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Location and type of isocitrate dehydrogenase mutations influence clinical characteristics and disease outcome of acute myeloid leukemia

Magdalena Koszarska,[1] Andras Bors,[1] Angela Feczko,[1] Nóra Meggyesi,[1] Arpad Batai,[2] Judit Csomor,[2] Emma Adam,[2] Andras Kozma,[2] Tamas I. Orban[3] Nora Lovas,[2] Zoltan Matrai,[2] Andrea Sipos,[2] Eva Karaszi,[2] Janos Dolgos,[2] Sandor Fekete,[2] Judit Reichardt[2], Eniko Lehoczky[2], Tamas Masszi,[2] Attila Tordai,[1] and Hajnalka Andrikovics[1]

[1]Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, Budapest, Hungary, [2]Department of Hematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital, Budapest, Hungary;[3]Institute of Molecular Pharmacology, Research Centre of National Science, Hungarian Academy of Sciences, Budapest, Hungary

Correspondence: Magdalena Koszarska, Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, Budapest, Hungary, Karolina út 19-21, Budapest, 1113, Hungary.

Phone/FAX:+36-1-3724449/4448

e-mail: m.koszarska@yahoo.pl

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ABSTRACT

Background: Mutations of isocitrate dehydrogenase 1 and 2 are novel common genetic alterations identified in acute myeloid leukemia.

Aims: To investigate the frequency, clinical associations and prognostic effect of isocitrate dehydrogenase 1 and 2 mutations together, followed by a detailed investigation of particular mutations.

Methods: A consecutive cohort of 376 patients diagnosed with acute myeloid leukemia were enrolled to compare clinical characteristics. Prognostic impact was analyzed for 314 patients younger than 60 years treated with curative intention. Isocitrate dehydrogenase 1 and 2 mutations were screened using allele-specific PCR and high resolution melting, followed by a confirmatory sequencing.

Results*:* Isocitrate dehydrogenase (*IDH*) 1 and 2 mutations were mutually exclusive, detected in 8.5% and 7.5% of the cases respectively. Presence of mutations was associated with older age (p=0.001), higher platelet count (p=0.001), intermediate risk karyotype (p<0.0001), nucleophosmin1 mutation (p=0.022), and with lower mRNA expression level of *ABCG2* gene (p=0.006), as compared to mutation negative cases. Remission, relapse rates and overall survival were not different in *IDH*-mutation positive patients. Interestingly, particular mutations differred in association with nucleophosmin1 mutation: co-occurrence was observed in 14.3% of R132C vs. 70% of R132H carriers (p=0.02); and in 47.4% of R140Q vs. 0% R172K carriers (p=0.02) of *IDH1* and *IDH2* genes, respectively. R132H negatively influenced overall survival compared to isocitrate dehidrogenase 1 and 2 negative (p=0.02) or to R132C (p=0.019) patients.

Conclusions: IDH mutations are frequent recurrent mutations in acute myeloid leukemia. Although a general common pathogenetic role is proposed, our results indicate that

differences in clinical characteristics and treatment outcome may exist among disctinct mutations of both genes.

INTRODUCTION

Acute myeloid leukemia (AML) has a highly heterogeneous genetic background.(1) The number of known genetic alterations increases steadily and newly identified mutations may provide a deeper insight into the pathogenesis of AML.(2) Mutational profiling helps to improve risk stratification and to bring better founded therapeutic decisions.(3) *IDH1* somatic mutation (R132C) was initially described in colon cancer.(4) Later, *IDH1* mutations affecting codon R132 and *IDH2* mutations affecting codon R172 were also discovered in gliomas.(5, 6) Overlapping arrays of mutations occur in around 15% of all AML cases.(7)

Under normal circumstances, *IDH* enzymes catalyze the conversion of isocitrate to α–ketoglutarate (αKG). The presence of *IDH* mutant enzymes results in aberrant production of 2-hydroxyglutarate (2HG), the structural analogue and competitive inhibitor of αKG. The production of 2HG is a common neomorphic activity of all *IDH1* and 2 mutations resulting in the block of αKG dependent enzymes such as tet methylcytosine dioxygenases 1 and 2 (*TET1 and 2*), or histone demethylases causing aberrant DNA and histone methylation, altered gene expression profiles and consecutively impaired stem cell differentiation.(2, 8, 9) In line with the common pathogenic background, *IDH1* and *IDH2* mutation positive AML cases share several common clinical characteristics, including an older age of onset, higher platelet count, and association with intermediate cytogenetic risk.(10-23) However, distinctive differences between *IDH1* and *IDH2* mutations or even between particular *IDH2* substitutions (R140 and R172) have recently been reported to affect morphology, or to associate with nucleophosmin 1 (*NPM1*) mutation and treatment outcome.(14, 16)

In this study, we analyzed the impact of *IDH* mutations on clinical characteristics (age, AML etiology, morphology, hematological laboratory parameters, karyotype, molecular genetic alteration at presentation) and prognostic outcome (remission, relapse rates, overall

and disease free survival). Following the combined analysis, we investigated the role of the particular *IDH1* and *IDH2* substitutions separately in a Hungarian cohort of AML patients.

PATIENTS AND METHODS

Patients

Our cohort consisted of 376 consecutive AML patients [180 males/196 females; median age: 48.6 years (range: 16-93)]. The patients were diagnosed and treated at the Department of Hematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital (formerly National Medical Center) between 2001 and 2009. The minimal follow up was 12 months (maximum: 107 months). Clinical data were collected retrospectively. Complete remission, early death (less than 28 days after the start of therapy), resistant disease, disease-free survival (DFS) and overall survival (OS) were defined according to recommended criteria.(1) Immunophenotyping was performed by a panel of monoclonal antibodies (CD7, CD13, CD14, CD33, CD34, HLA-DR). Cytogenetic abnormalities, based on at least 20 cells in metaphase were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2005). Fms-like tyrosine kinase 3 (*FLT3*) internal tandem duplication (*ITD*) and nucleophosmin 1 (*NPM1*) mutations were analyzed by PCR followed by capillary gel electrophoresis. *ABCG2* mRNA expression in the bone marrow at diagnosis was tested by real-time quantitative PCR by pre-developed TaqMan Gene Expression Assay (Hs01053790_m1, Life Technologies, Carslbad, USA) using LightCycler 480. ABL was used as a reference gene.(24) Patients signed informed consents in agreement with the Regional Ethics Committee approval.

IDH1 and IDH2 mutation analysis

IDH1 and *IDH2* mutation analyses were performed on genomic DNA isolated from bone marrow samples at the time of diagnosis. Allele-specific PCR (AS-PCR) for *IDH1* R132

codon mutations was adapted from Chou et al.(25) A similar single-tube, multiplex AS-PCR method was developed for the simultaneous detection of *IDH2* R140 and R172 mutations. High resolution melting (HRM) analysis was performed using LightCycler 480 Real-Time PCR System. AS-PCR was used as a primary screening method in all AML patients, and HRM was performed in parallel in patients with normal karyotype and in patients with positive AS-PCR screen. Sequencing using Beckman Coulter CEQ 8000 Genetic Analyser was performed in cases of *IDH1* codon 132 mutantion detected by AS-PCR and HRM to determine the exact amino acid substitution.

Statistical analyses

Continuous variables are presented as median and range. Mann-Whitney or Kruskal-Wallis tests were used to compare continuous variables in subgroups according to *IDH1/2* mutations. Fischer's exact test and $\chi 2$ test were performed to compare dichotomous variables. Log-rank test was used to compare DFS and OS between groups separated by *IDH* mutation status. A Cox proportional hazards model was computed for multivariate analysis of OS and DFS with the calculation of hazard ratio (HR) and 95% confidence interval (95%CI). The statistics was performed using Statistical Package for the Social Science [(SPSS) version 13.0].

RESULTS

To characterize the frequencies of *IDH1* and *IDH2* mutations, we screened 376 AML patients by AS-PCR and HRM. 32 patients had a mutation in *IDH1* (8.5%) and 28 patients in *IDH2* (7.5%). *IDH1* and *IDH2* mutations were mutually exclusive. In *IDH1,* R132C (n=14, 43.8%) was the most frequent alteration, in addition, R132H (n=10, 31.3%), R132G (n=5, 15.6%), R132L (n=2, 6.2%) and R132S (n=1, 3.1%) were detected. In the *IDH2* gene, twenty R140Q (71.4%) and eight R172K (28.6%) substitutions were identified.

Clinical features of IDH mutation positive AML patients

First, we investigated *IDH1* and *IDH2* mutation positive patients combined (*IDH1/2*^{mut}), which was followed by the analysis of *IDH1*^{mut} and *IDH2*^{mut} subtypes separately (analyses for the entire AML group are shown in Table 1). A further stratification was performed for the separate analysis of *IDH1* R132C and R132H or *IDH2* R140Q and R172K mutations (separated analyses for the entire AML group are shown in Table 2). We compared *IDH* mutation positive to *IDH1/2* double negative patients (*IDH1/2*^{neg}).

In the entire AML group, *IDH1/2* mutations together, as well as *IDH1* and *IDH2* separately including R132C and R140Q subtypes presented at older age (medians and ranges are listed) [*IDH1*^{mut}: 54.5 (30-93 years); *IDH2*^{mut}: 56.5 (31-77 years) compared to *IDH1/2*^{neg} :49.0 (16-86 years); p=0.013 and p=0.009, respectively]. *IDH* mutations showed no associaton with sex, etiology of AML or white blood cell (WBC) count at diagnosis. Higher platelet count (PLT) was observed in *IDH1/2*^{mut}, *IDH1*^{mut} and *IDH2*^{mut} as well as in R132C, R132H and R140Q mutants [*IDH1*^{mut}: 75 (10-326 G/L); *IDH2*^{mut}: 72 (5-215 G/L); vs. *IDH1/2*^{neg}: 39 (5-684 G/L); p=0.039, p=0.005]. R172K was associated with a lower lactate dehydrogenase (LDH) level [R172K: 411 (217-1398 U/L) vs. *IDH1/2*^{neg}: 734 (136-15418 U/L), p=0.043]. No difference could be observed in morphological distribution according to FAB.

IDH1 and *IDH2* mutation positive patients had intermediate risk karyotype more frequently [*IDH1*^{mut}*:* 80.6%, *IDH2*^{mut}*:* 81.5% vs. *IDH1/2*^{neg}*:* 52.7%; p=0.004, p=0.004]. None of the *IDH* substitutions were preferentially associated with *FLT3* ITD. *IDH1/2* and *IDH1* positive patients had predominantly *NPM1* mutation (*IDH1*^{mut}*:* 42.0% vs. *IDH1/2*^{neg}*:* 23.2%; p=0.029), while *IDH2* was not associated with *NPM1* mutations (33.3%, p=0.24).

IDH1/2, *IDH2* as well as R132C and R140Q mutants showed lower mRNA expression of *ABCG2* gene at diagnosis (*ABCG2* transcript/*ABL* transcript %) [*IDH2*^{mut}*:* 0.51 (0.05-3.24%)

vs. *IDH1/2*^{neg}: 1.35 (0.02-18.05%); p=0.012]. *IDH1*^{mut} also showed a tendency toward lower expression [0.52 (0.01-23.01%); p=0.062]. On the other hand, *ABCG2* expression was significantly lower in *NPM1* mutation(?) positive as compared to *NPM1* negative AML samples [*NPM1*^{mut}: 0.035 (0.01-2.02%) vs. *NPM1*^{neg}: 1.028 (0.02-23.01%); p<0.001]. To test whether the association of *IDH* mutations with lower *ABCG2* expression was independent from *NPM1*, we divided patients into three groups according to their IDH1/2 and NPM1 mutational status (Figure 1). *ABCG2* mRNA expression was the highest in the double negative group [*IDH1/2*^{neg}/*NPM1*^{neg}: 1.87 (0.02-18.05%)] comparing to the single positive group [*IDH1/2*^{neg}/*NPM1*^{mut} and *IDH1/2*^{mut}/*NPM1*^{neg}: 0.62 (0.07-23.01%); p=0.013] and to the double positive group [*IDH1/2*^{mut}/*NPM1*^{mut}: 0.22 (0.01-1.61%); p<0.001).

Interestingly, marked differences in the clinical presentation could be observed between *IDH1* R132H and R132C mutant AML patients. R132H and R132C comparisons are noted with p* in Table 2, while p values reflect comparisons to *IDH1/2*^{neg}. R132H mutant AMLs were more likely to have de novo origin (90%), while R132C positive AMLs were secondary to MDS, or therapy related in 50% of cases (p=0.08). FAB M1 was more common in R132C (50% vs. 0%, p=0.02). PLT at diagnosis was higher in R132H (136 vs. 45 G/L; p=0.050). R132H mutated AML was more likely to associate with *NPM1* mutations/expression(?) than R132C (70 vs.14%; p=0.02). Several distinctive features were detected also between *IDH2* R140Q and R172K mutation carriers at diagnosis (comparisons are noted with p# in Table 2). R172K showed lower WBC (12.4 T/L vs. 22.0 T/L; p=0.028), a tendency to lower LDH level (411 U/L vs. 586 T/L; p=0.09) compared to R140Q carriers. R172K mutation was mutually exclusive with *NPM1* mutations(?) (0% vs. R140Q: 47.4%; p=0.02).

In the intermediate cytogenetic risk group consisting of 205 patients, 25 *IDH1* (12.2%) and 22 *IDH2* mutations (10.7%) were identified (Supplementary Tables 1 and 2). Similarly to the total AML group, *IDH* mutations occured more often at older age and were associated with

higher PLT count at diagnosis. R172K was associated with lower WBC value/count(?) and LDH level as compared not only to the R140Q but also to the *IDH1/2*^{neg} subgroup. R140Q positive tumors preferentially showed FAB M1 subtype [R140Q: 53.3% vs. *IDH1/2*^{neg}*:* 24.8%, p=0.03]. *ABCG2* mRNA expression level was lower at diagnosis in patients both with *IDH1* and *IDH2* compared to *IDH1/2*^{neg}. Similarly to the total AML group, we noticed a higher *ABCG2* mRNA level in the double/triple negative? *IDH1/2*^{neg}/*NPM1*^{neg} group compared to *IDH1/2*^{neg}/*NPM1*^{mut} or *IDH1/2*^{mut}/*NPM1*^{neg} single positive and *IDH1/2*^{mut}/*NPM1*^{mut} double positive subgroups (3.53 (0.15-18.05%) vs. 0.66 (0.07-2.02%); 0.35 (0.05-1.61%), p=0.002; 0.001 respectively). HLA-DR expression was lower in *IDH1* mutant patients [*IDH1*^{mut}: 36 (0-92%) vs. *IDH1/2*^{neg}: 45 (0-96%), p=0.041]. CD34 was significantly higher in R132C vs. R132H [38 (3-89%) vs. 4 (1-68%); p=0.03]. R172K cases were more likely to have an intermediate risk and abnormal karyotype than R140Q samples (28.6% vs. 80.0 %; p=0.052). *IDH1* R132H preferentially associated with *NPM1* mutations(?) (87.5%) as compared to *IDH1/2*^{neg} (38.9%; p=0.009) or to R132C cases (11.1%; p=0.003). *IDH2* R172K was mutually exclusive with *NPM1* mutations(?) (0% vs. IDH1/2^{neg}: 38.9%, R140Q: 53.3%; p=0.047, 0.022 respectively). *IDH1/2*^{mut} patients were less likely to carry FLT3 ITD mutations (*IDH1/2*^{mut}: 19.1% vs. *IDH1/2*^{neg}: 35.0%, p=0.048).

Impact of IDH mutations on clinical outcome

Clinical outcome was evaluated in 314 patients younger than 60 years and treated with curative intention in the entire AML group, including 45 *IDH1/2* ^{mut} and 269 *IDH1/2*^{neg} patients (Supplementary Tables 3 and 4). *IDH1*^{mut} and *IDH2*^{mut} patients had similar remission and relapse rates compared to IDH1/2^{neg} patients. OS and DFS were not altered in *IDH1*^{mut} or *IDH2*^{mut} AML. On the other hand, a detailed analysis of the prognostic impact of different mutations revealed differences between particular *IDH* substitutions (Figure 2). Patients

harboring *IDH1* R132H had a higher early death rate (R132H: 44.4% vs. *IDH1/2*^{neg}*:* 12.6%; p=0.023), resulting in shorter OS for R132H patients compared to *IDH1/2*^{neg} (p=0.02) or R132C carriers (p=0.019). The 4-year OS was 0% in R132H, 33% in R132C, and 31% in IDH1/2^{neg} AML patients. In multivariate analyses (Table 3), *IDH1* R132H was associated with shorter OS independently of age, WBC count, cytogenetic risk, and *NPM1-FLT3* ITD status [HR (95%CI): 2.92 (1.38-6.16)], as compared to *IDH1/2*^{neg} AML cases.

In the intermediate cytogenetic risk group, we evaluated 177 patients for clinical outcome, including 38 *IDH1/2*^{mut} and 139 *IDH1/2*^{neg} patients (Supplementary Tables 5 and 6). Similarly to the entire AML cohort, there were no significant differences in remission and relapse rates, OS and DFS between patients with or without *IDH1/2* mutations. Patients harboring R132H had a higher early death rate (42.9% vs. 9.4%; p=0.029). R132H also showed a tendency toward adverse OS compared to *IDH1/2*^{neg} (p=0.09) and to R132C (p=0.052) groups.

DISCUSSION

IDH1 and *IDH2* mutations have been described as new frequent recurrent aberrations in AML. In our study, we found similar mutational frequencies (*IDH1*: 8.5% and *IDH2* 7.5%) as reported previously (*IDH1*: 2.0-9.6 % and *IDH2*: 5.0-10.0%) in adult total AML groups (not excluding acute promyelocytic leukemia).(10-13, 16, 18, 20, 26) Affecting *IDH1*, R132C (3.8%) and R132H (2.7%) were the most prevalent substitutions similarly to other studies. In case of *IDH2*, R140Q occurred more frequently (5.4%) compared to R172K (2.1%).

We confirmed that AML patients with *IDH1* or *IDH2* mutation share several common clinical characteristics like manifestation at older age (10, 12, 15, 17, 19, 26) or higher PLT count (10, 14, 17, 19, 21) at diagnosis. *IDH* mutations also occurred significantly more often in the cytogenetically intermediate risk AML in our cohort similarly to other reports. (10, 11, 13-16, 18, 20, 26) Interestingly, in our cohort, *IDH* mutations were not associated with normal

karyotype. *IDH* mutations occurred more frequently in AML with normal karyotype in all reports, except for a single study.(12) Similarly to other studies, we also observed an association between *IDH* and *NPM1* mutations. (10-20) As a novel common feature of *IDH1* and *IDH2* mutated AML, we described that *IDH* mutant AML showed a reduced *ABCG2* mRNA expression. The *ABCG2* multidrug transporter protein is a stem cell marker (and also known as a marker of cancer stem cells) and plays an important role in stem cell proliferation.(27) *IDH* mutations were shown to induce DNA and histone hypermethylation (2, 8) and the methylation of *ABCG2* promoter may lie behind the lower transcript level of this transporter.(28, 29) The expression of HLA-DR, an early hematopoesis-associated antigen, was also lower in $IDH1^{mut}$ compared to the $IDH1/2^{neg}$ AML subgroup within the intermediate karyotype risk group, similarly to a previous report by Chou et al.(13) Other reports discovered a specific association between *NPM1* and *IDH* mutations by clustering samples according to their methylation profile similarity. (30, 31)

Despite the strikingly similar clinical features of $IDH1^{mut}$ and $IDH2^{mut}$ AML, recently a few studies demonstrated differences between *IDH2* mutations occuring at sites R140 and R172 in AML cases.(14, 16) Similarly to their reports, we confirmed that R172K mutation showed lower WBC, lower LDH, higher likelihood for having intermediate risk abnormal karyotype compared to R140Q, as well as the lack of co-occurrence with mutant *NPM1*. As a novel finding, we observed distinct clinical characteristics of *IDH1* mutations affecting the same codon, R132H and R132C. There was a tendency that R132H mutation associated more frequently with de novo AML etiology compared to R132C mutation (90% vs. 50%, p=0.08). In *IDH1* R132H positive AML, acute myeloblastic leukemia without maturation (FAB M1) morphology was less frequent (p=0.02); PLT count at diagnosis was higher (p=0.05) and *NPM1* co-occured more frequently (p=0.02).

Contrary to the similar clinical characteristics of *IDH1* and *IDH2* mutations, data on the prognostic impact of different *IDH* mutations were reported to be more controversial. Grouping together the mutations with different prognostic impact may be one reason of the inconsistent reports. Several studies have found no prognostic impact of *IDH2* mutations,(17, 19, 21, 26) while others suggested that R140 confers good and R172 adverse prognosis.(3, 11, 14, 16, 17) In our patient cohort, no prognostic difference was detected with respect to the *IDH2* subgroup, possibly due to the low number of cases. *IDH1* was generally considered as a weak prognostic factor exerting its adverse effect only in special AML subgroups (like *FLT3* ITD negative,(15) *NPM1* negative (18, 20) or *NPM1* positive (11)). In our study, *IDH1* R132H was an independent adverse prognostic factor affecting early death rate and OS, while R132C did not differ from IDH^{neg} AML samples.

In the central nervous system (CNS), the vast majority (80-90%) of *IDH1* mutations is R132H, while R132C is more frequent in the haematopoietic clonal disorders. Scientific literature data reveals that R132H occurs less frequently in $IDH1^{mut}$ MPN (0%, $p<0.0001$) and in $IDH1^{mut}$ MDS ($p=0.085$) compared to $IDH1^{mut}$ AML (Table 4). Differences in the observed frequencies of R132H and R132C in CNS tumors, AML, and MPN suggest possible functional variations among *IDH1* codon R132 mutants. Although the ability to produce 2HG was similar in both R132 variants, kinetic analyses showed that the R132C substitution impairs the oxidative decarboxylation of isocitrate to αKG more severely as compared to R132H.(32-34)

In summary, we identified *IDH1* and *IDH2* mutations in 16% of AML. Although we confirmed the previously reported common clinical characteristics (older age at presentation, higher platelet count, association with intermediate risk karyotype and nucleophosmin mutation), we observed distinct clinical features among *IDH1* R132C and R132H or *IDH2* R140 and R172 mutations. This is the first report to draw attention that different mutations

affecting the same codon of *IDH1* might associate with distinct features and prognostic impact. Further studies with larger numbers of AML patients could extend our results and might reveal other unexpected genotype-phenotype correlations.

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Magdalena Koszarska participated in the design of the study, carried out molecular genetic investigations, performed the statistical analysis and drafted the manuscript. Andras Kozma, Emma Adam, Judit Csomor, Angela Feczko, Andras Bors, Tamas I. Orban, Eva Karaszi, participated in the acquisition and analysis of morphological, cytogenetic and molecular genetic data. Arpad Batai, Nora Lovas, Judit Reichardt, Eniko Lehoczky, Zoltan Matrai, Sandor Fekete, Andrea Sipos, Janos Dolgos, Tamas Masszi participated in the acquisition and analysis of clinical data. Tamas Masszi, Attila Tordai and Hajnalka Andrikovics conceived of the study, participated in its design and coordination, helped in interpretation of data and revising the manuscript. All authors read, revised and approved the final manuscript. The authors declare no conflict of interest.

FIGURES

Figure 1. Boxplot expression (median and quartiles) of ABCG2 expression in AML according to IDH1/2 mutation status alone (Panel A), according to NPM1 mutation status alone (Panel B) and IDH1/2 and NPM1 mutation status combined (Panel C).

Figure 2.

Panel A. Overall survival analysis of AML patients according to the different *IDH1* and *IDH2* mutations. R132H, R132C, R140Q, R172K vs. IDH1/2^{neg} p=**0.02**, 0.742, 0.357, 0.197 respectively; R132C vs. R132H **p=0.019**; R140Q vs. R172K p=0.455.
Panel B. Disease free survival analysis of AML patients according to the different *IDH1* and *IDH2* mutations. R132H, R132C, R140Q, R172K vs. IDH1/2^{neg} p=0.091, 0.892, 0.545, 0.253 respectively; R132C vs. R132H p=0.122; R140Q vs. R172K p=0.399

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Table 1. Pre-treatment, clinical and molecular characteristics according to IDH1 and IDH2 mutation status. Comparisons are presented between IDH1 and IDH2 double negative and IDH1 or IDH2 positive patients in the entire AML group.

Characteristics in the entire AML cohort	**IDH 1/2 negative n=316 (84.1%)**		**IDH1 mutant n=32 (8.5%)**		***P***	**IDH2 mutant n=28 (7.4%)**		***P***
	Number/ median	**%/ range**	**Number/ median**	**%/ range**		**Number/ median**	**%/ range**	
Age *median, (range)* (n=376)	49.0	(16-86)	54.5	(30-93)	**0.013**	56.5	(31-77)	**0.009**
Sex *(male/ female; %)*	156/160	(49.4%/50.6%)	11/21	(34.4/65.6%)	0.137	13/15	(46.4/53.6%)	0.845
Type of AML *(n, %)*								
De novo	190/314	(60.5%)	22/32	(68.8%)	0.447	20/26	(76.9%)	0.140
MDS	101/314	(32.2%)	6/32	(18.8%)	0.159	6/26	(23.1%)	0.388
t-AML	23/314	(7.3%)	4/32	(12.4%)	0.296	0/26	(0.0%)	0.238
FAB *(n, %)*								
M0	11/282	(3.9%)	0/29	(0.0%)	0.608	0/25	(0.0%)	0.609
M1	66/282	(23.4%)	8/29	(27.6%)	0.648	10/25	(40.0%)	0.088
M2	37/282	(13.1%)	6/29	(20.7%)	0.261	0/25	(0.0%)	0.055
M3	31/282	(10.9%)	0/29	(0.0%)	0.094	2/25	(8.0%)	1
M4	78/282	(27.7%)	11/29	(37.9%)	0.281	9/25	(36.0%)	0.364
M5	53/282	(18.8%)	4/29	(13.8%)	0.621	4/25	(16.0%)	1
M6	3/282	(1.1%)	0/29	(0.0%)	1	0/25	(0.0%)	1
M7	3/282	(1.1%)	0/29	(0.0%)	1	0/25	(0.0%)	1
Laboratory data*, median (range)*								
WBC, T/L (n=347)	10.5	(0.3-368)	10.7	(0.09-301)	0.832	6.91	(0.8-300)	0.294
PLT, G/L (n=278)	39	(5-684)	75	(10-326)	**0.039**	72	(5-215)	**0.005**
LDH, U/L (n=347)	734	(136-15418)	730	(260-4040)	0.710	571	(217-4500)	0.139
ABCG2, % (n=80)	1.35	(0.02-18.05)	0.52	(0.01-23.01)	0.062	0.51	(0.05-3.24)	**0.012**
HLA-DR, % (n=235)	45	(0-96)	32	(0-92)	0.241	23	(0-89)	0.182
CD13, % (n=229)	54	(0-99)	56	(7-91)	0.943	55	(14-92)	0.668
CD33, % (n=238)	68	(1-98)	68.5	(8-96)	0.414	60	(17-94)	0.236
CD34, % (n=238)	22	(0-95)	13	(0-89)	0.923	10	(0-88)	0.735
CD14, % (n=188)	2	(0-95)	1.5	(0-20)	0.310	2	(0-25)	0.861
CD7, % (n=231)	12	(0-93)	8.5	(0-60)	0.733	10	(4-68)	0.514
Cytogenetics *(n, %)*								
Favourable	61/300	(20.3%)	2/31	(6.5%)	0.089	2/27	(7.4%)	0.128
Intermediate	158/300	(52.7%)	25/31	(80.6%)	**0.004**	22/27	(81.5%)	**0.004**
Adverse	81/300	(27.0%)	4/31	(12.9%)	0.128	3/27	(11.1%)	0.105
Mutations *(n, %)*								
FLT3 ITD +	70/315	(22.2%)	6/32	(18.8%)	0.823	4/28	(14.3%)	0.472
NPM1 +	73/315	(23.2%)	13/31	(42.0%)	**0.029**	9/28	(33.3%)	0.244

Table 2. Pre-treatment, clinical and molecular characteristics of the most common IDH1 R132C, R132H and IDH2 R140Q, R172K mutations in the entire AML group. Comparisons are presented between IDH1 and IDH2 double negative (shown in Table 1.) and the individual mutations (p values). Comparisons between IDH1 R132C and R132H are presented with p* and comparisons between IDH2 R140Q and R172K are presented with p# values.

Characteristics in the entire AML cohort	**R132C+ n=14 (3.7%)**		***P***	**R132H+ n=10 (2.7%)**		***P***	***P****	**R140Q+ n=20 (5.3%)**		***P***	**R172K+ n=8 (2.1%)**		***P***	***P#***
	Number/ median	**%/ range**		**Number/ median**	**%/ range**			**Number/ median**	**%/ range**		**Number/ median**	**%/ range**		
Age *median,(range)*	57	(33-93)	**0.024**	52	(30-66)	0.561	0.285	56.5	(40-77)	**0.009**	56.5	(31-66)	0.415	0.746
Sex *(male/ female; %)*	5/9	(35.7/64.3%)	0.416	3/7	(30.0/ 70.0%)	0.338	0.56	11/9	(55.0/ 45.0%)	0.652	2/6	(25.0/ 75.0%)	0.284	0.16
Type of AML(n, %)														
De novo	7/14	(50.0%)	0.578	9/10	(90.0%)	0.095	0.08	15/18	(83.3%)	0.078	5/8	(62.5%)	1	0.33
MDS	4/14	(28.6%)	1	1/10	(10.0%)	0.179	0.36	3/18	(16.7%)	0.201	3/8	(37.5%)	0.716	1
t-AML	3/14	(21.4%)	0.089	0/10	(0.0%)	1	0.24	0/18	(0.0%)	0.624	0/8	(0.0%)	1	1
FAB (n, %)														
M0	0/12	(0.0%)	1	0/9	(0.0%)	1	-	0/20	(0.0%)	1	0/5	(0.0%)	1	-
M1	6/12	(50.0%)	0.078	0/9	(0.0%)	0.217	**0.02**	9/20	(45.0%)	0.056	1/5	(20.0%)	1	0.61
M2	1/12	(8.3%)	1	3/9	(3.3%)	0.112	0.27	0/20	(0.0%)	0.149	0/5	(0.0%)	1	-
M3	0/12	(0.0%)	0.623	0/9	(0.0%)	0.604	-	2/20	(10.0%)	1	0/5	(0.0%)	1	1
M4	4/12	(33.3%)	0.744	4/9	(44.4%)	0.275	0.67	5/20	(25.0%)	1	4/5	(80.0%)	**0.025**	**0.04**
M5	1/12	(8.3%)	0.702	2/9	(22.2%)	0.680	0.55	4/20	(20.0%)	1	0/5	(0.0%)	0.588	0.55
M6	0/12	(0.0%)	1	0/9	(0.0%)	1	-	0/20	(0.0%)	1	0/5	(0.0%)	1	-
M7	0/12	(0.0%)	1	0/9	(0.0%)	1	-	0/20	(0.0%)	1	0/5	(0.0%)	1	-
Laboratory data*, median(range)*														
WBC, T/L	8.4	(1-301)	0.685	14.5	(0.09-100)	0.946	0.794	12.4	(0.8-300)	0.846	22.0	(1.2-25.1)	**0.019**	**0.028**
PLT, G/L	45	(10-154)	0.982	136	(25-326)	**0.017**	**0.050**	68.5	(5-215)	**0.015**	79	(12-140)	0.132	0.929
LDH, U/L	700	(260-4040)	0.349	1204	(484-2079)	0.438	0.164	586	(304-4500)	0.571	411	(217-1398)	**0.043**	0.092
ABCG2, %	0.52	(0.01-1.94)	**0.045**	0.79	(0.22-23.01)	0.738	0.513	0.38	(0.05-1.61)	**0.008**	0.69	(0.22-3.24)	0.481	0.304
HLA-DR, %	24	(9-92)	0.289	26.5	(0-75)	0.225	0.867	21.5	(0-89)	0.139	44.5	(22-67)	0.976	0.606
CD13, %	71	(9-91)	0.569	51.5	(7-82)	0.484	0.463	55	(14-92)	0.564	47.5	(20-75)	0.821	0.606
CD33, %	54	(18-89)	0.414	69	(8-87)	0.469	1	61	(17-94)	0.391	45.5	(31-60)	0.295	0.513
CD34, %	38	(3-89)	0.151	3.5	(1-68)	0.340	0.054	1	(0-88)	0.700	65	(51-79)	0.070	0.513
CD14, %	1	(1-4)	0.433	2	(0-20)	0.726	0.710	2	(1-25)	0.645	4	(0-8)	0.646	0.667
CD7, %	8	(3-56)	0.784	15	(3-50)	0.920	0.779	10	(4-68)	0.634	12.5	(4-21)	0.602	0.909
Cytogenetics *(n, %)*														
Favourable	0/13	(0.0%)	0.080	2/10	(20.0%)	1	0.18	2/19	(10.5%)	0.386	0/8	(0.0%)	0.364	1
Intermediate	9/13	(69.2%)	0.271	8/10	(80.0%)	0.113	0.66	15/19	(79.0%)	0.583	7/8	(87.5%)	0.072	1
Adverse	4/13	(30.8%)	0.755	0/10	(0.0%)	0.069	0.10	2/19	(10.5%)	0.175	1/8	(12.5%)	0.686	1
Mutations *(n, %)*														
FLT3 ITD +	2/14	(14.3%)	0.742	2/10	(20.0%)	1	0.82	4/20	(20.0%)	1	0/8	(0.0%)	0.209	0.240
NPM1 +	2/14	(14.3%)	0.744	7/10	(70.0%)	**0.003**	**0.02**	9/19	(47.4%)	**0.026**	0/8	(0.0%)	0.206	**0.020**

Table 3. Multivariate analysis for overall and disease-free survival in all AML patients. IDH1 R132H mutation is an independent adverse prognostic factor of age, karyotype (in the entire AML cohort), and NPM1-FLT3 risk.

Discovery cohort	**Entire AML**						**Intermediate AML**					
	OS			**DFS**			**OS**			**DFS**		
	HR	**95%Cl**	**p**	**HR**	**95%Cl**	**p**	**HR**	**95%Cl**	**p**	**HR**	**95%Cl**	**p**
Age	1.02	1.00-1.03	**0.002**	1.02	1.00-1.03	**0.002**	1.02	1.00-1.04	**0.023**	1.02	1.00-1.04	**0.046**
Karyotype	2.09	1.68-2.62	**0.000**	2.04	1.64-2.54	**0.000**						
NPM1-FLT3 risk*	0.63	0.39-1.04	0.069	0.60	0.37-0.97	**0.041**	0.511	0.29-0.88	**0.016**	0.49	0.28-0.84	**0.010**
R132H mutation**	2.92	1.44-5.89	**0.003**	2.28	1.13-4.58	**0.021**	2.33	1.05-5.15	**0.037**	1.83	0.83-4.04	0.132

**NPM1-FLT3* risk: low risk (*NPM1* positive and *FLT3* negative) vs. high risk group (*NPM1* negative and *FLT3* negative, *NPM1* positive and *FLT3* positive, *NPM1* negative and *FLT3* positive combined). ** R132H positive patients vs. *IDH1/2* negative patients.

Remarks and abbreviations for Tables 1-3:

Significant p values are shown in bold. P values present comparisons between IDH mutation positive and IDH1/2 double negative ($IDH1/2^{neg}$) patients. P* values present comparisons between IDH1 R132C and R132H, p# values present comparisons between IDH2 R140Q and R172K mutation positive patients.

Abbreviations: ABCG2: ABCG2: ATP-binding cassette sub-family G member 2 expression at diagnosis; AML: acute myeloid leukemia; FAB: morphology according to French-American British classification; FLT3 ITD+: fms-like tyrosine kinase internal tandem duplication positive; IDH: isocitrate dehydrogenase; LDH: lactate dehydrogenase at diagnosis; MDS: myelodysplastic syndrome; MDS-AML: AML evolving from a primary documented myelodysplastic syndrome; MPN: myeloproliferative diseases; N.A. not applicable; NPM1+: nucleophosmin 1 positive; PLT: platelet count at diagnosis; t-AML: therapy-related myeloid neoplasm; WBC: white blood cell count at diagnosis; OS:overall survival; DFS: disease free survival; HR:hazard ratio; 95%Cl: 95% confidence interval.

Table 4. Recent studies on the frequency of *IDH1* R132H and R132C mutations in MPN, MDS and AML.

Author	Disease	Clinical correlates	Number of patients in the study [n]	Number of IDH1 R132 mutants in the study [n]	Number of R132H mutants in the study [n]	R132H vs IDH1 R132 total [%]	Number of R132HC mutants in the study [n]	R132C vs IDH1 R132 total [%]
Tefferi et al.* (35)	MPN	includes post MPN AML	1473	18	0	0,0%	7	38,9%
Green et al. (36)	MPN		16	3	0	0,0%	3	100,0%
Pardanani et al.* (37)	MPN	includes post MPN AML	200	5	0	0,0%	4	80,0%
		SUMMARY		**27**	**0**	**0%**	**14**	**51,9%**
Rocquain et al. (38)	MDS		65	2	0	0,0%	2	100,0%
Kosmider et al. (39)	MDS		100	2	0	0,0%	0	0,0%
Thol et al.** (22)	MDS		193	7	1	14,3%	6	85,7%
Lin et al. (26)	MDS		82	2	1	50,0%	0	0,0%
		SUMMARY		**13**	**2**	**15,4%**	**8**	**61,5%**
Ho et al. (43)	AML	children	257	0	0	na	0	na
Kosmider et al. (39)	AML	secondary	41	2	0	0,0%	2	100,0%
Zou et al. (40)	AML		68	5	0	0,0%	2	40,0%
Schnittger et al. (41)	AML		1414	93	6	6,5%	51	54,8%
Chou et al. (13)	AML	adult AML	493	27	7	25,9%	10	37,0%
Chotirat et al. (12)	AML	newly diagnosed AML	230	20	8	40,0%	6	30,0%
Boissel et al. (11)	AML	de novo, adult AML	520	50	22	44,0%	21	42,0%
Paschka et al. (19)	AML		805	61	28	45,9%	20	32,8%
Marcucci et al. (17)	AML	de novo, CN-AML	358	49	23	46,9%	15	30,6%
Thol et al.** (22)	AML	secondary AML (arising from MDS)	53	4	2	50,0%	1	25,0%
Green et al. (42)	AML		1333	107	54	50,5%	35	32,7%
Abbas et al. (10)	AML	newly diagnosed AML	893	55	31	56,4%	15	27,3%
Ho et al. (43)	AML	young adult	274	12	8	66,7%	1	8,3%
Rocquain et al. (38)	AML	including 46 primary cases and 18 arising from MDS	64	3	2	66,7%	1	33,3%
Wagner et al.** (23)	AML	CN-AML	275	29	20	69,0%	5	17,2%
Lin et al. (26)	AML		198	4	3	75,0%	0	0,0%
		SUMMARY		**521**	**214**	**41,1%**	**185**	**35,5%**

Possible overlapping in patients cohorts between *Tefferi and Pardanani, **Thol and Wagner

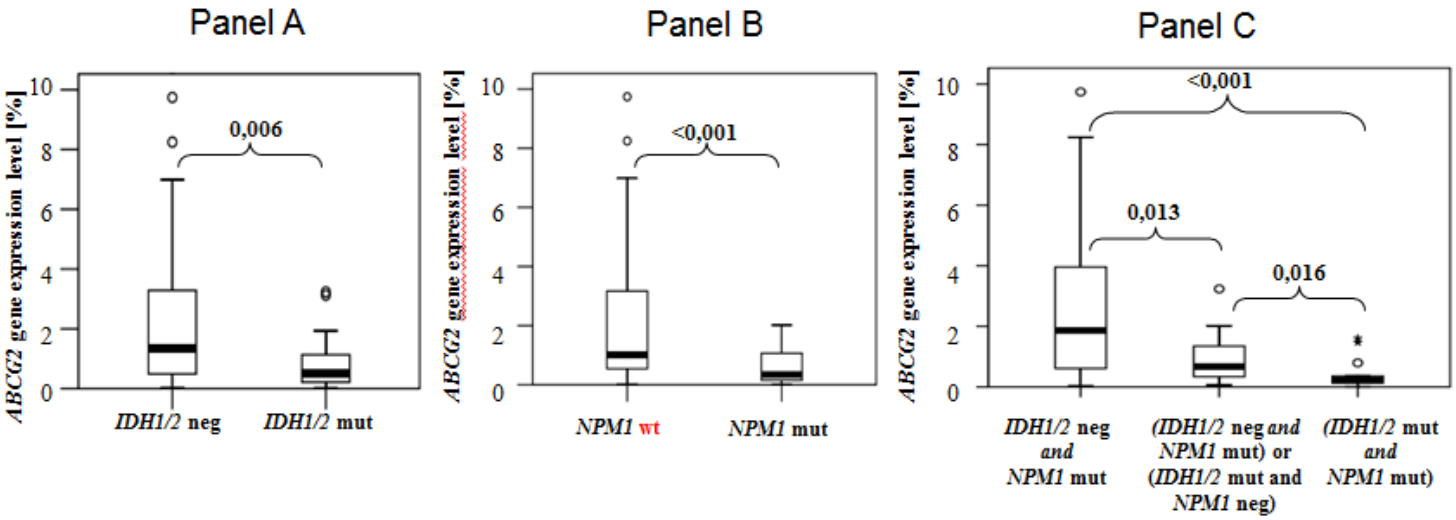
Panel A
Panel B
Panel C
ABCG2 gene expression level [%]
0,006
<0,001
0,013
0,016
IDH1/2 neg
IDH1/2 mut
NPM1 wt
NPM1 mut
IDH1/2 neg and NPM1 mut
(IDH1/2 neg and NPM1 mut) or (IDH1/2 mut and NPM1 neg)
(IDH1/2 mut and NPM1 mut)

Fig. 1.

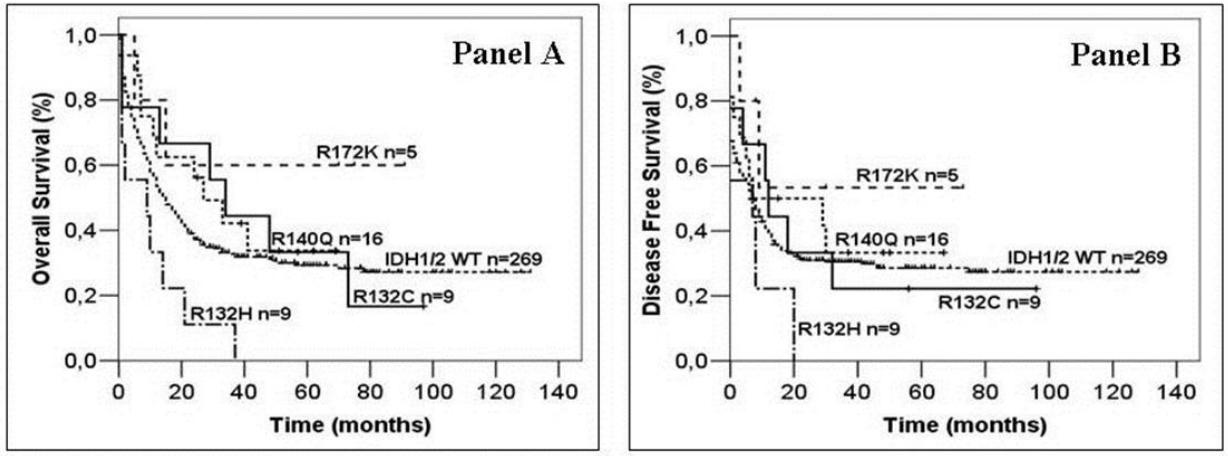
Panel A
Overall Survival (%)
R172K n=5
R140Q n=16
IDH1/2 WT n=269
R132C n=9
R132H n=9
Time (months)
Panel B
Disease Free Survival (%)
R172K n=5
R140Q n=16
IDH1/2 WT n=269
R132C n=9
R132H n=9
Time (months)

Fig. 2.

Supplementary Table 1. Pre-treatment, clinical and molecular characteristics according to IDH1 and IDH2 mutation status in the intermediate cytogenetic risk AML group. Comparisons are presented between IDH1 and IDH2 double negative and IDH1 or IDH2 positive patients.

Characteristics in the intermedier cytogenetic risk AML group	**IDH 1/2 negative (n=158; 77.0=%)**		**IDH1 mutant (n=25, 12.3%)**		***P***	**IDH2 mutant (n=22, 10.7%)**		***P***
	Number/ median	**%/ range**	**Number/ median**	**%/ range**		**Number/ median**	**%/ range**	
Age *median,(range)*(n=205)	48	(18-86)	53	(30-72)	**0.041**	54	(31-66)	**0.052**
Sex (male/ female; %)	75/83	(47.5%/52.5%)	9/16	(36.0%/64.0%)	0.388	9/13	(40.9%/59.1%)	0.651
Type of AML*(n, %)*								
De novo	117/158	(74.0%)	19/25	(76.0%)	1	17/20	(85.0%)	0.411
MDS	26/158	(16.5%)	3/25	(12.0%)	0.771	3/20	(15.0%)	1
t-AML	15/158	(9.5%)	3/25	(12.0%)	0.717	0/20	(0.0%)	0.225
FAB (n, %)								
M0	5/145	(3.5%)	0/23	(0.0%)	1	0/20	0 (0.0%)	1
M1	36/145	(24.8%)	6/23	(26.1%)	1	9/20	9 (45.0%)	0.066
M2	16/145	(11.0%)	4/23	(17.4%)	0.484	0/20	0 (0.0%)	0.223
M3	0/145	(0.0%)	0/23	(0.0%)	na	0/20	0 (0.0%)	na
M4	50/145	(34.5%)	9/23	(39.1%)	0.647	8/20	8 (40.0%)	0.626
M5	37/145	(25.5%)	4/23	(17.4%)	0.601	3/20	3 (15.0%)	0.409
M6	1/145	(0.7%)	0/23	(0.0%)	1	0/20	0 (0.0%)	1
M7	0/145	(0.0%)	0/23	(0.0%)	na	0/20	0 (0.0%)	na
Laboratory data, *median (range)*								
WBC, T/L (n=194)	18.8	(0.3-368)	14.5	(0.09-180)	0.356	8.61	(0.8-300)	0.072
PLT, G/L (n=149)	44.5	(5-365)	81	(12-326)	0.118	73	(5-215)	**0.029**
LDH, U/L (n=190)	917	(187-7630)	753	(260-2079)	0.223	592	(217-4500)	0.072
ABCG2, % (n=48)	1.37	(0.07-18.05)	0.37	(0.11-3.13)	**0.041**	0.35	(0.05-1.61)	**0.019**
HLA-DR, % (n=134)	45	(0-96)	36	(0-92)	**0.041**	22	(0-89)	0.323
CD13, % (n=134)	54	(0-99)	35	(7-91)	0.471	48	(14-92)	0.519
CD33, % (n=136)	70	(1-98)	73	(8-96)	0.150	60.5	(17-94)	0.071
CD34, % (n=136)	22	(0-95)	6	(0-89)	0.941	5.5	(0-88)	0.204
CD14, % (n=112)	2	(0-95)	1.5	(0-20)	0.172	2	(0-25)	0.695
CD7, % (n=129)	12	(0-93)	21	(0-56)	1	9	(4-22)	0.914
Mutations *(n, %)*								
FLT3 ITD +	55/157	(35.0%)	5/25	(20.0%)	0.172	4/22	(18.2%)	0.148
NPM1 +	61/157	(38.9%)	12/24	(50.0%)	0.372	8/22	(36.4%)	1

Supplementary Table 2. Pre-treatment, clinical and molecular characteristics of the most common IDH1 R132C, R132H and IDH2 R140Q, R172K mutations in the intermediate cytogenetic risk AML group. Comparisons are presented between IDH1 and IDH2 double negative (shown in Table 1.) and the individual mutations (p values). Comparisons between IDH1 R132C and R132H are presented with p* and comparisons between IDH2 R140Q and R172K are presented with p# values.

Characteristics	**R132C (n=9, 4.4%)**		***P***	**R132H (n=8, 3.9%)**		***P***	***P****	**R140Q (n=15, 7.3%)**		***P***	**R172K (n=7, 3.4%)**		***P***	***P#***
	Number/ median	**%/ range**		**Number/ median**	**%/ range**			**Number/ median**	**%/ range**		**Number/ median**	**%/ range**		
Age *median (,range)*	51	(33-72)	0.362	54	(30-66)	0.276	0.815	54	(40-64)	**0.036**	54	(31-66)	0.648	0.731
Sex *(male/ female; %)*	3/6	(33.3%/ 66.7%)	0.505	3/5	(37.5%/ 62.5%)	0.724	1	7/8	(46.7%/ 53.3%)	1	2/5	(28.6%/ 71.4%)	0.0451	0.648
Type of AML*(n, %)*														
De novo	6/9	(66.7%)	0.699	7/8	(87.5%)	0.681	0.577	12/13	(92.3%)	0.191	5/7	(71.4%)	1	0.270
MDS	1/9	(11.1%)	1	1/8	(12.5%)	1	1	1/13	(7.7%)	0.695	2/7	(28.6%)	0.339	0.270
t-AML	2/9	(22.2)	0.229	0/8	(0.0%)	1	0.471	0/13	(0.0%)	0.608	0/7	(0.0%)	1	na
FAB (n, %)														
M0	0/8	(0.0%)	1	0/7	(0.0%)	1	na	0/15	(0.0%)	1	0/5	(0.0%)	1	na
M1	4/8	(50.0%)	0.207	0/7	(0.0%)	0.198	0.077	8/15	(53.3%)	**0.030**	1/5	(20.0%)	1	0.319
M2	0/8	(0.0%)	1	2/7	(28.6%)	0.194	0.200	0/15	(0.0%)	0.367	0/5	(0.0%)	1	na
M3	0/8	(0.0%)	na	0/7	(0.0%)	na	na	0/15	(0.0%)	na	0/5	(0.0%)	na	na
M4	3/8	(37.5%)	0.169	3/7	(42.7%)	0.695	1	4/15	(26.7%)	0.775	4/5	(80.0%)	**0.057**	0.109
M5	1/8	(12.5%)	0.680	2/7	(28.6%)	1	0.569	3/15	(20.0%)	0.763	0/5	(0.0%)	0.334	0.539
M6	0/8	(0.0%)	1	0/7	(0.0%)	1	na	0/15	(0.0%)	1	0/5	(0.0%)	1	na
M7	0/8	(0.0%)	na	0/7	(0.0%)	na	na	0/15	(0.0%)	na	0/5	(0.0%)	na	na
Laboratory data*, median (range)*														
WBC, T/L	6.335	(1-180)	0.103	18.1	(0.09-100)	0.687	0.442	14.7	(0.8-300)	0.702	2.4	(1.2-25.1)	**0.006**	**0.047**
PLT, G/L	45	(12-118)	0.581	136	(25-326)	**0.032**	0.082	71	(5-215)	**0.038**	76	(12-140)	0.342	0.765
LDH, U/L	711.5	(260-1693)	0.298	1067	(484-2079)	0.944	0.574	680	(320-4500)	0.399	452	(217-1398)	**0.031**	0.112
ABCG2, %	0.38	(0.11-1.94)	0.092	0.57	(0.22-1.47)	0.253	1	0.34	(0.05-1.61)	**0.041**	0.65	(0.22-0.74)	0.192	0.905
HLA-DR, %	28	(9-92)	0.083	36	(14-75)	0.042	0.931	19	(0-89)	0.287	44.5	(22-67)	0.918	0.889
CD13, %	35	(9-91)	0.706	49	(7-82)	0.332	0.931	48	(14-92)	0.384	47.5	(20-75)	0.781	0.533
CD33, %	54	(18-89)	0.223	65	(8-87)	0.294	1	63.5	(17-94)	0.170	45.5	(31-60)	0.188	0.582
CD34, %	38	(3-89)	0.316	4	(1-68)	0.243	0.030	1	(0-88)	0.566	65	(51-79)	0.062	0.727
CD14, %	1	(1-2)	0.380	2	(0-20)	0.513	1	2	(1-25)	0.946	4	(0-8)	0.548	0.857
CD7, %	23	(6-56)	0.483	21	(3-50)	0.740	0.429	9	(4-22)	0.940	12.5	(4-21)	0.704	0.711
Mutations *(n, %)*														
FLT3 ITD +	1/9	(11.1%)	0.275	2/8	(25.0%)	0.716	0.576	4/15	(26.7%)	0.583	0/7	(0.0%)	0.096	0.263
NPM1 +	1/9	(11.1%)	0.156	7/8	(87.5%)	**0.009**	**0.003**	8/15	(53.3%)	0.285	0/7	(0.0%)	**0.047**	**0.022**

Supplementary Table 3. Treatment outcome according to IDH1 and IDH2 mutations in the entire AML group. Comparisons are presented between IDH1 and IDH2 double negative and IDH1 or IDH2 positive patients.

Characteristics in the entire AML	**IDH 1/2 negative**		**IDH1 mutant**		***P***	**IDH2 mutant**		***P***
	n	**%**	**n**	**%**		**n**	**%**	
Complete remission	184/269	(68.4%)	17/24	(70.8%)	1	18/21	(85.7%)	0.138
Early death	34/269	(12.6%)	5/24	(20.8%)	0.361	1/21	(4.8%)	0.487
Resistant disease	51/269	(19.0%)	2/24	(8.3%)	0.272	2/21	(9.5%)	0.387
Relapse	98/184	(53.3%)	9/17	(52.9%)	1	11/18	(61.1%)	0.624
Alive	81/269	(30.1%)	5/24	(20.8%)	0.483	9/21	(42.9%)	0.229

Supplementary Table 4. Treatment outcome of the most common IDH1 R132C, R132H and IDH2 R140Q, R172K mutations in the total AML group. Comparisons are presented between IDH1 and IDH2 double negative (shown in Table 3.) and the individual mutations (p values). Comparisons between IDH1 R132C and R132H are presented with p* and comparisons between IDH2 R140Q and R172K are presented with p# values.

Characteristics in the entire AML	**R132C**		***P***	**R132H**		***P***	***P****	**R140Q**		***P***	**R172K**		***P***	***P#***
	N	**%**		**n**	**%**			**n**	**%**		**n**	**%**		
Complete remission	7/9	(77.8%)	0.725	5/9	(55.6%)	0.474	0.619	13/16	(81.3%)	0.406	5/5	(100.0%)	0.329	0.549
Early death	1/9	(11.1%)	1	4/9	(44.4%)	**0.023**	0.294	1/16	(6.3%)	0.702	0/5	(0.0%)	1	1
Resistant disease	1/9	(11.1%)	1	0/9	(0.0%)	0.373	1	2/16	(12.5%)	0.745	0/5	(0.0%)	0.588	1
Relapse	4/7	(57.1%)	1	4/5	(80.0%)	0.376	0.576	8/13	(61.5%)	0.775	3/5	(60.0%)	1	1
Alive	2/9	(22.2%)	1	0/9	(0.0%)	0.063	0.471	6/16	(37.5%)	0.579	3/5	(60.0%)	0.169	0.611

Supplementary Table 5. Treatment outcome according to IDH1 and IDH2 mutations in the intermedier cytogenetic risk AML group. Comparisons are presented between IDH1 and IDH2 double negative and IDH1 or IDH2 positive patients.

Characteristics in intermedier cytogenetic risk AML	**IDH 1/2 negative**		**IDH1 mutant**		***P***	**IDH2 mutant**		***P***
	n	**%**	**n**	**%**		**n**	**%**	
Complete remission	100/139	(71.9%)	17/25	(68.0%)	0.810	15/18	(83.3%)	0.403
Early death	13/139	(9.4%)	4/25	(16.0%)	0.298	1/18	(5.6%)	1
Resistant disease	26/139	(18.7%)	4/25	(16.0%)	1	2/18	(11.1%)	0.743
Relapse	58/100	(58.0%)	10/17	(58.8%)	1	8/15	(53.3%)	0.784
Alive	39/139	(28.1%)	5/25	(20.0%)	0.472	7/18	(38.9%)	0.410

Supplementary Table 6. Treatment outcome of the most common IDH1 R132C, R132H and IDH2 R140Q, R172K mutations in the intermedier cytogenetic risk AML group. Comparisons are presented between IDH1 and IDH2 double negative (shown in Table 3.) and the individual mutations (p values). Comparisons between IDH1 R132C and R132H are presented with p* and comparisons between IDH2 R140Q and R172K are presented with p# values.

Characteristics in intermedier cytogenetic risk AML	**R132C**		***P***	**R132H**		***P***	***P****	**R140Q**		***P****	**R172K**		***P****	***P****
	N	**%**		**n**	**%**			**n**	**%**		**n**	**%**		
Complete remission	5/7	(71.4%)	1	4/7	(57.1%)	0.411	1	10/13	(76.9%)	1	5/5	(100.0%)	0.324	0.522
Early death	1/7	(14.3%)	0.514	3/7	(42.9%)	**0.029**	0.559	1/13	(7.7%)	1	0/5	(0.0%)	1	1
Resistant disease	1/7	(14.3%)	1	0/7	(0.0%)	0.353	1	2/13	(15.4%)	1	0/5	(0.0%)	0.585	1
Relapse	2/5	(40.0%)	0.649	4/4	(57.1%)	0.146	0.167	5/10	(50.0%)	0.742	3/5	(60.0%)	1	1
Alive	2/7	(28.6%)	1	0/7	(0.0%)	0.189	0.462	4/13	(30.8%)	1	3/5	(60.0%)	0.148	0.326

Remarks and abbreviations for Supplementary Tables 1-6:
Significant p values are shown in bold. P values present comparisons between IDH mutation positive and IDH1/2 double negative (wild type, WT) patients. P* values present comparisons between IDH1 R132C and R132H, p# values present comparisons between IDH2 R140Q and R172K mutation positive patients.
Abbreviations: ABCG2: ABCG2: ATP-binding cassette sub-family G member 2 expression at diagnosis; AML: acute myeloid leukemia; FAB: morphology according to French-American British classification; FLT3 ITD+: fms-like tyrosine kinase internal tandem duplication positive; IDH: isocitrate dehydrogenase; LDH: lactate dehydrogenase at diagnosis; MDS-AML: AML evolving from a primary documented myelodysplastic syndrome; N.A. not applicable; NPM1+: nucleophosmin 1 positive; PLT: platelet count at diagnosis; t-AML: therapy-related myeloid neoplasm; WBC: white blood cell count at diagnosis.