Towards personalised medicine for cancer

From initial therapy to follow-up

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Chapter 10: Clinical and biological implications of ancestral and non-ancestral IDH1 and IDH2 mutations in myeloid neoplasms


Abstract

Mutations in isocitrate dehydrogenase 1/2 (IDH1/2) are drivers of a variety of myeloid neoplasms. As they yield the same oncometabolite, D-2-hydroxyglutarate (D2HG), 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 mutations and IDH2 mutations are ancestral in up to 14/74 (19%) versus 34/99 (34%; P = 0.027) of cases, respectively, illustrating the pathogenic role of these lesions in myeloid neoplasms. IDH1/2 mutations were associated with poor overall survival, particularly in lower-risk myelodysplastic syndromes. Ancestral IDH1-mutated cases were associated with a worse prognosis than subclonal IDH1-mutated cases, whereas the position of IDH2 mutations within clonal hierarchy did not impact survival. This may relate to distinct mutational spectra with more DNMT3A and NPM1 mutations associated with IDH1-mutated cases, and more ASXL1, SRSF2, RUNX1, STAG2 mutations associated with IDH2-mutated cases. Our data demonstrate important clinical and biological differences between IDH1-mutated and IDH2-mutated myeloid neoplasms. These mutations should be considered separately as their differences could have implications for diagnosis, prognosis, and treatment with IDH1/2-mutant inhibitors of IDH1/2-mutated patients.

Introduction

Mutations in IDH1 and IDH2 are implicated in the development of numerous types of cancer, including glioma, chondrosarcoma, cholangiocarcinoma, angioimmunoblastic T-cell lymphoma, melanoma and certain myeloid neoplasms.15 Within myeloid malignancies, IDH1/2 mutations are present in a significant proportion of acute myeloid leukaemia (AML) and, while less common, in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN).18,32

IDH1/2 mutations impart a gain of function by causing single amino acid changes in the active sites of the enzymes. Whereas wild-type IDH1/2 convert isocitrate and NADP+ to α-ketoglutarate (αKG) and NADPH, IDH1/2 mutants convert NADPH and αKG to NADP+ and D2HG.13 D2HG and αKG have similar structures; resultant D2HG accumulation in IDH1/2-mutated cells inhibits many αKG-dependent dioxygenases, such as TET2 DNA hydroxyl demethylases30 and Jumanji domain-containing histone demethylases (e.g. KDM6A/UTX and KDM3B/IMJD1B).29 Dysfunction of these enzymes is considered to be responsible for global DNA hypermethylation, inhibition of differentiation, and preservation of stemness.35,38 In addition, D2HG activates Egg Laying Defective Nine (EGLN), which induces HIF1α degradation and growth factor-independent proliferation.31,96

As direct evidence that IDH1/2 mutations are oncogenic, introduction of IDH2R140Q or IDH2R172K into 10T1/2 mesenchymal progenitor cells yielded an AML-like disease in mice.92,94 IDH2R140Q was also necessary for AML maintenance in mice,93 indicating that IDH1/2 mutants may be useful therapeutic targets. These findings motivated the development of specific inhibitors of IDH1/2-mutant enzymes.130,158,281,382,383 The currently available inhibitors, AG-221 that targets IDH2R140Q and AG-120 that targets IDH1R132H, restrict the production of D2HG and thereby induce differentiation of AML cells ex vivo.281 Both agents have shown promising preliminary results in phase I clinical trials.384
Genomic studies of molecular landscapes in human cancer have frequently combined IDH1 mutations and IDH2 mutations as a single functional group despite physiological differences: IDH1 is localised in the cytosol and IDH2 in the mitochondrial matrix. Remarkably, the spectrum of cancers and their subtypes differ in the distribution of IDH1/2 mutations. IDH1 mutations predominate in glioma (95%), chondrosarcoma (95%) and cholangiocarcinoma (80%), whereas the IDH1 mutation:IDH2 mutation ratio is more balanced or even skewed towards a higher frequency of IDH2 mutations in AML. In addition, only IDH2 mutations have been reported in angioimmunoblastic T-cell lymphoma, osteosarcoma and gastric cancer, again suggesting that there are pathophysiologic differences between IDH1 mutations and IDH2 mutations. Biochemical investigations have shown that the specific amino acid substitutions IDH1R132H, IDH1R132C, IDH2R140Q and IDH2R172K differ in D2HG production potency. IDH2R172K is the most potent, followed by IDH1R132H, IDH1R132C and IDH2R140Q. There are more than 60 different αKG-dependent dioxygenases that can theoretically be inhibited by D2HG with likely distinct IC50 values for D2HG. Thus, different IDH1/2 mutants inhibit different sets of αKG-dependent enzymes and this may partially explain differing distributions of IDH1/2 mutations between cancers.15

To investigate the clinical impact of IDH1/2 mutations 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-mutated and IDH2-mutated samples, their clinical associations, overall patient survival, and clonal hierarchies. Our aim was to provide insights into IDH1/2-mutated myeloid malignancy pathogenesis, especially with respect to the clonal architecture of IDH1/2-mutated cases, and to determine whether IDH1 mutations 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 Organisation 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; eTable 1) 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 Leukaemia Laboratory, diagnosis was confirmed on the basis of World Health Organisation classification criteria. Patients with refractory anaemia (RA), RA with ringed sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), RCMD with ringed sideroblasts (RCMD-RS) and MDS-unclassifiable (MDS-u) were classified as lower-risk MDS. Those with refractory anaemia 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 S1. Clinical details of the patients, including blood counts, demographics, and survival times, were obtained from medical records.

Sequencing technology. DNA was obtained from tumour and germline paired samples. For WES, the 50 Mb of protein-coding sequences was enriched from total genomic DNA by liquid-phase hybridisation using SureSelect (version 4; Agilent Technology), followed by massively parallel sequencing with HiSeq 2000 (Illumina). Somatic mutations were called as previously described, using the GATK algorithm (Broad Institute). To minimise false positives and focus on
Clinical and biological implications of IDH1/2 mutations in myeloid neoplasms

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 analysed 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. 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. 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 versus subclonal events were determined using a copy number-adjusted VAF difference between two events, with a higher VAF indicating ancestral origin. We used a VAF threshold of 5% (absolute) to reliably discriminate ancestral from subclonal events. Competing genetic events below this threshold were considered to be of undeterminable ancestry. This threshold was chosen based on previous studies 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 χ² 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 α < 0.05. These analyses were performed using SPSS (IBM), Prism 6 (Graphpad Software) and the R statistical programming language.

Results

Clinical characterisation 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-mutated cases, IDH1R132C (49%, n = 36) and IDH1R132H (38%, n = 28) were the most common; 12% (n = 9) harboured IDH1R132X. Amongst IDH2-mutated cases, IDH2R140Q (85%, n = 84) was the most frequent mutation, followed by IDH2R140X (5%, n = 5), IDH2R172K (9%, n = 9) and IDH2R172W (1%, n = 1; Figure 1A). IDH1-mutated patients were younger than IDH1/2 wild-type patients (P = 0.01), an observation similar to that reported for IDH1-mutated glioblastoma patients. Across the spectrum of MDS, the frequency of IDH1/2 mutations increased with progression, as IDH1 mutations occurred in 2% of lower-risk MDS cases, in 3% of higher-risk MDS cases and in 7% of sAML cases. The frequency of IDH2 mutations increased as MDS progressed (in 2% of lower-risk MDS, in 7% of higher-risk MDS and in 6% 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 (Figure 1). These findings suggest that in IDH1/2-mutated MDS cases, a high proportion of IDH1/2 mutations 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-mutated cases were enriched for a normal karyotype, IDH1-mutated patients more often had a deletion of chromosome Y in comparison with IDH1/2 wild-type patients. Other clinical characteristics were similar between IDH1/2-mutated and IDH1/2 wild-type patients (Table 1 and eTable 2). More detailed clinical
information on IDH1-mutated and IDH2-mutated patients with myeloid malignancies is shown in eTables 3 and 4.

**Molecular characterisation of IDH1 and IDH2-mutated myeloid neoplasms.** To characterise the molecular features of patients with myeloid neoplasms with an IDH1 mutation or IDH2 mutation, we analysed associations between IDH1/2 mutations and other molecular events (Figure 2A and eTable 5). Overall, NPM1 mutations were more frequent in both IDH1-mutated cases and IDH2-mutated cases, compared with IDH1/2 wild-type patients (Figure 2B and eTables 5-7). Other somatic events were not significantly associated with both IDH1-mutated cases and IDH2-mutated cases compared with IDH1/2 wild-type cases.

However, when IDH1-mutated cases were compared with IDH1/2 wild-type cases, they were enriched for DNMT3A (P < 0.0001), PHF6 (P = 0.0006) and FLT3 (P = 0.02) mutations, whereas IDH2-mutated cases were enriched for ASXL1 (P < 0.0001), SRSF2 (P < 0.0001), RUNX1 (P = 0.003) and STAG2 (P < 0.0001) compared with IDH1/2 wild-type cases. NPM1 mutations occurred more frequently in IDH1-mutated patients than in IDH2-mutated patients (P = 0.045), whereas IDH2-mutated patients were enriched for ASXL1 (P = 0.02), SRSF2 (P = 0.007) and STAG2 (P = 0.02) mutations compared with IDH1-mutated patients. Cohesin complex mutant carriers (STAG2, RAD21, SMC3) were more frequent in IDH1-mutated cases (P = 0.03) and IDH2-mutated (P < 0.0001) cases compared with IDH1/2 wild-type cases. Conversely, SF3B1 and TET2 mutations occurred less frequently in IDH1-mutated cases (P < 0.0001 and P = 0.02) and in IDH2-mutated cases (P < 0.0001 and P < 0.0001) than in IDH1/2 wild-type cases. Although we observed a negative correlation between IDH1 mutations and TET2 mutations, TET2 mutations, if present, were less infrequent in IDH1-mutated patients than in IDH2-mutated patients (P = 0.03; Figure 2C).

### Table 1. Clinical characterisation of the IDH1/2 mutation in 2119 patients.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>IDH1\textsuperscript{MUT} (n = 78)</th>
<th>IDH2\textsuperscript{MUT} (n = 101)</th>
<th>IDH1/2\textsuperscript{WT} (n = 1940)</th>
<th>Total (n = 2119)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>60</td>
<td>65</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Sex: Male</td>
<td>46 (59%)</td>
<td>64 (63%)</td>
<td>1154 (59%)</td>
<td>1264</td>
</tr>
<tr>
<td>Sex: Female</td>
<td>30 (38%)</td>
<td>33 (33%)</td>
<td>761 (39%)</td>
<td>824</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk MDS</td>
<td>21 (2%)</td>
<td>15 (2%)</td>
<td>0.001</td>
<td>832</td>
</tr>
<tr>
<td>High-risk MDS</td>
<td>18 (3%)</td>
<td>37 (7%)</td>
<td>0.02</td>
<td>481</td>
</tr>
<tr>
<td>sAML</td>
<td>11 (7%)</td>
<td>8 (6%)</td>
<td>0.69</td>
<td>134</td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>2 (1%)</td>
<td>7 (4%)</td>
<td>0.85</td>
<td>156</td>
</tr>
<tr>
<td>MPN</td>
<td>0</td>
<td>2 (3%)</td>
<td>0.76</td>
<td>61</td>
</tr>
<tr>
<td>pAML</td>
<td>26 (8%)</td>
<td>32 (10%)</td>
<td>0.001</td>
<td>276</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>46 (59%)</td>
<td>65 (64%)</td>
<td>0.01</td>
<td>1010 (52%)</td>
</tr>
<tr>
<td>Aberrant</td>
<td>27 (35%)</td>
<td>29 (29%)</td>
<td>0.02</td>
<td>792 (41%)</td>
</tr>
<tr>
<td>Complex</td>
<td>5 (6%)</td>
<td>7 (7%)</td>
<td>1</td>
<td>136 (7%)</td>
</tr>
</tbody>
</table>

Overview of clinical characteristics of the IDH1-mutated, IDH2-mutated and IDH1/2 wild-type patients in the study cohort. Percentages in the diagnosis rows are calculated as percentages within a certain diagnosis. Percentages in the karyotype rows are calculated as percentage of IDH1-mutated or IDH2-mutated 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). *Sex NA: IDH1-mutated patients: 2, IDH2-mutated patients: 4, IDH1/2 wild-type patients: 23; **Karyotype NA: IDH1-mutated patients: 6, IDH2-mutated patients: 0, IDH1/2 wild-type patients: 2. Abbreviations: IDH1\textsuperscript{MUT}, IDH1-mutated; IDH2\textsuperscript{MUT}, IDH2-mutated; IDH1/2\textsuperscript{WT}, IDH1/2 wild-type.
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 leukaemia; sAML, secondary acute myeloid leukaemia.

Analysis of ancestry and clonal architecture of IDH1/2-mutated patients. Using copy number-adjusted VAF, we reconstructed the clonal architecture of IDH1/2-mutated patients to establish whether an IDH1 mutation or IDH2 mutation was an ancestral or subclonal mutation. Clonal hierarchy was further confirmed and refined by serial analyses, performed in 60 exemplary cases (Figure 3C). IDH1 mutations and IDH2 mutations were ancestral in 19% and 34% of IDH1/2-mutated 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 versus subclonal status of some IDH1/2 mutations remained “undetermined” when there were small VAF differences between IDH1/2 mutations and other mutations (see Methods). A lower proportion of IDH1-mutated cases were of ancestral origin compared to IDH2-mutated cases (P = 0.03). We also observed differences in the clonal succession between specific IDH1/2-mutant variants (Figure 3B). Whereas IDH1R132H and IDH1R132C mutations were ancestral in equal frequencies (22% and 21%), other IDH1 mutations (IDH1T98I [n = 1], IDH1R132L [n = 1] and IDH1R132S [n = 5]) occurred only as subclonal events. Similarly, IDH2R140K mutations were ancestral in 39% of IDH2R140K-mutated cases, whereas other IDH2R140 mutations (IDH2R140H [n = 2] and IDH2R140W [n = 3]) were always subclonal. IDH2R172 mutations were ancestral in only 10% of IDH2R172-mutated patients. In the different types of myeloid neoplasms we did not observe differences in the distribution between ancestral IDH1/2 mutations versus subclonal IDH1/2 mutations and there was no difference in the mean VAF of IDH1 mutations versus IDH2 mutations (eFigure 2A-C). We observed higher VAFs of IDH1/2 mutations in patients with ancestral IDH1/2 mutations than in patients with subclonal IDH1/2 mutations (Figure 3C and eFigure 2D). VAFs of IDH1/2 mutations were highest in patients with IDH1/2 mutations of undeterminable ancestry, which may reflect a higher disease burden in patients when IDH1/2 mutations cooperate with other mutations. WES analyses revealed that IDH1 mutations and IDH2 mutations were ancestral in 1/21 (5%) and 2/24 (8%) of IDH1/2-mutated cases, respectively, whereas TS suggested that IDH1 mutations and IDH2 mutations were ancestral in 13/53 (25%) and 32/75 (43%) of cases (difference between ancestral cases in WES versus TS; P = 0.0002). This difference probably reflects missed ancestral mutations in the TS samples (eTable 8). The clonal architecture of representative IDH1/2-mutated patients (out of 60 studied) that were serially sequenced is shown in Figure 3D and eFigure 2E. In subclonal IDH1/2-mutated 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 mutations or IDH2 mutations, respectively. Subclonal IDH1 mutations were most often preceded by an ancestral DNMT3A mutation (30%; eFigure 2F), whereas ancestral RUNX1 mutations most frequently preceded a subclonal IDH2 mutation (17%; eFigure 2G). We observed a RUNX1 mutation preceding a subclonal IDH1 mutation only once. Hierarchical clonal analyses of neoplasms with an undeterminable place of an IDH1/2 mutational within the clonal architecture (small VAF differences between IDH1/2 mutations and other mutations) may provide insight into which mutations cooperate (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 mutations. DNMT3A mutations were most frequently the "main competitor" of IDH1 mutations and IDH2 mutations of undetermined ancestry (eFigure 2H-I). We did not observe a single RUNX1 mutation that competed with IDH2 mutations, indicating that VAF differences between ancestral RUNX1 mutations and subclonal IDH2 mutations, and vice versa, are rather large.

**Prognostic effect of IDH1/2 mutations in myeloid neoplasms.** In our cohort, IDH1/2 mutations were associated with worse overall survival (Figure 4A). In subset analyses, we observed that IDH1/2 mutations were significantly associated with reduced median overall survival in lower-risk MDS patients (median overall survival: 41 versus 66 months, \( P = 0.03 \)), but not in higher-risk MDS (median overall survival: 34 versus 30 months, \( P = 0.91 \)), sAML (median overall survival: 26 versus 19 months, \( P = 0.74 \)) or pAML patients (median overall survival: 20 versus 16 months, \( P = 0.79 \); Figure 4B-E). This finding further illustrates the role of IDH1/2 mutations in progression to a more malignant disease. Patients with ancestral IDH1 mutations tended to have a worse survival than patients with subclonal IDH1 mutations (median overall survival: 23 versus 32 months, \( P = 0.09 \); Figure 4F), whereas there was no survival difference between patients with ancestral IDH2 mutations versus subclonal IDH2

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**Figure 2. Mutational spectrum and co-occurring mutations in IDH1/2-mutated cases.**

(A) Mutational spectrum and clinical spectrum of IDH1-mutated cases (left half) and IDH2-mutated cases (right half) with myeloid neoplasms. (B) Prevalence of co-occurring mutations in IDH1/2-mutated cases, compared with IDH1/2 wild-type cases. Abbreviations: del, deletion; IDH1WT, IDH1-mutated, IDH2MT, IDH2-mutated, IDH1/2WT, IDH1/2 wild-type; hrMDS, hemimut, hemizygous mutation; high-risk MDS; lrMDS, low-risk MDS; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; pAML, primary acute myeloid leukaemia; sAML, secondary acute myeloid leukaemia; smut, somatic mutation.
Figure 3. Analysis of clonal architecture of IDH1/2-mutated cases.

(A) Percentages of IDH1/2-mutated 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-mutated 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-mutated patients.
between IDH1 mutations or IDH2 mutations and overall survival was investigated separately in low-risk MDS to assess the impact on subsequent outcomes in early disease. Compared with IDH1/2 wild-type patients, we found that IDH2-mutated patients (median overall survival: 30 versus 66 months, \( P = 0.003 \)), but not IDH1-mutated patients (median overall survival: 42 versus 66 months, \( P = 0.64 \)), had a worse prognosis (Figure 4H). Because IDH1 mutations were less frequently ancestral than IDH2 mutations in lower-risk MDS patients (22\% versus 40\%), we analysed whether ancestry determines to what extent IDH1/2 mutations impacted overall survival. Indeed, patients with ancestral IDH1 mutations had a worse survival than patients with myeloid neoplasms that had a subclonal IDH1 mutations (median overall survival: 23 versus 42 months, \( P = 0.05 \)) or IDH1/2 wild-type patients (median overall survival: 23 versus 66 months, \( P = 0.006 \); Figure 4I). Ancestral IDH2 mutations may also be associated with worse prognosis, compared with subclonal IDH2 mutations. However, definitive conclusions from this subset analysis were hindered by the limited number of cases (Supplementary Figure S1).

**Discussion**

In this chapter, we describe the clinical and molecular characterisation of IDH1-mutated and IDH2-mutated patients with myeloid neoplasms. The size of the cohort allowed for the most comprehensive

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**Figure 4. IDH1/2 mutations and overall patient survival in myeloid neoplasms.**

(A) Survival data of IDH1/2-mutated 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-mutated 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; IDH1\(^{MT}\), IDH1-mutated; IDH2\(^{MT}\), IDH2-mutated; IDH1/2\(^{MT}\), IDH1/2 wild-type.
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 mutations and IDH2 mutations can occur as ancestral or subclonal defects. In a substantial proportion of cases, IDH1 mutations (19%) and IDH2 mutations (34%) represent ancestral lesions, but more often IDH1/2 mutations 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 mutations are associated with a poor prognosis, while in higher-risk myeloid neoplasms, prognosis could not be further stratified. Patients with ancestral an IDH1 mutation exhibit worse survival than those with a subclonal IDH1 mutation, particularly in lower-risk MDS. Such a difference was not found when ancestral IDH2 mutations versus subclonal IDH2 mutations were compared, indicating that IDH2 mutations more rapidly dominate the clonal hierarchy.

Analyses of mutations that positively or negatively correlate with IDH1/2 mutations in myeloid neoplasms have heretofore treated IDH1/2 mutations cases as a single functional entity. In our cross-sectional analysis, this approach of pooling IDH1/2 mutations 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 mutations and IDH2 mutations separately. In addition, IDH1-mutated cases were significantly enriched for DNTM3A and PHF6 mutations, and IDH2-mutated cases for ASXL1, RUNX1, SRSF2 and STAG2 mutations. When IDH1/2-mutated cases would be pooled, all these six mutations are significantly more frequently occurring in IDH1/2-mutated cases versus IDH1/2 wild-type cases. This would falsely suggest that they occur more frequently in both IDH1-mutated and IDH2-mutated cases, whereas the significant correlation is only true for either IDH1-mutated cases or IDH2-mutated cases. “pAML-associated” DNMT3A and NPM1 mutations occurred significantly more frequently in IDH1-mutated cases than in IDH2-mutated cases, whereas the reverse was true for “MDS/sAML/(MPN)-associated” ASXL1, RUNX1, SRSF2 and STAG2 mutations. Of note, there were no significant proportional differences with respect to diagnosis between IDH1-mutated cases and IDH2-mutated cases, with only a trend (P = 0.051) towards higher-risk MDS patients among IDH2-mutated cases than among IDH1-mutated cases. Apparently, the functional cooperation between certain mutations (e.g. DNTM3A mutations and IDH1 mutations or RUNX1 mutations and IDH2 mutations) 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-mutated cases and IDH2-mutated cases may stem from the differences in the biochemical consequences of these lesions. For instance, the various IDH1/2 mutants differ in D2HG 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 D2HG. Whereas IDH1 is cytoplasmic, IDH2 is mitochondrial and αKG levels may not be completely interchangeable between these subcellular compartments, generating different conditions for IDH1 mutants and IDH2 mutants to synthesise D2HG. Furthermore, nuclear DNA and histone demethylases (e.g. TET2, Jumonji) and cytoplasmic prolyl hydroxylases (EGLN) are considered to be drivers of IDH1/2 mutant-induced oncogenesis, suggesting that D2HG mainly functions oncogenically outside mitochondria. It is unknown to what extent D2HG passes through the mitochondrial membrane. Therefore, the impact of the compartmentalisation of αKG and D2HG on the downstream effects of IDH2 mutations versus IDH1 mutations is unclear.

D2HG functions as an oncometabolite that inhibits various αKG-dependent dioxygenases. Whereas the effects of D2HG on DNA demethylase TET2, histone demethylase Jumonji and the HIF1α degrader EGLN have been described thoroughly, 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 D2HG. All these enzymes have specific IC50 values of D2HG for inhibition of


Thus, each D2HG concentration (i.e. each IDH1/2 mutant 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 mutations. D2HG inhibits TET2 and results in a genome-wide DNA hypermethylation in IDH1/2-mutated cancers that induces stemness and inhibits differentiation. In our cohort, and that of others, 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-mutated cases (14%) than in IDH2-mutated cases (4%). It is possible that IDH1 mutations result in less TET2 inhibition than IDH2 mutations and that a synergistic/additive effect of co-occurring 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 mutations. 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 mutations is UTX/KDM6A. However, we did not observe mutual exclusivity between IDH1/2 and UTX mutations, suggesting that D2HG-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.

Our study provides the first comprehensive subclassification of IDH1/2-mutated cases with myeloid neoplasms based on their rank within clonal hierarchy, and thus their timing in clonal ontogeny. While cases initiated by IDH1/2 mutations are a distinct subset of myeloid neoplasms, in many instances IDH1/2 mutations 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 mutations are related to poor clinical outcomes, whereas this association is weaker for subclonal IDH1/2 mutations. These findings illustrate that patients with myeloid neoplasms carrying ancestral IDH1/2 mutations are the best candidates for therapy with IDH1/2-mutant inhibitors. Thus, sequencing that targets mutations frequently co-occurring with IDH1/2 mutations, in particular those which are often ancestral, may aid rational clinical treatment of IDH1/2-mutated patients with IDH1/2-mutant inhibitors.

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

In summary, we present results that demonstrate distinct differences between IDH1 mutations and IDH2 mutations. 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-mutated myeloid neoplasms with IDH1/2-mutant inhibitors. We conclude that the minority of patients with myeloid neoplasms carrying ancestral IDH1/2 mutations are the best candidates for therapy with IDH1/2-mutant inhibitors.
Supplementary Figure S1. IDH1/2 mutations, clonal hierarchy and patient survival.

(A) Survival data of patients with myeloid neoplasms carrying IDH1/2 mutations, with IDH1-mutated patients and IDH2-mutated patients shown separately. (B-E) Subset survival analyses with IDH1-mutated patients and IDH2-mutated patients shown separately in low-risk MDS (B), high-risk MDS (C), secondary AML (D) and primary AML (E). (F) Survival data of patients with low-risk MDS carrying IDH2 mutations, stratified on the ancestral importance of the IDH2 mutation. Abbreviations: 1°, ancestral genetic event; 2°, subclonal genetic event; ?°, genetic event of undeterminable ancestry; IDH1mt, IDH1-mutated; IDH2mt, IDH2-mutated; IDH1/2wt, IDH1/2 wild-type.

Supplementary Table S1. Clinical characterisation of the study cohort

<table>
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<th>Diagnosis</th>
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Abbreviations: RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; MDS-u, myelodysplastic syndrome unclassified; RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts; t-MDS, treatment-induced myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; CMML, chronic myelomonocytic leukaemia; MDS/MPN-u, myelodysplastic syndrome/myeloproliferative neoplasm unclassified; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis; CML, chronic myelogenous leukaemia; PV, polycythaemia vera; ET, essential thrombocytosis; aCML, atypical chronic myelogenous leukaemia; PMF, primary myelofibrosis; pAML, primary acute myeloid leukaemia; sAML, secondary acute myeloid leukaemia.