *IDH*$^-$ patients was 80% and 63% in *IDH*$^+$ (p = 0.086).

We were able to demonstrate that *IDH* mutations act as an adverse prognostic marker of OS in AML-NK patients. That is, patients with *IDH* mutations had significantly worse OS, with a tendency for shorter DFS. This also confirmed earlier findings.[6-8,11,16,17,20] Among the *IDH*$^+$ patients, OS rate in those who received allogeneic SCT was significantly higher than that in those not given it. This was also observed by Yamaguchi *et al*.[11] and suggests that allogeneic SCT may improve OS in younger patients with *IDH* mutations.

The emergence of new molecular markers in AML-NK has contributed to a better and more precise classification of patients. This group is identified as an intermediate risk group, but because of its heterogeneity in terms of clinical outcome of the disease, more precise allocation is necessary. In addition to the *FLT3* and *NPM1* gene mutations that have already found significance as valuable prognostic factors, the detection of *IDH* mutations has contributed to refined risk classification of AML-NK patients.

When we applied molecular classification based on the presence/absence of *NPM1* and *FLT3* mutations in our cohort of patients, we observed that the presence of *IDH* mutations had an adverse impact on OS in the intermediate risk subgroup (*NPM1*$^-$/*FLT3-ITD*$^-$). This finding, already reported by others[11,16], argues in favour of testing for *IDH* mutations among AML-NK patients.

The frequent co-occurrence of *IDH* mutations with *NPM1* and less often with *FLT3* mutations, indicates that such mutations cooperate in the process of leukemogenesis. *IDH1* and *IDH2* are epigenetic modifier genes involved in DNA methylation and histone modification, and do not completely fit into our current definition of type-I and type-II aberrations, as suggested by the 2-hit theory of cancerogenesis.[34,35] Nevertheless, it has been suggested that *IDH* mutations are an early event in a variety of myeloidneoplasias like myelodisplastic syndrome and myeloproliferative neoplasms (MPN).[35,36] In patients with MPN, the acquisition of *IDH* mutations predicts an increased risk of progression to secondary AML, potentially serving as a marker for early stage transformation.[37-39] Also, the fact that *IDH* mutations are stable during the course of the disease supports the presumption that their emergence is an early event in malignant transformation.

Even though the prognostic significance of *IDH* mutations has been extensively studied, there are only few reports about their value in MRD monitoring. Thus, Gross *et al*.[40] and Jeziskova *et al*.[23] each presented four patients with *IDH1* and *IDH2* mutations, followed by the investigations of Chou *et al*.[15,21] In all three studies, as in our nine *IDH*$^+$ patients who were available for sequential analysis, the mutation was lost during CR and reappeared at relapse of the disease as the same type of mutation. Moreover, none of the patients acquired new *IDH* mutations during relapse.[15,21,23,40] In our study, we registered two *IDH*$^+$ patients retaining the mutation in CR and during the whole follow-up. Chou *et al*.[22] explained a similar finding through the hypothesis that *IDH* mutations are important in maintaining the leukaemia phenotype through cooperation with other oncogenic mutations, but alone are not sufficient for leukaemogenesis *in vivo*.

IDH1 and *IDH2* mutations have significant potential as MRD markers, assuming that the method applied meets the sensitivity criteria for MRD detection. The usual method for discovering *IDH* mutations is PCR-followed by direct sequencing, with a sensitivity of about 20%.[8,14,15,18] Based on this and the fact that *IDH* mutations are heterozygous,

the application of more sensitive methods, such as real-time PCR specific for a given mutation, should be considered for monitoring therapy response and early relapse.

In conclusion, acquired *IDH* mutations are common abnormalities in AML-NK. They confer an adverse effect, especially in patients lacking *NPM1* mutations. In combination with other molecular markers, *IDH* mutational status can lead to an improved risk stratification approach for AML-NK patients. Moreover, *IDH* mutations are stable during the course of the disease and can be potentially used as markers for MRD detection. This could be especially important if specific treatment with *IDH* inhibitors is introduced in everyday practice.

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