CLINICAL AND BIOLOGICAL IMPLICATIONS OF ANCESTRAL AND NON-ANCESTRAL *IDH1* AND *IDH2* MUTATIONS IN MYELOID NEOPLASMS

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Abstract

Mutations in isocitrate dehydrogenase 1/2 ($IDH1/2^{MT}$) are drivers of a variety of myeloid neoplasms. As they yield the same oncometabolite, D-2-hydroxyglutarate, they are often treated as equivalent, and pooled. We studied the validity of this approach and found *IDH1/2* mutations in 179 of 2119 myeloid neoplasms (8%). Cross-sectionally, the frequencies of these mutations increased from lower- to higher-risk disease, thus suggesting a role in clinical progression. Variant allelic frequencies indicated that $IDH1^{MT}$ and $IDH2^{MT}$ are ancestral in up to 14/74 (19%) *vs.*

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SUPPLEMENTARY INFORMATION

'Supplementary information is available at Leukemia's website'

34/99 (34%; P=0.027) of cases, respectively, illustrating the pathogenic role of these lesions in myeloid neoplasms. $IDH1/2^{MT}$ was associated with poor overall survival, particularly in lower-risk myelodysplastic syndromes. Ancestral $IDH1^{MT}$ cases were associated with a worse prognosis than subclonal $IDH1^{MT}$ cases, whereas the position of $IDH2^{MT}$ within clonal hierarchy did not impact survival. This may relate to distinct mutational spectra with more *DNMT3A* and *NPM1* mutations associated with $IDH1^{MT}$ cases, and more *ASXL1, SRSF2, RUNX1, STAG2* mutations associated with $IDH2^{MT}$ cases. Our data demonstrate important clinical and biological differences between $IDH1^{MT}$ and $IDH2^{MT}$ myeloid neoplasms. These mutations should be considered separately as their differences could have implications for diagnosis, prognosis, and treatment with IDH1/2^{MT} inhibitors of $IDH1/2^{MT}$ patients.

Keywords

isocitrate dehydrogenase; acute myeloid leukemia; myelodysplastic syndromes; 2-hydroxyglutarate; clonal hierarchy

INTRODUCTION

Mutations in isocitrate dehydrogenases 1 and 2 ($IDH1^{MT}$ and $IDH2^{MT}$) are implicated in the development of numerous types of cancer, including glioma, chondrosarcoma, cholangiocarcinoma, angioimmunoblastic T-cell lymphoma and certain myeloid neoplasms[1]. Within myeloid malignancies, $IDH1/2^{MT}$ are present in a significant proportion of acute myeloid leukemia (AML) and, while less common, in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN)[2, 3].

$IDH1/2^{MT}$ impart a gain of function by causing single amino acid changes in the active sites of the enzymes. Whereas (wild-type) IDH1/2^{WT} convert isocitrate and NADP$^+$ to α-ketoglutarate (αKG) and NADPH, mutant IDH1/2 convert NADPH and αKG to NADP$^+$ and *D*-2-hydroxyglutarate (*D*-2HG)[4]. *D*-2HG and αKG have similar structures; resultant *D*-2HG accumulation in $IDH1/2^{MT}$ cells inhibits many αKG-dependent dioxygenases, such as TET2 DNA hydroxyl demethylases[5, 6] and Jumanji domain-containing histone demethylases (*e.g.*, KDM6A/UTX and KDM3B/JMJD1B). Dysfunction of these enzymes is considered to be responsible for global DNA hypermethylation, inhibition of differentiation, and preservation of stemness[7, 8]. In addition, *D*-2HG activates, rather than inhibits Egg Laying Defective Nine (EGLN), which induces HIF1α degradation and growth factor-independent proliferation[9, 10].

As direct evidence that $IDH1/2^{MT}$ are oncogenic, introduction of $IDH2^{R140Q}$ or $IDH2^{R172K}$ into 10T1/2 mesenchymal progenitor cells yielded an AML-like disease in mice[11–13]. $IDH2^{R140Q}$ was also necessary for AML maintenance in mice[12], indicating that $IDH1/2^{MT}$ may be useful as therapeutic targets. These findings motivated the development of specific inhibitors of IDH1/2-mutant enzymes[14–18]. The currently available inhibitors, AG-221 that targets IDH2^{R140Q} and AG-120 that targets IDH1^{R132H}, restrict the production of *D*-2HG and thereby induce differentiation of AML cells *ex vivo*[15]. Both agents have shown promising preliminary results in phase I clinical trials[19].

Genomic studies of molecular landscapes in human cancer have frequently combined $IDH1^{MT}$ and $IDH2^{MT}$ as a single functional group despite physiological differences: IDH1 is localized in the cytosol and IDH2 in the mitochondrial matrix. Remarkably, the spectrum of cancers and their subtypes differ in the distribution of $IDH1/2^{MT}$. $IDH1^{MT}$ predominates in glioma (95%), chondrosarcoma (95%) and cholangiocarcinoma (80%), whereas the $IDH1^{MT}$:$IDH2^{MT}$ ratio is more balanced or even skewed towards a higher frequency of $IDH2^{MT}$ in AML[1]. In addition, only $IDH2^{MT}$ have been reported in angioimmunoblastic T-cell lymphoma, osteosarcoma and gastric cancer, again suggesting that there are pathophysiologic differences between $IDH1^{MT}$ and $IDH2^{MT}$. Biochemical investigations have shown that the specific amino acid substitutions IDH1^{R132H}, IDH1^{R132C}, IDH2^{R140Q} or IDH2^{R172K} differ in D-2HG production potency. IDH2^{R172K} is the most potent, followed by IDH1^{R132H}, IDH1^{R132C} and IDH2^{R140Q}. There are more than 60 different αKG-dependent dioxygenases that can theoretically be inhibited by D-2HG with likely distinct IC$_{50}$ values for D-2HG. Thus, different $IDH1/2^{MT}$ inhibit different sets of αKG-dependent enzymes and this may partially explain differing distributions of $IDH1/2^{MT}$ between cancers[1].

To investigate the clinical impact of $IDH1/2^{MT}$ in myeloid neoplasms, we studied a cohort of 2119 patients with myeloid neoplasms. We performed whole-exome/targeted multi-amplicon sequencing on the samples obtained from these patients from different institutions. We compared the mutational landscapes of $IDH1^{MT}$ and $IDH2^{MT}$ samples, their clinical associations, overall patient survival, and clonal hierarchies. Our aim was to provide insights into $IDH1/2^{MT}$ myeloid malignancy pathogenesis, especially with respect to the clonal architecture of $IDH1/2^{MT}$ cases, and to determine whether $IDH1$ and $IDH2$ mutations should be grouped or considered separately, particularly with respect to the potential benefits of IDH1/2-mutant inhibitors in various clinical contexts.

METHODS

Patient population

Blood and bone marrow samples were obtained from 2119 patients diagnosed with lower-risk MDS (868) and higher-risk MDS (536), defined per World Health Organization classification; secondary AML (sAML; 153); myeloproliferative neoplasms (MPN; 63); MDS/MPN (165); or primary AML (pAML; 334). From this cohort, 418 samples from 409 patients were subjected to whole-exome sequencing (WES). Furthermore, 1815 samples from 1761 patients were tested for a subset of genes (including $IDH1$ and $IDH2$; Supplementary Table S1) using targeted sequencing (TS). The total of 2179 samples listed above is greater than our total of 2119 samples because serial samples of 60 patients were included in the analysis. The sum of 409 patients subjected to WES and 1761 patients subjected TS is larger than 2119 because the WES and TS cohorts are partially overlapping.

Informed consent was obtained from patients according to protocols approved by the institutional review boards and in accordance with the Declaration of Helsinki. These patients were seen and treated at the Cleveland Clinic, the University of Tokyo and the Munich Leukemia Laboratory, diagnosis was confirmed on the basis of World Health Organization classification criteria. Patients with refractory anemia (RA), RA with ringed

sideroblasts (RARS), refractory cytopenias with multilineage dysplasia (RCMD), RCMD with ringed sideroblasts (RCMD-RS) and MDS-unclassifiable (MDS-u) were classified as lower-risk MDS. Those with refractory anemia with excess blasts (RAEB-1 and RAEB-2) were classified as higher-risk MDS. Details of MDS/MPN and MPN subcategories are provided in Supplementary Table S2. Clinical details of the patients, including blood counts, demographics, and survival times, were obtained from medical records.

Sequencing technology

For WES, DNA was obtained from tumor and germline paired samples. The 50 Mb of protein-coding sequences was enriched from total genomic DNA by liquid-phase hybridization using SureSelect (version 4) (Agilent Technology, Santa Clara, CA), followed by massively parallel sequencing with HiSequation 2000 (Illumina, San Diego, CA). Somatic mutations were called as previously described[20, 21], using the GATK algorithm (Broad Institute). To minimize false positives and focus on the most prevalent or relevant somatic events, we implemented a rational bioanalytic filtering approach and applied heuristic bioanalytic pipelines. For confirmation of somatic mutations, we analyzed paired germline DNA from CD3^{+} lymphocytes. For TS of a specific panel of genes we applied multi-amplicon deep sequencing (TrueSeq; Illumina) to frequently-affected exons of 60 selected genes. The sequencing libraries were generated according to an Illumina paired-end library protocol and subjected to deep sequencing on MiSeq (Illumina) instrumentation according to standard protocol. Copy number information at the locus of each mutation was assessed as previously reported[22]. For samples sequenced in Munich, 104 known or putative mutational gene targets in MDS were examined for mutations from the cohort using massively parallel sequencing (Illumina) or SureSelect (Agilent) captured target sequences[23]. High-probability oncogenic mutations were called by eliminating sequencing/mapping errors and known/possible SNPs based on available databases and frequencies of variant reads. Genomic copy number status was calculated by directly enumerating corresponding sequencing reads in each exon.

Analysis of clonal architecture

Variant allelic frequencies (VAF) were calculated as the fraction of mutated reads of the total number of reads of a certain gene. VAFs were adjusted using copy number information at the locus of each mutation. We recapitulated the clonal architecture of a patient using these copy number-adjusted VAFs. Ancestral *vs* subclonal events were determined using a copy number-adjusted VAF difference between two events, with a higher VAF indicating ancestral origin. We used an VAF threshold of 5% (absolute) to reliably discriminate ancestral from subclonal events. Events below this threshold were considered to be of undeterminable ancestry. This threshold was chosen based on previous studies[24] and statistical calculations of our own data, based on the average depth of sequencing in our samples.

Statistical analysis

Comparisons of proportions were performed using the χ^2 and Fisher's exact tests and differences in values and in ranks were assessed by Student t tests and Mann-Whitney U tests, respectively. Cox models were used to identify correlates with overall survival. Kaplan-Meier curves were generated to graphically depict survival differences. Throughout,

2-sided tests were used with significance defined as $\alpha<.05$. These analyses were performed using SPSS, GraphPad Prism 6 and the R statistical programming language.

RESULTS

Clinical characterization of the *IDH1/2* mutation

Among the 2119 patient samples tested (Table 1), we identified *IDH1* and *IDH2* mutations in 78 (4%) and 101 (5%) cases, respectively, in a mutually exclusive fashion. Of the *IDH1*^{MT} cases, *IDH1*^{R132C} (49%, n=36) and *IDH1*^{R132H} (38%, n=28) were the most common; 12% and 1% harbored *IDH1*^{R132X} and *IDH1*^{T98I}, respectively. Amongst *IDH2*^{MT} cases, *IDH2*^{R140Q} (85%, n=84) was the most frequent mutation, followed by *IDH2*^{R140X} (5%, n=5), *IDH2*^{R172K} (9%, n=9) and *IDH2*^{R172W} (1%, n=1) (Figure 1A). *IDH1*^{MT} patients were younger than *IDH1/2*^{WT} patients (*P*=.01), an observation similar to that reported for *IDH1*^{MT} glioblastoma patients[25]. Across the spectrum of MDS, the frequency of *IDH1/2*^{MT} increased with progression, as *IDH1*^{MT} occurred in 2% of lower-risk MDS cases, in 3% of higher-risk MDS cases and in 7% of sAML cases. The average number of mutations in each of these categories as determined by WES did not increase as much with 21, 30, and 27, respectively (Supplementary Figure S1). *IDH2*^{MT} frequency increased as MDS progressed (in 2% of lower-risk MDS, in 7% of higher-risk MDS and in 6% of sAML cases). These findings suggest that in *IDH1/2*^{MT} MDS cases, a high proportion of *IDH1/2*^{MT} are associated with progression to advanced disease. In addition, *IDH1* and *IDH2* mutation frequencies were relatively lower in MDS/MPN and MPN patients, and showed the highest frequency in pAML patients (Figure 1B). While *IDH2*^{MT} were enriched for a normal karyotype, *IDH1*^{MT} patients more often had a deletion of chromosome Y in comparison with *IDH1/2*^{WT} patients. Other clinical characteristics were similar between *IDH1/2*^{MT} and *IDH1/2*^{WT} patients (Table 1; Supplementary Table S3). More detailed clinical information on *IDH1*MT and *IDH2*MT patients with myeloid malignancies is shown in Supplementary Tables S4 and S5.

Molecular characterization of *IDH1* and *IDH2*-mutated myeloid neoplasms

To characterize the molecular features of patients with myeloid neoplasms with *IDH1*^{MT} *or IDH2*^{MT}, we analyzed associations between *IDH1/2*^{MT} and other mutational events (Figure 2A; Supplementary Table S6). Overall, *NPM1* mutations were more frequent in both *IDH1*^{MT} and *IDH2*^{MT} cases, compared with *IDH1/2*^{WT} patients (Figure 2B and Supplementary Tables S6-8). Other somatic events were not significantly associated with both *IDH1*^{MT} and *IDH2*^{MT} cases compared with *IDH1/2*^{WT} cases.

However, when *IDH1*^{MT} cases were compared with *IDH1/2*^{WT} cases, they were enriched for *DNMT3A* (*P*<.0001), *PHF6* (*P*=.0006) and *FLT3* (*P*=.0195) mutations, whereas *IDH2*^{MT} cases were enriched for *ASXL1* (*P*<.0001), *SRSF2* (*P*<.0001), *RUNX1* (*P*=.0034) and *STAG2* (*P*<.0001) compared with *IDH1/2*^{WT} cases. *NPM1* mutations occurred more frequently in *IDH1*^{MT} patients than in *IDH2*^{MT} patients (*P*=0.045), whereas *IDH2*^{MT} patients were enriched for *ASXL1* (*P*=0.016), *SRSF2* (*P*=0.007) and *STAG2* (*P*=0.019) mutations compared with *IDH1*^{MT} patients. Cohesin complex mutant carriers (*STAG2, RAD21, SMC3*) were more frequent in *IDH1*^{MT} (*P*=.025) and *IDH2*^{MT} (*P*<.0001) cases

compared with $IDH1/2^{WT}$ cases. Conversely, *SF3B1* and *TET2* mutations occurred less frequently in $IDH1^{MT}$ (*P*<.0001 and *P*=.023) and in $IDH2^{MT}$ (*P*<.0001 and *P*<.0001) cases than in $IDH1/2^{WT}$ cases. Although we observed a negative correlation between *IDH1* and *TET2* mutations, *TET2* mutations, if present, were less infrequent in $IDH1^{MT}$ patients than in $IDH2^{MT}$ patients (Figure 2C).

Analysis of ancestry and clonal architecture of *IDH1/2*-mutated patients

Using copy number-adjusted VAF, we reconstructed the clonal architecture of $IDH1/2^{MT}$ patients to establish whether an $IDH1^{MT}$ or $IDH2^{MT}$ was an ancestral or subclonal mutation. Clonal hierarchy was further confirmed and refined by serial analyses, performed in 60 exemplary cases (Figure 3C). $IDH1^{MT}$ and $IDH2^{MT}$ were ancestral in 19% and 34% of $IDH1/2^{MT}$ patients and subclonal in 55% and 45%, respectively (Figure 3A). As we used a cut-off value of 5% (absolute) in VAF difference, the ancestral *vs.* subclonal status of some $IDH1/2^{MT}$ remained "undetermined" when there were small VAF differences between *IDH1/2* mutations and other mutations (see Methods). A lower proportion of $IDH1^{MT}$ cases were of ancestral origin compared to $IDH2^{MT}$ cases (*P*=.027). We also observed differences in the clonal succession between specific $IDH1/2^{MT}$ variants (Figure 3B). Whereas $IDH1^{R132H}$ and $IDH1^{R132C}$ mutations were ancestral in equal frequencies (22% and 21%), other *IDH1* mutations ($IDH1^{T98I}$ (n=1), $IDH1^{R132L}$ (n=1) and $IDH1^{R132S}$ (n=5)) occurred only as subclonal events. Similarly, $IDH2^{R140Q}$ mutations were ancestral in 39% of $IDH2^{R140Q}$-mutated cases, whereas other $IDH2^{R140}$ mutations ($IDH2^{R140L}$ (n=2) and $IDH2^{R140W}$ (n=3)) were always subclonal. $IDH2^{R172}$ mutations were ancestral in only 10% of $IDH2^{R172}$-mutated patients. In the different types of myeloid neoplasms we did not observe differences in the distribution between ancestral *vs.* subclonal mutations in *IDH1/2* and there was no difference in the mean VAF of $IDH1^{MT}$ vs $IDH2^{MT}$ (Supplementary Figure S2A-C). We observed higher $IDH1/2^{MT}$ VAFs in patients with ancestral $IDH1/2^{MT}$ than subclonal $IDH1/2^{MT}$ (Figure 3C and Supplementary Figure S2D). $IDH1/2^{MT}$ VAFs were highest in patients with $IDH1/2^{MT}$ of undeterminable ancestry, which may reflect a higher disease burden in patients when $IDH1/2^{MT}$ cooperate with other mutations. WES analyses revealed that $IDH1^{MT}$ and $IDH2^{MT}$ were ancestral in 1/21 (5%) and 2/24 (8%) of $IDH1/2^{MT}$ cases, respectively, whereas TS suggested that $IDH1^{MT}$ and $IDH2^{MT}$ were ancestral in 13/53 (25%) and 32/75 (43%) of cases (*P*=.0002). This difference probably reflects missed ancestral mutations in the TS samples (Supplementary Table S9). The clonal architecture of representative $IDH1/2^{MT}$ patients (out of 60 studied) that were serially sequenced is shown in Figure 3D and Supplementary Figure S2E.

In subclonal $IDH1/2^{MT}$ cases, we investigated the corresponding ancestral events. These analyses showed heterogeneity, with 19 and 20 different ancestral mutations in 35 and 38 cases with subclonal $IDH1^{MT}$ or $IDH2^{MT}$, respectively. Subclonal $IDH1^{MT}$ were most often preceded by an ancestral *DNMT3A* mutation (30%; Supplemental Figure S2F), whereas ancestral *RUNX1* mutations most frequently preceded a subclonal $IDH2^{MT}$ (17%; Supplemental Figure S2G). We observed a *RUNX1* mutation preceding a subclonal $IDH1^{MT}$ only once. Hierarchical clonal analyses of neoplasms with indeterminable $IDH1/2^{MT}$ clonal architecture (small VAF differences between *IDH1/2* mutations and other mutations) may provide insight into which mutations co-operate (in the case of enrichment of co-occurring

mutations; Figure 2) or compete (in the case of mutual exclusivity with co-occurring mutations) with $IDH1/2^{MT}$. *DNMT3A* mutations were most frequently the "main competitor" of $IDH1^{MT}$ and $IDH2^{MT}$ of undetermined ancestry (Supplemental Figure S2H-I). We did not observe a single *RUNX1* mutation that competed with $IDH2^{MT}$, indicating that VAF differences between ancestral *RUNX1* mutations and subclonal $IDH2^{MT}$, and *vice versa*, are rather large.

Prognostic effect of *IDH1/2* mutations in myeloid neoplasms

In our cohort, $IDH1/2^{MT}$ were associated with worse overall survival (Figure 4A). In subset analyses, we observed that $IDH1/2^{MT}$ were significantly associated with reduced median overall survival in lower-risk MDS patients (41 *vs.* 66 months, *P*=.03), but not in higher-risk MDS (34 vs 30 months, *P*=.91), sAML (26 vs. 19 months, *P*=.74) or pAML patients (20 *vs.* 16 months, *P*=.79; Figure 4B-E). This finding further illustrates the role of $IDH1/2^{MT}$ in progression to a more malignant disease. Patients with ancestral $IDH1^{MT}$ tended to have a worse survival than patients with subclonal $IDH1^{MT}$ (23 *vs.* 32 months, P=.09; Figure 4F), whereas there was no survival difference between patients with ancestral *vs.* subclonal $IDH2^{MT}$ (33 vs. 30 months, *P*=.35; Figure 4G).

The association between $IDH1^{MT}$ or $IDH2^{MT}$ and overall survival was investigated separately in low-risk MDS to assess the impact on subsequent outcomes in early disease. Compared with $IDH1/2^{WT}$ patients, we found that $IDH2^{MT}$ patients (30 *vs.* 66 months, *P*=.003), but not $IDH1^{MT}$ patients (42 *vs.* 66 months, *P*=.64), had a worse prognosis (Figure 4H). Because $IDH1^{MT}$ were less frequently ancestral than $IDH2^{MT}$ in lower-risk MDS patients (22% *vs.* 40%), we analyzed whether ancestry determines to what extent $IDH1/2^{MT}$ impacts overall survival. Indeed, patients with ancestral $IDH1^{MT}$ had a worse survival than patients with myeloid neoplasms that had a subclonal $IDH1^{MT}$ (23 *vs.* 42 months, P=.05) or $IDH1/2^{WT}$ patients (23 *vs.* 66 months, *P*=.006; Figure 4I). Ancestral $IDH2^{MT}$ may also be associated with worse prognosis, compared with subclonal $IDH2^{MT}$. However, definitive conclusions from this subset analysis were hindered by the limited number of cases (Supplementary Figure S3).

DISCUSSION

In this report, we describe the clinical and molecular characterization of $IDH1^{MT}$ and $IDH2^{MT}$ patients with myeloid neoplasms. The size of the cohort allowed for the most comprehensive analysis to date of molecular, morphologic and clinical features associated with *IDH1/2* mutations, separately and combined. For the first time, a comprehensive analysis of clonal architecture distinguished ancestral from subclonal somatic lesions and determined differences in their clinical and biological impact. We demonstrate that $IDH1^{MT}$ and $IDH2^{MT}$ can occur as ancestral or subclonal defects. In a substantial proportion of cases, $IDH1^{MT}$ (19%) and $IDH2^{MT}$ (34%) represent ancestral lesions, but more often $IDH1/2^{MT}$ follow other ancestral mutations as subclonal events, likely explaining higher percentages of subclonal events in advanced myeloid disease. When present in lower-risk/early MDS, $IDH1/2^{MT}$ are associated with a poor prognosis, while in higher-risk myeloid neoplasms, prognosis could not be further stratified. Patients with ancestral $IDH1^{MT}$ exhibit worse

survival than those with a subclonal $IDH1^{MT}$ mutation, particularly in lower-risk MDS. Such a difference was not found when ancestral *vs.* subclonal $IDH2^{MT}$ were compared, indicating that $IDH2^{MT}$ more rapidly dominate the clonal hierarchy.

Analyses of mutations that positively or negatively correlate with $IDH1/2^{MT}$ in myeloid neoplasms have heretofore treated $IDH1/2^{MT}$ cases as a single functional entity. In our cross-sectional analysis, this approach of pooling $IDH1/2^{MT}$ was only adequate for their association with *NPM1* mutations, and their mutual exclusivity with *TET2* mutations, as these are the only lesions that were significant for both $IDH1^{MT}$ and $IDH2^{MT}$ separately. In addition, $IDH1^{MT}$ cases were significantly enriched for *DNTM3A* and *PHF6* mutations, and $IDH2^{MT}$ cases for *ASXL1, SRSF2, RUNX1* and *STAG2* mutations. When $IDH1/2^{MT}$ cases would be pooled, all these six mutations are significantly more frequently occurring in $IDH1/2^{MT}$ *vs. IDH1/2*^{WT} cases. This would falsely suggest that they occur more frequently in both $IDH1^{MT}$ and $IDH2^{MT}$ cases, whereas the significant correlation is only true for either $IDH1^{MT}$ or $IDH2^{MT}$ cases. "pAML-associated" *DNMT3A* and *NPM1* mutations occurred significantly more frequently in $IDH1^{MT}$ cases than in $IDH2^{MT}$ cases, whereas the reverse was true for "MDS/sAML/(MPN)-associated" *ASXL1, SRSF2, STAG2* and *RUNX1* mutations. Of note, there were no significant proportional differences with respect to diagnosis between $IDH1^{MT}$ and $IDH2^{MT}$ cases, with only a trend (P=0.051) towards higher-risk MDS patients among $IDH2^{MT}$ cases than among $IDH1^{MT}$ cases. Apparently, the functional cooperation between certain mutations (e.g. *DNTM3A* mutations and $IDH1^{MT}$ or *RUNX1* mutations and $IDH2^{MT}$) in the clonal hierarchy is driving the aforementioned positive correlations without resulting in disease phenotypes that are typical for these lesions.

The differences in at least some of the biologic/clinical features between $IDH1^{MT}$ and $IDH2^{MT}$ cases may stem from the differences in the biochemical consequences of these lesions. For instance, the various IDH1/2^{MT} differ in D-2HG levels they produce, which is likely due to the impact of the specific amino acid substitutions on the catalytic site and thereby the conversion rate of αKG to D-2HG. Whereas IDH1 is cytoplasmic, IDH2 is mitochondrial and αKG levels may not be completely interchangeable between these subcellular compartments, generating different conditions for IDH1^{MT} and IDH2^{MT} to synthetize D-2HG. Furthermore, nuclear DNA and histone demethylases (e.g. TET2, Jumonji) and cytoplasmic prolyl hydroxylases (EGLN) are considered to be drivers of $IDH1/2^{MT}$-induced oncogenesis[1], suggesting that D-2HG mainly functions oncogenically outside mitochondria. It is unknown to what extent D-2HG passes the mitochondrial membrane. Therefore, the impact of the compartmentalization of αKG and D-2HG on the downstream effects of $IDH2^{MT}$ *vs. IDH1*^{MT} is unclear.

D-2HG functions as an oncometabolite that inhibits various αKG-dependent dioxygenases. Whereas the effects of D-2HG on DNA demethylase TET2, histone demethylase JumonjiC and the HIF1α degrader EGLN have been described thoroughly[9, 26, 27], there are in fact over 60 different αKG-dependent human dioxygenases involved in a plethora of cellular functions that may be inhibited, or possibly activated, as in the case of EGLN, by D-2HG. All these enzymes have specific IC_{50} values of D-2HG for inhibition of αKG-dependent enzymes. Thus, each D-2HG concentration (*i.e.* each IDH1/2^{MT} variant) is expected to

inhibit a specific subset of αKG-dependent dioxygenases and alter cellular functions in a variety of ways.

It has been proposed that the αKG-dependent DNA demethylase TET2 is one of the most important downstream targets of *IDH1/2*MT [26, 28]. *D*-2HG inhibits TET2 and results in a genome-wide DNA hypermethylation in *IDH1/2*MT cancers that induces stemness and inhibits differentiation[7]. In our cohort, and that of others[29], *IDH1/2* and *TET2* mutations are mostly mutually exclusive, supporting the notion of similar cellular downstream effects. *TET2* mutations, albeit rare, were more frequent in *IDH1*MT cases (14%) than in *IDH2*MT cases (4%). It is possible that *IDH1*MT result in less TET2 inhibition than *IDH2*MT and that a synergistic/additive effect of co-occuring *IDH1* and *TET2* mutations augments TET2 inhibition. Of note, TET2 is widely held to be a downstream element of the pathogenic cascade induced by *IDH1/2*MT. Despite this, the profoundly distinct nosologic and morphologic spectra associated with *TET2* and *IDH1/2* mutations speak against this notion. Another putative important downstream target of *IDH1/2*MT is UTX/KDM6A[7, 27]. However, we did not observe mutual exclusivity between *IDH1/2* and *UTX* mutations, suggesting that *D*-2HG-mediated UTX inhibition has a different effect than the *UTX* mutations that we observed. Notably, we did not observe a negative correlation between *IDH1/2* and *WT1* mutations, as previously reported[30].

Our study provides the first comprehensive subclassification of *IDH1/2*MT cases with myeloid neoplasms based on their rank within clonal hierarchy, and thus their timing in clonal ontogeny. While cases initiated by *IDH1/2*MT are a distinct subset of myeloid neoplasms, in many instances *IDH1/2*MT are subclonal. The impact of the corresponding ancestral lesions, then, may more profoundly shape the individual neoplastic biology. This observation has clinical implications because, at least theoretically, therapeutic targeting of subclonal lesions is less likely to be curative as the ancestral clone cannot be eliminated. Remissions achieved in such cases thus have a greater likelihood of relapse, and durable clinical benefit may depend on combination therapies that also target the ancestral event(s). In addition, we report that ancestral *IDH1/2*MT are related to poor clinical outcomes, whereas this association is weaker for subclonal *IDH1/2*MT. These findings illustrate that patients with myeloid neoplasms carrying ancestral *IDH1/2*MT are the best candidates for therapy with IDH1/2MT inhibitors. Thus, sequencing that targets mutations frequently co-occurring with *IDH1/2*MT, in particular those which are often ancestral may aid rational clinical treatment of *IDH1/2*MT patients with IDH1/2MT inhibitors.

*IDH1/2*MT occur in a myriad of cancer types. Their biology in glioma and acute myeloid leukemia has been studied most intensively. The distribution of *IDH1/2*MT differs between gliomas and myeloid neoplasms, and fundamental differences exist in *IDH1/2*MT biology between these 2 tumor types. Whereas *IDH1/2*MT are very early events in gliomagenesis and has even been proposed as a canonical ancestral event[31, 32], our findings show that this is in stark contrast to myeloid neoplasms, in which *IDH1/2*MT are ancestral in a minority of cases. In addition, *IDH1/2*MT associate with a strikingly prolonged overall survival in glioma[25, 33], whereas our study, and that of others[34], showed that these mutations are associated with worse prognosis in myeloid neoplasms.

In summary, we present results that demonstrate distinct differences between *IDH1*^{MT} and

IDH2^{MT}. The position in the clonal hierarchy may be important for understanding the impact on the biology and clinical consequences of these mutations, and may refine future treatment of *IDH1/2*^{MT} myeloid neoplasms with IDH1/2^{MT} inhibitors. We conclude that the minority of patients with myeloid neoplasms carrying ancestral *IDH1/2*^{MT} are the best candidates for therapy with IDH1/2^{MT} inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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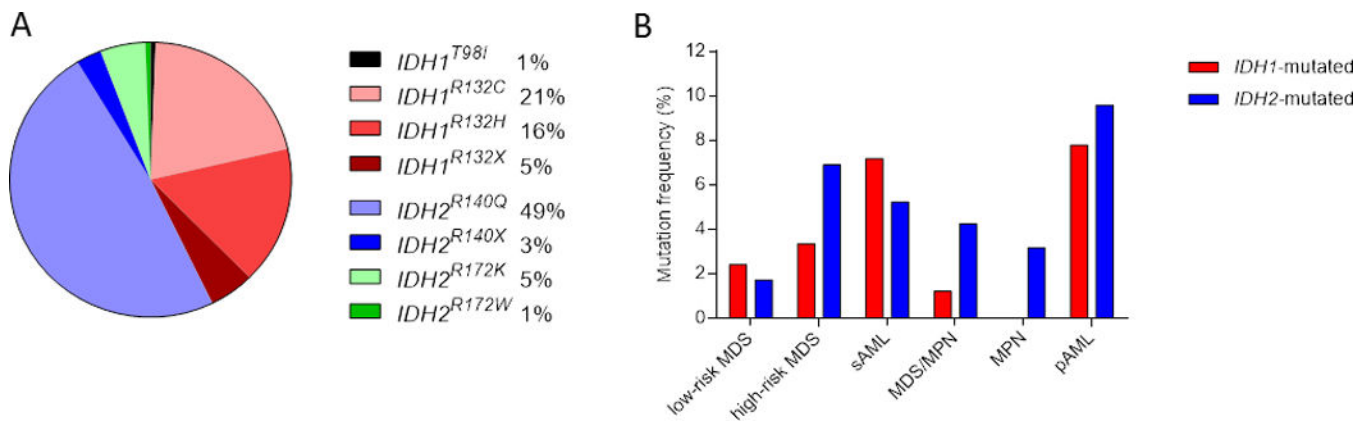
Figure 1. Breakdown of *IDH1/2* mutations among disease types and specific amino acid substitutions

(A) Frequencies of *IDH1* and *IDH2* mutations in various myeloid neoplasms. (B) Pie chart showing the percentages of the specific IDH1/2 mutational amino acid substitutions in the cohort.

Abbreviations: pAML, primary acute myeloid leukemia; sAML, secondary acute myeloid leukemia.
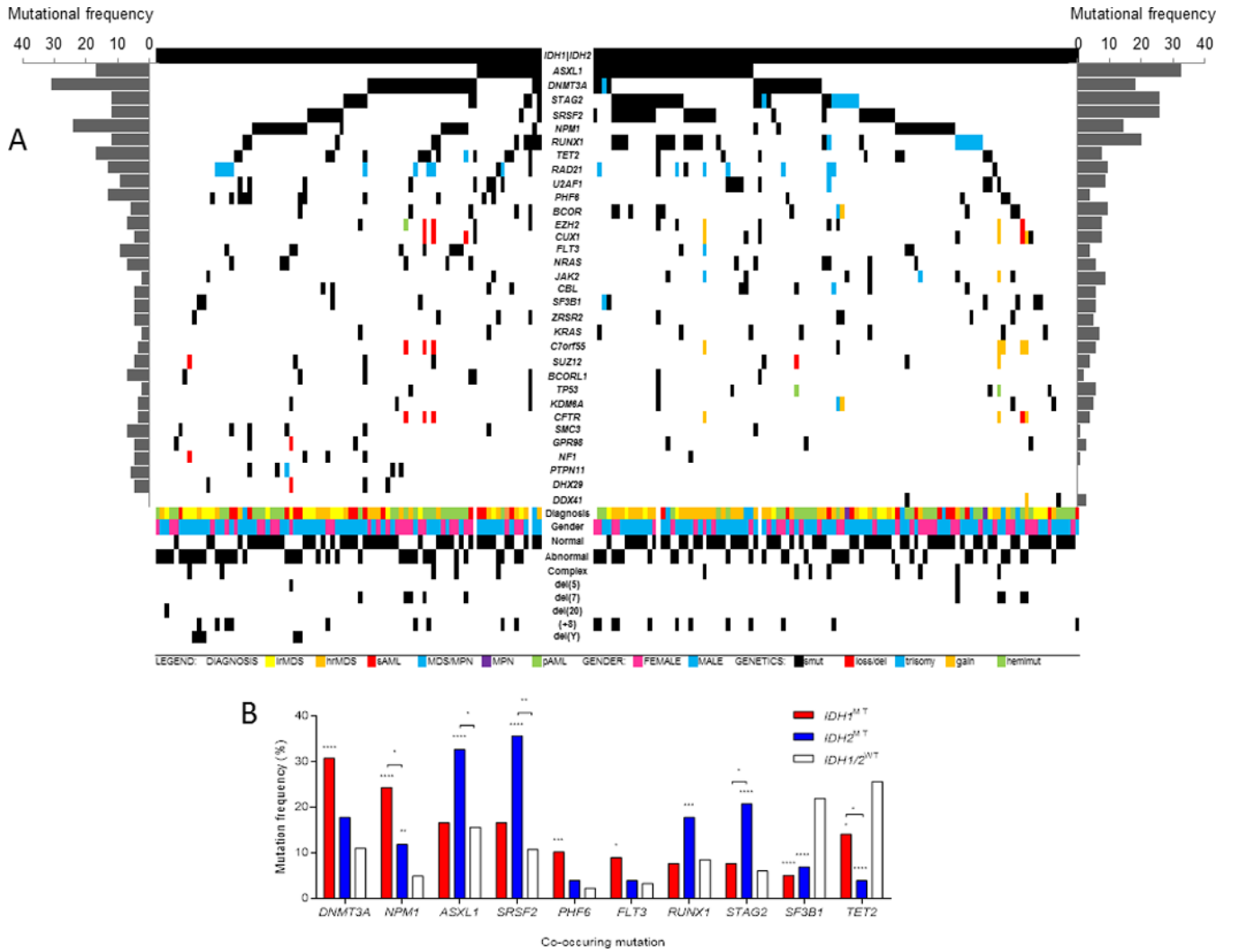
Figure 2. Mutational spectrum and most and least frequently co-occurring mutations in *IDH1/2*-mutated cases

(A) Mutational spectrum and clinical spectrum of *IDH1*^MT cases (left half) and *IDH2*^MT cases (right half) with myeloid neoplasms. (B) Prevalence of co-occurring mutations in *IDH1/2*^MT cases, compared with *IDH1/2*^WT cases.

Abbreviations: lrMDS, low-risk MDS; hrMDS, high-risk MDS; sAML, secondary acute myeloid leukemia; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; pAML, primary acute myeloid leukemia; smut, somatic mutation; del, deletion; hemimut, hemizygous mutation.
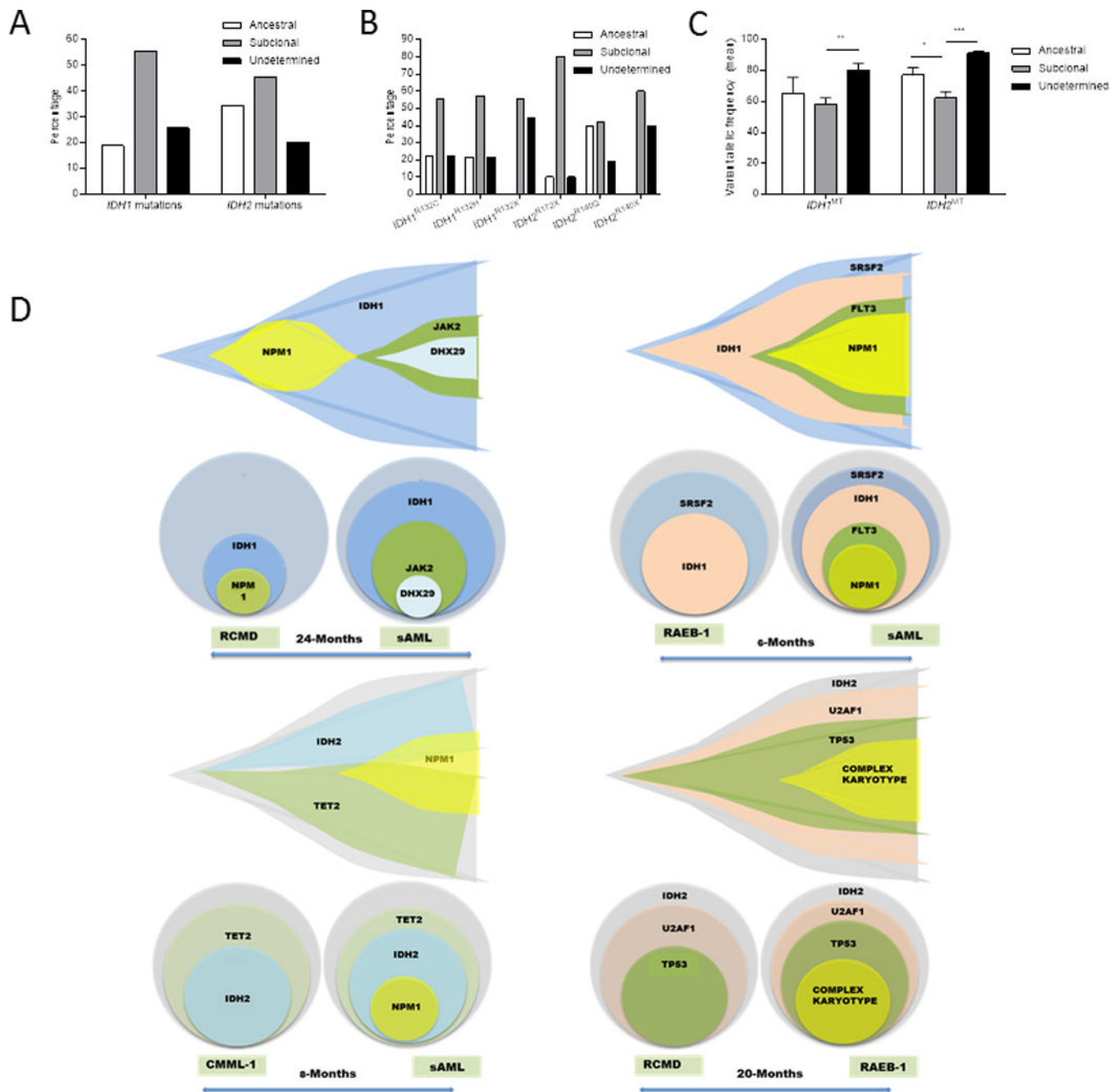
Figure 3. Analysis of clonal architecture of *IDH1/2*-mutated cases

(A) Percentages of *IDH1/2*^MT cases in which the *IDH1/2* mutation was an ancestral event, a subclonal event or an event of undeterminable ancestry, based on the variant allelic frequency of the *IDH1/2* mutations and other co-occurring mutations. (B) As in (A), but with specific IDH1/2 amino acid substitutions. (C) Mean variant allelic frequencies of *IDH1* mutations and *IDH2* mutations in *IDH1/2*^MT cases in which the *IDH1/2* mutation is an ancestral or subclonal event, or of undeterminable ancestry. (D) Fish plots of serially sequenced *IDH1/2*^MT patients.
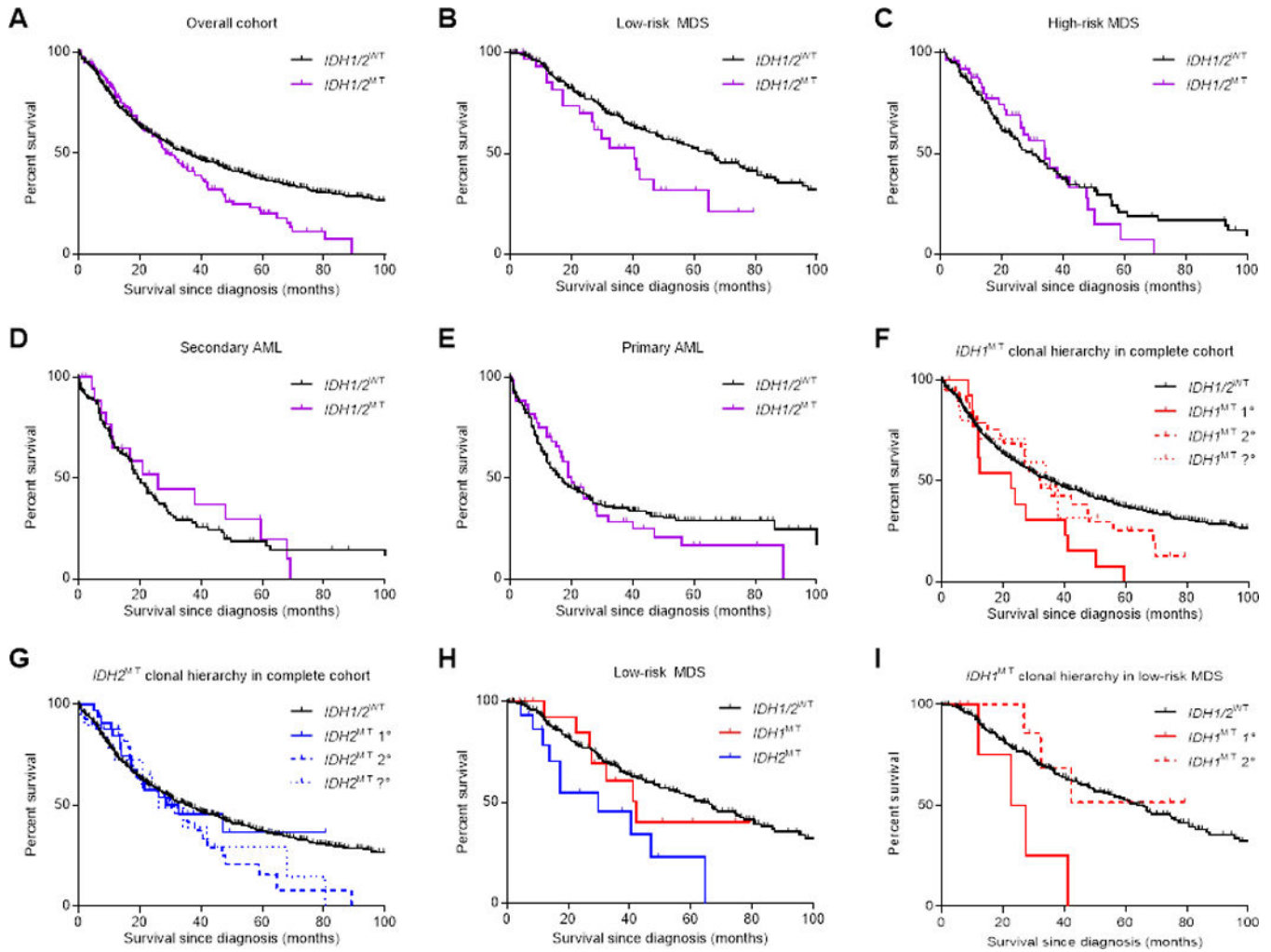
Figure 4. *IDH1/2* mutations and associations with overall patient survival in myeloid neoplasms
(A) Survival data of *IDH1/2*^MT patients with myeloid neoplasms. (B-E) Subset survival analyses in low-risk MDS (B), high-risk MDS (C), secondary AML (D) and primary AML (E). (F) Survival data of *IDH1*^MT patients with myeloid neoplasms, stratified on the ancestral importance of the *IDH1* mutation. (G) as in (F), but with *IDH2* mutations. (H) Subset survival analysis in low-risk MDS patients, with *IDH1* and *IDH2* mutations shown separately. (I) As in (F), but with *IDH1* mutations in low-risk MDS patients. Abbreviations: 1°, ancestral genetic event; 2°, subclonal genetic event; ?°, genetic event of undeterminable ancestry.

Table 1

Clinical characterization of the *IDH1/2* mutation in 2119 patients

	$IDH1^{MT}$ (N=78)	p-value	$IDH2^{MT}$ (N=101)	p-value	$IDH1/2^{WT}$ (N=1940)	Total (N=2119)
Age (median)	60	**0.01**	65	0.58	66	66
Sex [*]						
Male	46 (59%)		64 (63%)		1154 (59%)	1264
Female	30 (38)		33 (33%)		761 (39%)	824
Diagnosis						
Low-risk MDS	21 (2%)	0.0049	15 (2%)	0.0001	832	868
High-risk MDS	18 (3%)	0.5947	37 (7%)	**0.0186**	481	536
sAML	11 (7%)	**0.0239**	8 (6%)	0.6869	134	153
MDS/MPN	2 (1%)	0.08	7 (4%)	0.85	156	165
MPN	0	0.176	2 (3%)	0.76	61	63
pAML	26 (8%)	**0.0001**	32 (10%)	**0.0001**	276	334
Karyotype [**]						
Normal	46 (59%)	0.2789	65 (64%)	**0.0138**	1010 (52%)	1121 (53%)
Aberrant	27 (35%)	0.2917	29 (29%)	**0.0164**	792 (41%)	848 (40%)
Complex	5 (6%)	1	7 (7%)	1	136 (7%)	150 (7%)

[*] Sex NA: *IDH1*MT: 2, *IDH2*MT: 4, *IDH1/2*WT: 23

[**] Karyotype NA:*IDH1*MT: 0, *IDH2*MT: 0, *IDH1/2*WT: 2

Overview of clinical characteristics of the *IDH1*MT, *IDH2*MT and *IDH1/2*WT patients in the study cohort. Percentages in the diagnosis rows are calculated as percentage of patients with a certain diagnosis with *IDH1*MT or *IDH2*MT. Percentages in the karyotype rows are calculated as percentage of *IDH1*MT/*IDH2*MT patients with a certain karyotype of their myeloid neoplasm. *P* values are calculated using the Student's *t* test (Age) or Fisher's Exact test (all other characteristics).