Differential impact of IDH1/2 mutational subclasses on outcome in adult AML: Results from a large multicenter study

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Abstract:
Mutations of the isocitrate dehydrogenase-1 (IDH1) and IDH2 genes are amongst the most frequent alterations in acute myeloid leukemia (AML) and can be found in ~20% of patients at diagnosis. Among 4930 patients (median age 56 years, interquartile range 45-66) with newly diagnosed, intensively treated AML, we have identified IDH1 mutations (mIDH1) in 423 (8.6%) and IDH2 mutations (mIDH2) in 575 (11.7%) patients. Overall, there were no differences in response rates or survival for patients with mIDH1 or mIDH2 compared to patients without mutated IDH1/2. However, distinct clinical and co-mutational phenotypes of the most common subtypes of IDH1/2 mutations could be associated with differences in outcome. IDH1-R132C was associated with significantly increased age, lower white blood cell count (WBC), less frequent co-mutation of NPM1 and FLT3-ITD as well as lower rate of complete remissions and a trend for reduced overall survival (OS) compared to other mIDH1 variants and wtIDH1/2. In our analysis, IDH2-R172K was associated with significantly lower WBC, more karyotype abnormalities, and less frequent co-mutations of NPM1 and/or FLT3-ITD. Among patients within the ELM2017 intermediate- and adverse-risk groups, RFS and OS were significantly better for patients with IDH2-R172K compared to wtIDH, providing evidence that AML with IDH2-R172K could be a distinct entity with a specific co-mutation pattern and favorable outcome. In summary, the presented data from a large cohort of IDH1/2 mutant AML patients indicate novel and clinically relevant findings for the most common IDH1-mutation subtypes.

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Differential impact of IDH1/2 mutational subclasses on outcome in adult AML: Results from a large multicenter study

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Mutations of the isocitrate dehydrogenase-1 (IDH1) and IDH2 genes are amongst the most frequent alterations in acute myeloid leukemia (AML) and can be found in ~20% of patients at diagnosis. Among 4930 patients (median age 56 years, interquartile range 45-66) with newly diagnosed, intensively treated AML, we have identified IDH1 mutations (mIDH1) in 423 (8.6%) and IDH2 mutations (mIDH2) in 575 (11.7%) patients. Overall, there were no differences in response rates or survival for patients with mIDH1 or mIDH2 compared to patients without mutated IDH1/2. However, distinct clinical and co-mutational phenotypes of the most common subtypes of IDH1/2 mutations could be associated with differences in outcome.

IDH1-R132C was associated with significantly increased age, lower white blood cell count (WBC), less frequent co-mutation of NPM1 and FLT3-ITD as well as lower rate of complete remissions and a trend for reduced overall survival (OS) compared to other mIDH1 variants and wtIDH1/2.

In our analysis, IDH2-R172K was associated with significantly lower WBC, more karyotype abnormalities, and less frequent co-mutations of NPM1 and/or FLT3-ITD. Among patients within the ELN2017 intermediate- and adverse-risk groups, RFS and OS were significantly better for patients with IDH2-R172K compared to wtIDH, providing evidence that AML with IDH2-R172K could be a distinct entity with a specific co-mutation pattern and favorable outcome.

In summary, the presented data from a large cohort of IDH1/2 mutant AML patients indicate novel and clinically relevant findings for the most common IDH-mutation subtypes.
Key Points:
- Patients with IDH1-R132C have a lower CR rate and a trend for reduced OS
- Patients with IDH2-R172K in the ELN intermediate/adverse-risk group have a significantly better RFS and OS

Keywords: acute myeloid leukemia, IDH1 mutation, IDH2 mutation
INTRODUCTION

Isocitrate dehydrogenase-1 (IDH1), localized in the cytoplasm and IDH2, localized in mitochondria, belong to a group of catalytic enzymes involved in cellular metabolism and response to oxidative damage. They are encoded by the IDH1 and IDH2 genes located on chromosome 2 band q33 and chromosome 15 band q26, respectively. Physiologically, their main function is the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) as part of the citric acid cycle.

Somatic mutations of IDH1 and IDH2 genes are among the most frequent alterations in acute myeloid leukemia (AML). They can be found in ~20% of patients at diagnosis, with IDH2 mutations occurring more frequently, and appear to be early events in leukemogenesis.

There are inconsistent results regarding the impact of IDH1 and IDH2 mutations on patient outcomes with respect to complete remission (CR) rate, relapse-free survival (RFS) and overall survival (OS). These conflicting results are possibly explained by the differential effects of certain subtypes of mutations. While mutations at the “hotspots” IDH1 codon 132, IDH2 codon R140 and IDH2 codon R172 share the functional consequence of increased 2-HG production, several lines of evidence suggest that there are important differences in the biology of these mutation types.

For example, IDH1 gene mutations in glioma predominantly involve the R132H substitution (found in >80% of patients), while in AML the R132C and R132H mutations are found at comparable frequencies.

In addition, the co-mutation spectrum differs between different types of IDH1/IDH2 mutations. Consequently, IDH2-R172K has recently been suggested to define a
distinct genomic category of AML being mutually exclusive from NPM1 mutations and
other class-defining lesions and yielding favorable outcome²,⁶.

Recently, IDH inhibitors have been established as targeted therapies with Ivosidenib⁸
and Enasidenib⁹ showing promising results in AML patients with relapsed or refractory disease. They are currently under further investigation as mono-therapy as well as in combination with multiple other established treatments in AML.

A detailed analysis of clinical and genetic associations with prognosis is needed to thoroughly assess the impact of the different subtypes of leukemia-associated
IDH1/IDH2 gene mutations, which is only feasible in a large enough cohort of AML patients.

We therefore analyzed a large group of newly diagnosed AML patients receiving intensive treatment to investigate the impact of IDH1/2 mutations on outcome.

PATIENTS AND METHODS

Patient population

All AML patients consecutively enrolled into intensive AML treatment protocols or the patient registries of the SAL and AML-CG study groups with sufficient biomaterial available were included in this analysis. All patients received intensive chemotherapy based on anthracyclines in combination with cytarabine within the following clinical trials: AML96¹⁰, AML2003¹¹, AMLCG1999¹², AML60+¹³, AMLCG2008¹⁴, and SORAML¹⁵ or enrolled onto the prospective SAL AML registry (NCT03188874).

Detailed information on treatment regimens used are provided in the corresponding publications. Patients were not treated with IDH1/2-inhibitors. The study was conducted in accordance to the Declaration of Helsinki and approved by the
responsible ethics committees. Only data from patients who have signed informed consent on analyses of data were included.

**Molecular analysis**

Screening for *IDH1* and *IDH2* mutations was performed using genomic DNA isolated from pre-treatment bone marrow (BM) or peripheral blood (PB) samples. Patients enrolled in SAL trials were screened by denaturing high performance liquid chromatography (DHPLC) as described previously\(^{16}\). All samples with an aberrant DHPLC-chromatogram were analyzed by Sanger sequencing or by sensitive ultradeep NGS\(^{17}\). In addition, a subset of SAL patients was analyzed using an NGS panel-based approach focusing on genes frequently mutated in hematopoietic disease (TruSight Myeloid Panel, Illumina)\(^{18}\). Both methods were concordant in all samples analyzed with both procedures. The lower limit of detection (LLOD) of these methods was 0.1% (ultradeep NGS) and 1-5% (DHPLC, panel NGS). All patients enrolled in AML-CG trials were analyzed using a custom targeted NGS assay\(^{19}\). Mutations in *FLT3* and *NPM1* were analyzed as described in detail in previous work\(^{20,21}\).

**Definitions**

*De novo* AML excludes patients with previous malignancy and treatment with chemotherapy and/or radiotherapy. AML in patients with a documented history of myelodysplasia or myeloproliferative disorders were considered as secondary AML (sAML). Therapy-associated myeloid neoplasm (t-MN) comprised patients with prior exposure to chemotherapy and/or radiation therapy. CR and OS were defined according to the current ELN criteria\(^{22}\).
Statistical analysis

CR rate and OS are reported for the whole cohort. Cox regression, stratified for the different study protocols, was used to compare survival and to estimate univariate and adjusted hazard ratios. For the binary endpoint of complete remission, logistic regression models were fitted to estimate univariate and adjusted odds ratios.

To compare categorical variables between mutational groups, the Chi squared test was used. Continuous variables were compared with the Kruskal-Wallis test.

RESULTS

IDH1 and IDH2 mutations

In the entire cohort (n=4930) we found IDH1 mutations (mIDH1) in 423 (8.6%) and IDH2 mutations (mIDH2) in 575 (11.7%) patients. Fourteen patients (0.3%) harboured both, an IDH1 and IDH2 mutation. The median follow-up for patients alive was 88 months (95%-CI: 85.9-91.0). Table 1 summarizes the patients’ characteristics. The median age for all patients was 56 years (interquartile range (IQR), 45-66). NPM1, FLT3-ITD and CEBPA mutations were found in 32%, 22% and 7% (of which 54% were bi-allelic) of the patients, respectively.

The median variant allele fraction (VAF) for IDH mutations was 38% (IQR: 30-43) with no difference in VAF between mutational subgroups (Supplement Tbl.1).

Compared to wild-type IDH1/2 (wtIDH1/2), patients with mutated IDH1/2 showed significantly lower WBC (p=.002), were more likely to have a normal karyotype (p<.001) and were more often accompanied by mutated NPM1 (p<.001). A detailed table of differences between wtIDH1/2 and mIDH1/2 is provided in the Supplements.
Supplement Tbl.2. Overall, no significant differences were observed between patients with wt IDH and those with mIDH1 or mIDH2 regarding CR rate (73% [95%-CI: 72-75%], 69% [95%-CI: 64-73], and 73% [95%-CI: 69-77%] p=.17), median RFS (17 vs. 17 vs. 18 months, respectively, p=.52) and median OS (20 vs. 18 vs. 22 months respectively, p=.58) as shown in Fig. 1. However, IDH mutational status influenced OS in distinct ELN2017 subgroups (Fig. 2). In the ELN2017 favorable-risk category, mutations in IDH1/2 were associated with worse OS than wt IDH1/2 (mIDH1 HR=1.43 [95%-CI: 1.14-1.79], p<.01 and mIDH2 HR=1.39 [95%-CI: 1.13-1.72], p<.01). In ELN2017 adverse-risk category mIDH2 did not significantly affect OS, while there was a trend towards poorer survival for mIDH1 (HR=1.31 [95%-CI: 1.00-1.73], p=.0542), while mIDH2 did not associate with OS. There was no impact of mIDH1/2 on OS in the ELN2017 intermediate-risk category.

**IDH1 mutational variants**

The most common IDH1 variants were R132C, (n= 179 patients, 42%) and R132H, (n=177 patients, 42%). Other IDH1 mutations were R132G identified in 7%, R132S in 4%, and R132L in 5% of the IDH1 mutated patients. As previous analyses have suggested differences in outcome according to individual amino acid exchanges⁵, we analyzed these individual groups in more detail.

In IDH1-mutated patients, we observed significant differences in baseline characteristics (Table 2) between the two most common mutational subtypes – R132C and R132H. Patients carrying the R132C mutation were older (60 vs. 54 years, p<.001), had lower WBC (4.3 vs. 22.5 Gpt/L, p<.001) and were less likely to have additional NPM1 (24% vs. 71%, p<.001), and/or FLT3-ITD mutations (10% vs. 27%, p<.001) than those with the R132H variant. **Patients with the R132C mutation**
frequently showed frequent co-mutations in DNMT3A (53%), NPM1 (25%) and RUNX1 (21%). The R132H variant was frequently associated with mutations of NPM1 (78%), DNMT3A (50%), PTPN11 (25%) as well as FLT3-ITD (23%) and -TKD (19%) (Fig. 3A). Further, in patients with an FLT3-ITD mutation the median ITD-to-wildtype ratio was significantly lower in patients with an R132C mutation (0.3 vs. 0.7, \( p = .029 \)). Patients with R132C had more often secondary AML compared to R132H (16% vs. 7%) and were less likely to have a normal karyotype (63.5% vs. 83.5%, \( p < .001 \)). Given this, R132C mutations were underrepresented in ELN2017 favorable-risk category (21% vs. 63%, \( p < .001 \), but were more often grouped into ELN2017 intermediate- (51% vs. 28%, \( p < .001 \)) and adverse-risk categories (28% vs. 9%, \( p < .001 \)) compared to R132H mutations. In a univariate analysis, the CR rate was significantly lower in patients with IDH1-R132C compared to those with the R132H variant (62% [95%-CI: 54-69] vs. 77% [95%-CI: 70-83], OR 0.48 [95%-CI: 0.30-0.76], \( p = .002 \)) and wt IDH1/2 (62% [95%-CI: 54-69] vs. 73% [95%-CI: 72-75], \( p = .003 \)) while RFS and OS did not differ. In multivariate analysis including age, WBC, type of AML, and ELN2017 risk, the CR rate was significantly lower in patients with the IDH1-R132C compared to other mIDH1 (OR = 0.63 [95%-CI: 0.43-0.92], \( p = .016 \), Supplement Tbl. 3.1). For OS, univariate analysis showed reduced survival for R132C compared to R132H mutated patients, without reaching statistical significance (15 months [95%-CI: 12-22] vs. 23 months [95%-CI: 16-36], HR 1.18 [95%-CI: 0.91-1.53] \( p = .22 \)) (Fig. 4A). There was no significant impact of R132C or R132H mutations on OS within the different ELN2017 risk categories.

For the less common mIDH1 mutational variants - R132G, R132S, and R132L - we found significantly lower CR rates in a multivariate analysis including WBC, type of AML, FLT3-ITD, NPM1, and ELN2017 risk (OR = 0.52 [95%-CI: 0.28 to 0.96], \( p = .036 \)).
supplement Tbl. 3.2), with no differences in between the subgroups (Supplement Tbl. 43). For RFS and OS there was no significant difference compared to other mIDH1 variants.

**IDH2 mutational variants**

Among mIDH2, 438 patients had the R140Q (77%) and 110 patients the R172K (19%) substitution. Rarely found were R140G (1%), R140L (1%), and R172S (0.2%) mutations. For patients with mIDH2, R172K was associated with a significantly lower WBC at diagnosis ($p<.001$), higher platelet count ($p<.001$), a lower rate of normal karyotype ($p<.001$), higher rate of trisomy 8 ($p<.01$), and was less frequently accompanied by NPM1 ($p<.001$) and/or FLT3-ITD ($p<.001$) mutations compared to variants at R140. Patients with IDH2-R172K mutations were less likely to be in the ELN2017 favorable-risk category (2% vs. 43%, $p<.001$) and were more often in the intermediate- (59% vs. 35%, $p<.001$) and adverse-risk category (39% vs. 22%, $p<.001$) compared to those with R140 variants (Table 2). Patients with the R140Q variant often carried co-mutations in NPM1 (50%), DNMT3A (38%), SRSF2 (31%) and FLT3-ITD (28%), while the most frequent co-mutations in patients carrying the R172K variant were DNMT3A (76%) and ASXL1 (20%); (Fig. 3A).

Overall, there was no significant difference when we compared R172K to variants at R140 in CR rate (73% [95%-CI: 63-81] vs. 73% [95%-CI: 69-77], $p=.99$, OR=0.97 [95%-CI 0.61-1.55], $p=.90$). However, Likewise, RFS (28 months [95%-CI: 17-50] vs. 17 months [95%-CI: 14-24], $p=.22$, HR=0.92 [95%-CI: 0.68-1.23], $p=.57$) and OS (26 months [95%-CI: 22-46] vs. 19 months [95%-CI: 16-27], $p=.21$, HR=0.89 [95%-CI: 0.68-1.17], $p=.40$) were not significantly different between the groups. Likewise, for patients with R172K mutation, although this difference was not statistically
significant (Fig. 4B). However, in multivariate analysis including age, WBC, ELN risk, type of AML, and mutational variants of $\text{mIDH}_1$ and $\text{mIDH}_2$, $\text{IDH}_2$-R172K was identified as an independent predictor of improved RFS (HR= 0.675 [95%-CI: 0.50-0.92], $p= .013$) and OS (HR= 0.737 [95%-CI: 0.57-0.95], $p=.018$) compared to other $\text{mIDH}_1/2$ (supplemental Tbl. 3.3).

As only two patients with the $\text{IDH}_2$-R172K mutation were in the favourable-risk group, we focused on the ELN2017 intermediate- and adverse-risk groups in more detail to investigate the impact of different $\text{IDH}_2$ mutations. While again no difference was observed in the CR rate, OS was significantly longer in patients harbouring $\text{IDH}_2$-R172K mutations (n=105) in univariate testing (26 months [95%-CI: 22-49] vs. 13 months [95%-CI: 10-17], HR=0.68 [95%-CI 0.5-0.9], $p=.003$) compared to those with the R140Q mutation (n=231) (Fig. 4). In a multivariate analysis including age, WBC, type of AML, ELN2017 risk as well as the different subtypes of $\text{mIDH}_1/2$, we found that patients harbouring the R172 mutation had a significant improved OS compared to wt $\text{IDH}_1/2$ patients with a HR of 0.72 (95%-CI: 0.56-0.93, $p= .012$, supplemental Tbl. 3.3). In contrast, the R140Q mutation as well as $\text{IDH}_1$ mutations did not have a significant impact. This effect was more pronounced within the ELN2017 adverse-risk category where the $\text{IDH}_2$-R172K was associated with a significantly better OS (HR=0.59 [95%-CI: 0.41-0.86], $p<.015$) while within the ELN2017 intermediate-risk category there was a trend towards improved OS for $\text{IDH}_2$-R172K (HR=0.73 [95%-CI: 0.52-1.04], $p=.3108$) (Fig. 2B, Fig.5) in univariate analysis.

Based on the current ELN2017 classification, the treatment of patients with FLT3 and/or NPM1 mutation is clearly defined. Given the strong correlation between $\text{IDH}_2$ mutation subtypes and NPM1 and FLT3 mutations, we aimed to specific impact of
IDH1/2 mutations in investigated the subset of patients with mIDH2 but without NPM1 or FLT3-ITD mutations \((n=294)\). While again CR rate did not differ for R172K compared to R140Q, RFS (33 months [95%-CI: 17-50] vs. 12 months [95%-CI: 9-18], \(p<.01\)), and OS (27 months [95%-CI: 23-52] vs. 14 months [95%-CI: 10-19], \(p<.01\), \(OR=0.68\) [95%-CI:0.50-0.93], \(p=.02\)) were significantly better for R172K, irrespectively of ELN2017 risk groups (Supplement Fig.1).

**Co mutations in IDH1/2 patients and effect on outcome**

Due to the heterogenous co-mutation spectrum of the different IDH mutations subtypes, we investigated the impact of these mutations on outcome (restricted to a prevalence of >15% per subgroup). Next-Generation Sequencing showed frequent co-mutations of mIDH variants predominantly in epigenetic modifiers, especially DNMT3A for all variants, while mutations in genes affecting signaling pathway were most frequently found in mIDH1-R132H. NPM1 was frequently associated with mIDH1-R132C, -R132H and mIDH2-R140Q, however it was only very rarely found in patients with it was almost exclusive with mIDH2-R172K (Fig. 3A).

The results of this analysis clearly indicated a profound effect of the presence of NPM1 mutations on outcome, irrespective of the subbed accompaning mutational variant of mIDH. We also saw a negative prognostic effect of the presence of DNMT3a mutations in patients with IDH1 R312C. None of the other common co-mutations tested had a significant effect in any of the given subgroups (Fig. 3B).
DISCUSSION

We have analyzed a cohort of 4930 patients diagnosed with AML with respect to their IDH1/2 mutational status. In concordance with recent reports, we found IDH1/2 to be mutated in ~20% of AML cases with mIDH2 being slightly more common than mIDH1. Overall, mIDH1/2 was associated with a significantly lower WBC, a higher proportion of cases with normal karyotype and was more often accompanied by NPM1 mutations. In general, there was no difference in outcome between mIDH1/2 and wtIDH1/2 patients in our analysis. As previous reports showed conflicting results concerning the prognostic value of IDH1/2 mutational status on outcome with several reports suggesting an adverse impact, while others found a beneficial/favorable or no impact at all, we focused on the mutational variants of mIDH1 and mIDH2.

We found comparable proportions of different IDH gene variants as reported in previous cohorts. Patients with IDH1-R132C were significantly older, had fewer NPM1 and FLT3-ITD mutations and were less likely to have a normal karyotype. Hence, they were underrepresented in the favorable-risk group according to ELN2017 when compared to other mIDH1 variants. While CR rate for patients with mIDH1-R132C was lower in comparison to IDH1-R132H, RFS and OS did not differ. Wagner et al. also did not report an adverse outcome for IDH1-R132C, but identified an adverse impact on outcome for a single nucleotide polymorphism located in codon 105 in the same exon as the IDH1 R132 variant.

The mIDH2 variant R172 was recently suggested as a new provisional AML entity given its co-mutational landscape and improved outcome. Papaemmanuil et al. analyzed 1540 AML samples and found AML with IDH2-R172 (1%) to be mutually exclusive with NPM1 and other class-defining lesions, Meggendorfer et al.
demonstrated a favorable outcome for patients harboring the \textit{IDH2-R172} mutation in a study population of 306 \textit{mIDH1/2} de novo AML patients. These results, however, are not undisputed. The accumulation of the oncometabolite 2-HG leads to enhanced proliferation and blocked differentiation of immature hematopoietic cells and \textit{IDH2-R172} has been shown to induce higher levels of (R)-2-HG than \textit{IDH2-R140}. Serum-2-HG has been shown to be a prognostic indicator with higher levels of 2-HG yielding unfavorable outcome. DiNardo et al. found a trend for inferior OS for AML patients harboring \textit{IDH2-R172} (n=9/223) in CR after induction chemotherapy who showed higher levels of serum-2-HG. Regarding \textit{mIDH1-R132H}, Losman et al. demonstrated increased 2-HG levels compared to wtIDH in an in vitro model with TF-1 erythroleukemia cells and report a blockage of differentiation in hematopoiesis triggered by the R-enantiomer of 2-HG. However, further evidence is needed to provide a better molecular understanding of the interplay between \textit{IDH} mutational subtypes and 2-HG activity, especially with respect to clinical outcomes. Recently, Duchmann et al. reported the impact of \textit{IDH1}, \textit{IDH2-R140} and \textit{IDH2-R172} associated with different co-mutations. The proportions of different \textit{IDH} variants were comparable to our study. In line with our study, Duchmann et al. reported \textit{IDH2-R172} to be associated with fewer co-mutations and to be mutually exclusive with \textit{NPM1}. In their analysis, co-mutations of \textit{NPM1} and \textit{IDH2-R140} or \textit{IDH1-R132} were associated with higher rates of CR and co-mutations of \textit{NPM1} and \textit{IDH2-R140} had significantly prolonged OS, but in contrast to our findings and other recent studies they did not find an association with favorable outcomes for \textit{IDH2-R172}. While Duchmann et al. refer to the ELN2010 classification for subgroup analysis, we used the more recent ELN2017 classification.
In our study, patients with IDH2-R172K showed lower WBC, a lower rate of normal karyotype and were very rarely accompanied by NPM1 and FLT3-ITD mutations. Within the ELN2017 adverse-risk group, IDH2-R172K was associated with a significantly improved RFS and OS, while in ELN2017 intermediate-risk patients there was a trend towards improved RFS and OS, although statistical significance was not reached, even in this large data set. First, this provides further evidence for improved outcome of AML with IDH2-R172K without other class-defining lesions, thereby yielding potential implications in future patient care and treatment selection. Second, this highlights the need for coordinated multicenter ‘big data’ efforts like the HARMONY consortium⁴¹ to illuminate the clinical and biological importance of rare mutations in myeloid neoplasms.

It is important to be noted, that patients in our study were not treated with specific IDH inhibitors. The advent of targeted therapy with mIDH inhibitors like ivosidenib⁸ and enasidenib⁹ warrants new studies to evaluate the outcome for different mutational sub-variants of mIDH1/2 mutations in response to selective inhibitors.

Further, in older AML patients ineligible for intensive chemotherapy IDH mutational status has an impact on response to therapy with hypomethylating agents (HMA) and/or the BCL2 inhibitor venetoclax⁴². For venetoclax, as single agent or in combination with HMA, several recent studies found significantly improved response rates and OS in older AML patients harboring mIDH1/2, especially for mIDH2⁴³-⁴⁷.

A variety of ongoing trials is set to further illuminate the effects of targeted therapies and hypomethylating agents in mIDH AML with some specifically investigating the impact of different mutational variants on treatment response and outcome (NCT 03471260⁴⁸, NCT 02677929⁴⁹, NCT 03683433, NCT 03383575, NCT 02719574, NCT 03173248).
In conclusion, we have analyzed a large cohort of AML patients for the prevalence and prognostic impact of IDH mutations. A detailed analysis of different mutation variants revealed distinct clinical and co-mutational features of miIDH1-R132C and we provide additional evidence in support of delineating miIDH2-R172K as a distinct entity based on its co-mutational landscape and significant impact on outcome. The differences in outcomes of distinct mutation variants of miDH need to be considered in future trials. Our analysis serves as a benchmark for future studies incorporating novel agents to show improvements compared to conventional intensive regimens.

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Table 1. Patient characteristics

| Characteristic                  | All patients analyzed for IDH | N = 4930 |
|---------------------------------|-------------------------------|---------|
| Age, median (IQR)               | 56 (45-66)                    |         |
| Female sex, n/N (%)             | 2429/4930 (49.3)              |         |
| Disease status, n/N (%)         |                               |         |
Table 2. Patient characteristics (A) and outcome (B) by IDH mutation type

| IDH1/2 WT | IDH1 R132C | IDH1 R132H | IDH1 other | IDH2 R172C | IDH2 R140A | p-value |
|----------|------------|------------|------------|------------|------------|---------|
| N = 3946 | N = 179    | N = 177    | N = 67     | N = 110    | N = 446    |         |

| De novo | sAML | aMDS | WBC (G/L), median (IQR) | PLT (G/L), median (IQR) | Bone marrow blasts (%), median (IQR) | Normal karyotype, n/N (%) | Complex karyotype, n/N (%) | Trisomy 8, n/N (%) | ELN risk 2017, n/N (%) | NPM1 mutated, n/N (%) | FLT3-ITD mutated, n/N (%) | CEBPA mutated, n/N (%) | CEBPA, monoallelic mutations, n/N (%) | CEBPA, biallelic mutations, n/N (%) | IDH1 mutated, n/N (%) | IDH2 mutated, n/N (%) | IDH1/2 mutated, n/N (%) | IDH1 and IDH2 mutated, n/N (%) | IDH VAF, median (IQR) | IDH1 mutation type, n/N (%) | IDH2 mutation type, n/N (%) |
|---------|------|------|--------------------------|--------------------------|-------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------------|--------------------------|---------------------------------|---------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------------|--------------------------|
| 3988/4891 (81.5) | 626/4891 (12.8) | 277/4891 (5.7) | 14.7 (3.5-49.4) | 53 (29-84) | 66 (42-81) | 2538/4613 (55) | 452/3626 (12.5) | 387/3626 (10.6) | 1518/4515 (36) | 1628/4515 (36.1) | 1309/4515 (29) | 144/4515 (31.8) | 1688/4910 (22.2) | 324/4682 (6.7) | 108/4682 (24.6) | 128/4682 (26.4) | 425/4930 (8.6) | 515/4930 (10.7) | 14/4930 (0.3) | 38.3 (30-43.3) | 179/423 (42.3) |
| R132C   | R132Q  | R132H | R132S                  | R132S                   | R132S                              | R132S                     | R132S                    | R132S                     | R132S                     | R132S                     | R132S                           | R132S                    | R132S                           | R132S                           | R132S                     | R132S                     | R132S                     | R132S                     | R132S                     |
| 179/423 (42.3) | 28/423 (6.6) | 177/423 (41.8) | 18/423 (4.3) | 20/423 (4.7) | 4/572 (0.7) | 8/572 (1.4) | 438/572 (76.8) | 110/572 (19.3) | 1/572 (0.2) |
|                | 65 (44-65) | 62 (53-69) | 54 (44-66) | 60 (51-67) | 61 (50-66) | 59 (51-64) |
|----------------|------------|------------|------------|------------|------------|------------|
| Disease status, n/N (%) |            |            |            |            |            |            |
| De novo        | 318/3913 (81) | 149/179 (79.3) | 156/176 (88.6) | 57/67 (85.1) | 94/110 (85.5) | 268/441 (63.4) |
| sAML           | 511/3913 (13.1) | 26/179 (14.6) | 13/176 (7.4) | 9/67 (13.4) | 14/110 (12.7) | 51/441 (11.6) |
| AML            | 234/3913 (6) | 11/179 (6.1) | 7/176 (4) | 1/67 (1.5) | 2/110 (1.8) | 22/441 (5) |

**WBC (G/L), median (IQR)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 15.3 (3.9-50.5) | 4.3 (1.6-25.3) | 22.5 (3.8-67) | 15.2 (3.6-51.9) | 2.3 (1.2-9.2) | 18.8 (4.1-56.6) |
| FLT3 ratio, median (IQR) | 0.6 (0.2-0.8) | 0.3 (0.1-0.5) | 0.7 (0.3-0.9) | 0.4 (0.2-0.7) | 0.5 (0.2-0.7) | 0.5 (0.2-0.7) |
| NPM1           | 33/152 (21.7) | 86/155 (55.6) | 31/59 (52.5) | 2/8 (25) | 3/100 (3) | 177/405 (43.7) |

**PLT (G/L), median (IQR)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 343/3913 (8.8) | 71/179 (4.1) | 51/277 (5.6) | 53/152 (3.5) | 27/324 (8.5) | 243/441 (5.5) |
| FLT3 ratio, median (IQR) |            |            |            |            |            |            |

**Bone marrow blasts (%), median (IQR)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 63 (40.6) | 71 (55.46) | 70 (51.84) | 64 (60.88) | 70 (43.81) | 64 (44.46) |

**Normal karyotype, n/N (%)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 1891/3717 (51) | 101/159 (63.3) | 132/158 (83.5) | 44/61 (72.1) | 58/101 (57.4) | 304/412 (73.8) |

**Complex karyotype, n/N (%)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 424/2549 (14.4) | 8/127 (6.3) | 5/127 (3.9) | 1/48 (2.1) | 3/74 (4.1) | 11/256 (4.3) |

**Immature, n/N (%)**

|                | 393/3913 (10.1) | 71/179 (4.1) | 51/277 (5.6) | 53/152 (3.5) | 27/324 (8.5) | 243/441 (5.5) |

**ELN risk 2017, n/N (%)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| Favorable      | 1234/3940 (33.9) | 33/152 (21.7) | 98/155 (63.2) | 31/59 (52.5) | 2/8 (25) | 177/405 (43.7) |
| Intermediate   | 1280/3640 (35.2) | 78/152 (51.3) | 44/155 (28.4) | 21/55 (36.8) | 63/100 (63) | 141/405 (34.8) |
| Adverse        | 1126/3640 (30.9) | 41/152 (26.7) | 13/155 (8.4) | 6/38 (10.3) | 35/100 (35) | 87/405 (21.5) |

**APM4 mutated, n/N (%)**

|                | 1110/3914 (28.4) | 43/158 (24.2) | 125/176 (71) | 43/67 (64.2) | 2/110 (1.8) | 220/446 (49.4) |

**PLT/THF mutated, n/N (%)**

|                | 890/3926 (22.7) | 181/179 (10.1) | 47/176 (26.7) | 18/67 (26.9) | 5/110 (4.5) | 106/446 (24.2) |

**PLT ratio, median (IQR)**

|                | 0.6 (0.3-0.8) | 0.3 (0.1-0.5) | 0.7 (0.3-0.9) | 0.4 (0.2-0.7) | 0.5 (0.2-0.7) | 0.5 (0.2-0.7) |

**CEBPA4 mutated, n/N (%)**

|                | 268/3886 (7.4) | 7/177 (4) | 1/175 (0.6) | 4/67 (6) | 6/110 (5.5) | 17/442 (3.8) |

**IDH2 VAF, median (IQR)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 37.2 (27.6-41) | 31.5 (25.4-42) | 40 (28.6-47.6) | 38.3 (31.2-45) | 39 (32.8-45) | 35 (32.8-45) |

**Allogeneic HSC in CR1, n/N (%)**

|                | 732/3946 (18.8) | 25/179 (14) | 23/177 (13) | 9/67 (13.4) | 2/110 (19.1) | 65/446 (14.6) |

**B) Outcome by IDH mutation type**

|                | 2830/3946 (73.3) | 110/179 (61.8) | 136/177 (76.6) | 46/67 (68.7) | 80/110 (72.7) | 327/446 (73.3) |

**CR1, n/N (%)**

|                | 19.7 (18.1-21.4) | 14.7 (12.2-21.9) | 23 (16.4-36.1) | 18.7 (13.3-61.4) | 25.6 (21.6-46.3) | 18.9 (15.7-27.4) |

**OS (months), median (95% CI)**

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
| IDH            | 0.0674     |            |            |            |            |            |

**Figure legends**

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Fig. 1 Overall survival according to IDH mutations
Kaplan-Meier plot for overall survival of AML patients with mutated IDH1 (green), mutated IDH2 (blue), and wild-type IDH1/2 (orange); time in months.

Fig. 2 Overall survival according to different mutational subtypes of mIDH in ELN2017 risk categories
Kaplan-Meier plots for overall survival of AML patients according to ELN2017 favorable-, intermediate- and adverse-risk categories A) for patients with mutated IDH1 (green), mutated IDH2 (blue), and wild-type IDH1/2 (orange) and B) for respective mutational variants of IDH1/2: IDH1-R132H (green), IDH1-R132C (gold), IDH1-other (R132G, R132S, R132L; turquoise), IDH2-R140 (blue), IDH2-R172 (purple), wild-type IDH (orange). p-values were determined with the log-rank test; time in months.

Fig. 3 Heatmap of frequent co-mutations of mIDH mutational subtypes and impact on survival
A) Heatmap grouped for epigenetic, signaling, splicing, transcription, and cohesion pathways for the IDH1/2 mutational subtypes. B) OS analysis on the impact of frequent co-mutations

Fig. 4 Overall survival for all patients according to IDH1 and IDH2 mutation
Kaplan-Meier plots for overall survival of AML patients with A) mutated IDH1: IDH1-R132C (green), IDH1-R132H (blue), IDH1-other (R132G, R132S, R132L) and wild-type IDH (orange); B) mutated IDH2: IDH2-R140 (green), IDH2-R172 (blue), wild-type IDH (orange); time in months.
Fig. 5 Overall survival according to *IDH2* mutational status in ELN2017 intermediate- and adverse-risk patients

Kaplan-Meier plots for overall survival of AML patients with the ELN2017 intermediate- and adverse-risk group in regard to mutated *IDH2*-R140 (green), *IDH2*-R172 (blue) and wild-type-IDH (orange); time in months.
