Non-IDH1-R132H IDH1/2 mutations are associated with increased DNA methylation and improved survival in astrocytomas, compared to IDH1-R132H mutations

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Abstract: Somatic mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 occur at high frequency in several tumour types. Even though these mutations are confined to distinct hotspots, we show that gliomas are the only tumour type with an exceptionally high percentage of IDH1R132H mutations. Patients harbouring IDH1R132H mutated tumours have lower levels of genome-wide DNA-methylation, and an associated increased gene expression, compared to tumours with other IDH1/2 mutations ("non-R132H IDH1/2 mutations"). This reduced methylation is seen in multiple tumour types and thus appears independent of the site of origin. For 1p/19q non-codeleted glioma (astrocytoma) patients, we show that this difference is clinically relevant: in samples of the randomised phase III CATNON trial, patients harbouring tumours with IDH mutations other than IDH1R132H have a better outcome (hazard ratio 0.41, 95% CI [0.24, 0.71], p = 0.0013). Such non-R132H IDH1/2-mutated tumours also had a significantly lower proportion of tumours assigned to prognostically poor DNA-methylation classes (p < 0.001). IDH mutation-type was independent in a multivariable model containing known clinical and molecular prognostic factors. To confirm these observations, we validated the prognostic effect of IDH mutation type on a large independent dataset. The observation that non-R132H IDH1/2-mutated astrocytomas have a more favourable prognosis than their IDH1R132H mutated counterpart indicates that not all IDH-mutations are identical. This difference is clinically relevant and should be taken into account for patient prognostication. Keywords: Astrocytoma; Gene expression; Genome-wide DNA methylation; IDH1; IDH2.

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Non-IDH1-R132H IDH1/2 mutations are associated with increased DNA methylation and improved survival in astrocytomas, compared to IDH1-R132H mutations

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Abstract
Somatic mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 occur at high frequency in several tumour types. Even though these mutations are confined to distinct hotspots, we show that gliomas are the only tumour type with an exceptionally high percentage of IDH1R132H mutations. Patients harbouring IDH1R132H mutated tumours have lower levels of genome-wide DNA-methylation, and an associated increased gene expression, compared to tumours with other IDH1/2 mutations (“non-R132H IDH1/2 mutations”). This reduced methylation is seen in multiple tumour types and thus appears independent of the site of origin. For 1p/19q non-codeleted glioma (astrocytoma) patients, we show that this difference is clinically relevant: in samples of the randomised phase III CATNON trial, patients harbouring tumours with IDH mutations other than IDH1R132H have a better outcome (hazard ratio 0.41, 95% CI [0.24, 0.71], p = 0.0013). Such non-R132H IDH1/2-mutated tumours also had a significantly lower proportion of tumours assigned to prognostically poor DNA-methylation classes (p < 0.001). IDH mutation-type was independent in a multivariable model containing known clinical and molecular prognostic factors. To confirm these observations, we validated the prognostic effect of IDH mutation type on a large independent dataset. The observation that non-R132H IDH1/2-mutated astrocytomas have a more favourable prognosis than their IDH1R132H mutated counterpart indicates that not all IDH-mutations are identical. This difference is clinically relevant and should be taken into account for patient prognostication.

Keywords Astrocytoma · Genome-wide DNA methylation · Gene expression · IDH1 · IDH2

Introduction
Somatic mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 occur at high frequency in various tumour types including gliomas (primary malignant central nervous system tumours), intrahepatic cholangiocarcinomas (bile duct tumours), enchondromas and chondrosarcomas (bone tumours), sinonasal undifferentiated carcinomas and leukemias [12, 33]. More sporadic but similar mutations have been found in a wide variety of other tumour types including melanoma, and prostate and pancreatic cancer [54]. IDH1/2 mutations are causal for the disease and tumours often remain dependent on the mutation for growth [22, 42]. The importance of the mutation is confirmed by the activity of IDH-inhibitors: inhibiting the mutant activity of either IDH1 or IDH2 shows anti-tumour activity in relapsed/refractory IDH1/2 mutated acute myeloid leukemia [14, 45] and cholangiocarcinoma
patients [1]. The objective response rates in these trials are in the order of 40%, though patients eventually relapse. In gliomas, however, mutant IDH1/2 inhibitors have thus far not shown a survival benefit, but further studies on early-stage tumours are ongoing [32].

The IDH1/2 mutations are confined to defined gene hotspots and affect either arginine 132 (R132) in IDH1 or arginines R172 or R140 in IDH2. Although IDH1/2 mutations are confined to these three hotspots, several reports have shown that the IDH-mutation spectrum differs per tumour type [12, 15, 20, 37]. The hotspot mutations all change the activity of the wild-type (wt) protein from an enzyme that produces alpha-ketoglutarate (aKG) to an enzyme that produces D-2 hydroxyglutarate (D-2HG) [12, 27] which ultimately keeps cells in an undifferentiated state [19, 30], but individual IDH1/2 mutations differ in their ability to produce D-2HG [5, 40]. IDH1R132H, the IDH1/2 mutation with relatively low D-2HG production capacity, is the most common mutation in gliomas; other mutations such as IDH1R1132C have tenfold lower $K_M$ and have higher enzymatic efficiency [5, 40]. This difference may have biological implications as not all aKG-dependent enzymes are equally well inhibited by D-2HG [11, 53]. For example, tet methylcytosine dioxygenase 2 (TET2) enzymes that mediate the first step in DNA-demethylation, requires relatively high D-2HG levels for inhibition [31, 53].

Here, we have used data from six large and independent DNA methylation datasets (the randomised phase III CATNON clinical trial on anaplastic 1p/19q non-codeleted gliomas [49], the TCGA-LGG cohort [8], samples included in the TAVAREC randomised phase 2 clinical trial on astrocytomas [51], a large cohort of acute myeloid leukemias (AML) [48] and a cohort of chondrosarcomas [52]) derived from four different tumour types, to examine the molecular effects of different types of IDH1/2 mutations. We report that tumours harbouring IDH1R132H mutations, regardless of tumour type, have lower genome-wide DNA methylation levels compared to those harbouring other IDH1/2 hotspot mutations (‘non-IDH1-R132H IDH1/2-mutated tumours’). For astrocytoma patients, we show this difference has clinical relevance as patients harbouring such non-IDH1R132H IDH1/2-mutated tumours have improved survival compared to those harbouring IDH1R132H mutations. Our data support the notion that increased genome-wide DNA methylation levels are associated with improved outcome in this tumour type and indicate that the type of IDH1/2 mutation should be taken into account for prognostication of astrocytoma patients.

### Materials and methods

#### Datasets

The COSMIC database (Assessed 27 December 2019) was screened for hotspot IDH1 (R132) and IDH2 (R172 and R140) mutations. Mutations were stratified by tumour type; tumours with a low prevalence of mutations were concatenated (site of origin of ‘other tumours’: prostate $n = 11$, pancreas $n = 6$, skin $n = 32$, large intestine $n = 1$, soft tissue $n = 22$, endometrium $n = 1$, breast $n = 9$, urinary tract $n = 2$, liver $n = 7$, stomach $n = 1$, upper aerodigestive tract $n = 35$, salivary gland $n = 1$, thyroid $n = 1$). CATNON clinical data [49] and IDH1/2 mutation and DNA methylation data (Tesileanu, submitted) were reported previously. TCGA glioma data (DNA methylation and RNA-seq) [8], MSK-IMPACT data [9] and AML data [48] were downloaded from the TCGA data portal. Clinical data and mutation status for the chondrosarcoma data were reported previously [52]. Clinical data from the TAVAREC trial were derived from ref [51], and supplemented with DNA methylation data of 89 tumours. Most (80%) TAVAREC samples were derived from the initial tumour. Processing of CATNON and TAVAREC DNA methylation data was performed as described (Tesileanu, submitted). For the CATNON, TCGA-astrocytoma and TAVAREC datasets, we included only IDH1/2 mutated samples from non 1p/19q-codeleted tumours. Although all CATNON and TAVAREC samples were initially diagnosed as astrocytomas, DNA methylation analysis found 1p/19q codeletion in 8 samples included in the CATNON trial and 3 samples in the TAVAREC trial (Tesileanu, submitted). To ensure a molecularly homogenous sample cohort, all 1p/19q codeleted samples were removed prior to any analysis presented. For IDH1/2 mutated MSK-IMPACT samples, the distinction between astrocytic and oligodendrocytic tumours was made by absence or presence of telomerase reverse transcriptase (TERT) promoter mutations [26, 46]. In the Chinese Glioma Genome Atlas [CGGA] [23], the exact IDH1/2 mutation was not noted and therefore limited for the scope of this analysis. We used only the 1p/19q codeleted tumours in this dataset with IDH2 mutations being designated as “non-IDH1R132H IDH1/2-mutated” and all IDH1 mutations as “R132H”. In oligodendrogliomas, IDH1 mutations virtually always result in R132H [20]. RNA-seq data (raw read counts) were normalized and processed using DEseq2.

#### Statistical analysis

Survival curves were created using the Kaplan–Meier method. The log-rank test was used to determine survival differences. A Wilcoxon rank test on beta values (i.e. the
intensity of the methylated probe/sum of methylated and unmethylated probe intensity) was used to identify differentially methylated probes in CATNON and TCGA-astrocytoma datasets. To increase power in the smaller sized datasets, we performed an \( F \) test on \( M \) values (i.e. the log2 ratio of the methylated/unmethylated probe intensities) to identify differentially methylated CpGs using the dmpFinder function in the Minfi Bioconductor package [4]. To further increase statistical power in the chondrosarcoma dataset (required as this dataset had few samples), we first made a selection of the most variable probes (i.e. those with a standard deviation > 2; ~5% of the total number of probes) followed by an \( F \) test on the \( M \) values. In all differential methylation analysis, p-values were corrected for false discovery rate (adjusted p-value).

Differences in mutation frequencies were determined using a chi-squared test. Pathway analysis was performed using Ingenuity pathway analysis (Qiagen, Venlo, The Netherlands). An association model was made with the Cox proportional hazards method and included, next to \( IDH1/2 \) mutation type, factors that are known to be related to outcome from literature such as sex, treatment with temozolomide, age at randomization, WHO performance score, \( O^6 \)-methylguanine DNA methyltransferase (\( MGMT \)) promoter methylation status, use of corticosteroids at randomization, and DNA methylation profiling. All \( p \) values below 0.05 were considered significant. Statistical analysis was performed using R version 3.6.3 and packages minfi, stats, rms, survival.

### Results

**The \( IDH1^{R132H} \) mutation predominates in gliomas**

We screened the catalogue of somatic mutations in the cancer (COSMIC) database [16], extracted \( IDH1/2 \) hotspot mutation data (\( IDH1^{R132} \), \( IDH2^{R172} \) and \( IDH2^{R140} \)) and stratified them by tumour organ site. As expected, tumours with a high frequency of \( IDH1/2 \) mutations include the central nervous system (CNS), biliary tract, bone, haematopoietic and lymphoid tumours (leukemias). Interestingly, even if there are only three mutational hotspots, there are marked differences in the distribution of mutations between tumour sites (Fig. 1). For example, the \( IDH1^{R132H} \) mutation is by far the most predominant \( IDH1/2 \) mutation in CNS tumours (\( n = 7265/8026 \), 90.5%) whereas this mutation is present at much lower frequencies in bone (\( n = 49/361 \), 13.6%), leukemic (\( n = 519/2995 \), 17.3%) and other tumours (\( n = 14/129 \), 10.9%), and thus far has never been identified in biliary tract tumours (\( n = 212 \) (\( p < 0.001 \), chi-square test). In contrast, the mutation that results in \( IDH1^{R132C} \) is quite rare in gliomas (223/8026, 2.8%) but much more prevalent in all other tumour types: bone (\( n = 212/361 \), 67.1%), leukemic

![Fig. 1](image-url)  

**Fig. 1** \( IDH1 \) and \( IDH2 \) hotspot mutation distribution separated by site of origin. \( IDH1^{R132H} \) mutations are the most predominant mutation in gliomas. \( IDH2 \) mutations are most common to haematopoietic tumours.
DNA methylation is lower in IDH1R132H mutant glioma

We used genome-wide DNA methylation data from CATNON trial samples and compared profiles of IDH1R132H mutated tumours (n = 369) to those harbouring other “non-R132H” IDH1 and IDH2 hotspot mutations (n = 69). Our data shows that the overall level of DNA methylation was significantly lower in tumours harbouring IDH1R132H mutations compared to tumours harbouring non-IDH1R132H IDH1/2-mutations. For example, there are 2461 probes showing a reduction in beta values >0.2 in IDH1R132H mutated tumours (at p < 0.01) but there are no probes showing an increase >0.2. This is exemplified in the volcano plot where a strong skew towards increased DNA methylation in non-IDH1R132H IDH1/2- mutated samples is observed (Fig. 2a). Probes showing the largest increase in DNA methylation were those that were partially methylated in IDH1R132H mutated tumours (i.e. probes with beta values between 0.25 and 0.75); there were few probes that became (partially) methylated from an unmethylated state (Fig. 2b).

Gliomas with higher levels of genome-wide DNA methylation generally are associated with longer survival in adults [8, 13, 28, 35]. Since non-R132H IDH1/2-mutated gliomas have increased DNA methylation levels, we compared the overall survival of patients with different IDH mutations. In patients included in the CATNON randomised phase III clinical trial, those harbouring tumours with non-R132H IDH1/2-mutations indeed had longer overall survival compared to patients harbouring IDH1R132H mutated tumours (Fig. 2c). The hazard ratio for non-R132H IDH1/2-mutations compared to IDH1R132H mutations was 0.41, 95% CI [0.24, 0.71], p = 0.0013.

DNA methylation profiling can also assign tumours to specific (prognostic) methylation subclasses. In line with the poorer survival, IDH1R132H mutated tumours also had a significantly higher proportion assigned to the prognostically poorer subclass A_IDH_HG (“IDH-mutant, high-grade astrocytoma”, n = 100/366 vs. 9/71, p = 0.036, Chi-squared test) using the subclasses as defined by Capper et al. (“CNS-classifier”) [7]. They also have a higher proportion of G-CIMP low tumours (18/369 vs. 0/62) and G-CIMP-high tumours with risk to progression to G-CIMP low (111/335 vs. 2/62) in the classifier as defined by the TCGA and de Souza et al. (“glioma classifier”, p < 0.001, chi-squared test, Table 1) [8, 13].

A heatmap of the most differentially methylated CpGs of CATNON data (n = 677, selected on a beta value change > 0.25 and false discovery corrected p values < 10e−5) shows a gradient from high to low methylation levels. As expected, the non-R132H IDH1/2-mutated tumours cluster together at the high-methylation end of this spectrum. Interestingly, most of the tumours with less favourable molecular subtypes (A_IDH_HG, G-CIMP low, G-CIMP high with risk to progression) clustered together at the other, demethylated end (Fig. 2e). Although the clinical follow-up of CATNON patients is limited, the number of mortality events also tended to cluster at the demethylated end of the heatmap which suggests that there is a strong correlation between the level of methylation of these 677 probes and survival.

To determine whether the type of mutation is a prognostic factor independent of the DNA methylation subtypes, we stratified these subtypes by IDH1/2 mutation (IDH1R132H vs. non-R132H IDH1/2-mutated). Our data show that, even within the prognostic DNA methylation subtypes, patients harbouring non-R132H IDH1/2-mutated tumours had a significantly longer survival compared to those harbouring IDH1R132H-mutated tumours, regardless of the classifier used (Fig. 2d, supplementary Fig. 1, Online resource). The type of IDH1/2 mutation was also an independent prognostic factor in a multivariable analysis that included all known factors associated with survival in this trial (treatment, age, corticosteroid use and sex, supplementary Table 1, online resource). It remained significant when DNA methylation subclass was included in this analysis (Table 1, Supplementary Table 2, online resource). These data demonstrate that the type of IDH1/2 mutation is an independent factor associated with patient survival.

To confirm these observations, we performed a similar analysis on the IDH1/2 mutated, 1p/19q non-codeleted glioma patients included in the TCGA dataset [8]. Similar to observed in the CATNON dataset, a striking increase in DNA methylation levels was seen in non-R132H IDH1/2-mutated tumours compared to those harbouring a IDH1R132H mutation (Fig. 3a, b). Also similar was the observation that patients harbouring non-R132H IDH1/2-mutated tumours survived significantly longer; the hazard ratio (HR) of patients harbouring...
non-R132H IDH1/2-mutated tumours \( (n = 37) \) versus IDH1\(^{R132H}\)-mutated tumours \( (n = 177) \) was 0.20 (95% CI [0.047, 0.837], \( p = 0.028 \) Fig. 3c). Finally, IDH1\(^{R132H}\) mutated tumours also had a higher proportion of tumours assigned to the prognostically poorer G-CIMP low DNA methylation class \( (4/116 \text{ vs. } 0/27) \) and a higher number at risk of progression to G-CIMP low \( (29/111 \text{ vs. } 0/24, \ p = 0.016) \). The type of IDH mutation remained a factor significantly associated with survival in a multivariable model that contained tumour grade and patient age (supplementary Table 3, online resource).

DNA methylation generally shows a negative correlation with gene expression, especially when the methylated CpGs are located near the transcriptional start site [44, 50]. We, therefore, examined whether the reduction in DNA methylation in IDH1\(^{R132H}\) mutated tumours is associated with an increase in gene expression in the 1p/19q non-codeleted gliomas present in the TCGA dataset. Indeed, of the genes differentially expressed between IDH mutation types (with > twofold change in expression level at \( p < 0.01 \) significance level) in astrocytomas, most \( (157/183, 86\%) \) were upregulated in IDH1\(^{R132H}\) mutated tumours (Fig. 3d, Supplementary Table 4, online resource). Pathway analysis using these 183 genes indicates that genes upregulated in IDH1\(^{R132H}\) mutated tumours were involved in cellular movement, cell death and survival, cell-to-cell signalling and interaction and carbohydrate metabolism (Supplementary Fig. 2, online resource).

We performed a second validation using 1p/19q non-codeleted samples included in the randomised phase II TAVAREC clinical trial. Again, the vast majority of probes had lower DNA methylation levels in IDH1\(^{R132H}\) mutated tumours \( (n = 83) \) compared to non-R132H IDH1/2-mutated tumours \( (n = 11, \) Fig. 4a) and the most differentially methylated probes were those partially methylated in IDH1\(^{R132H}\) mutated tumours (Fig. 4b). Moreover, there was a large degree of overlap in differential DNA methylation between CATNON and TAVAREC samples (Fig. 4c). In TAVAREC, there was no significant difference in survival between patients harbouring IDH1\(^{R132H}\) and non-R132H IDH1/2-mutated tumours (HR 1.21, 95% CI [0.60, 2.45], \( p = 0.60) \). This, however, may be related to the specific inclusion criteria of this trial: patients were included only when the tumour showed signs of malignant progression at the time of progression (i.e. contrast enhancement on the MRI scan). In this respect, it is interesting to note that the percentage of non-R132H IDH1/2-mutated tumours was almost two-fold lower in TAVAREC trial samples (13%) compared to CATNON (19%) and TCGA (20%). Although this difference in frequency was not significant, these numbers are in line with the notion that non-R132H IDH1/2-mutated tumours have lower frequencies of malignant progression. The small number of patients harbouring non-R132H IDH1/2-mutated tumours \( (n = 11) \) may also mask potential survival differences. A heatmap of most differentially methylated probes shows that non-R132H IDH1/2-mutated tumours and tumours assigned to the prognostically poorer subclass A\(_{\text{IDH HG}}\) clustered at opposite ends of this heatmap (Fig. 4d).

A forest plot of the combined CATNON, TCGA and TAVAREC survival data shows a summary estimate HR for non-R132H IDH1/2-mutated tumours of 0.56 with 95% CI [0.37, 0.85], association \( p = 0.006 \) (Fig. 4e).

To test whether mutation-dependent DNA methylation differences were restricted to 1p/19q non-codeleted gliomas (astrocytomas), we analysed the genome-wide methylation profiles of (i) IDH1/2 mutated, 1p/19q codeleted gliomas (oligodendroglialomas, TCGA), (ii) acute myeloid leukemias (TCGA) and (iii) chondrosarcomas. Although the sample sizes of these datasets were relatively small in all tumour types \( (1p/19q \text{ codeleted gliomas } n = 135 \text{ vs. } 14; \text{ acute myeloid leukemias } n = 4 \text{ vs. } n = 24; \text{ chondrosarcomas } n = 3 \text{ vs. } n = 17 \text{ for IDH1}\(^{R132H}\) and non-R132H IDH1/2-mutated tumours respectively), there was less DNA methylation in IDH1\(^{R132H}\) vs. non-R132H IDH1/2-mutation tumours (Fig. 5a–c). These data demonstrate that the level of DNA methylation is lower in tumours harbouring IDH1/2 mutations with presumed low D-2HG production.

**Table 1** Multivariable model

| IDH mutation type     | HR    | 95% CI  | \( p \) value |
|-----------------------|-------|---------|--------------|
| Non-R132H v. R132H    | 0.486 | 0.278   | 0.852        | 0.012 |
| Sex                   | 1.465 | 1.033   | 2.076        | 0.032 |
| Treatment             |       |         |              |       |
| RT→TMZ vs. RT         | 0.410 | 0.257   | 0.653        | 0.000 |
| TMZ/RT vs. RT         | 0.802 | 0.520   | 1.237        | 0.319 |
| TMZ/RT→TMZ vs. RT     | 0.385 | 0.231   | 0.639        | 0.000 |
| Age                   | 1.121 | 0.656   | 1.914        | 0.677 |
| > 60 vs. < 40 years   | 3.824 | 1.812   | 8.069        | 0.000 |
| Performance score     | 1.404 | 0.991   | 1.990        | 0.056 |
| 2 vs. 0               | 2.282 | 0.704   | 7.401        | 0.169 |
| MGMT promoter methylation |   |         |              |       |
| UM vs. M              | 1.001 | 0.640   | 1.567        | 0.996 |
| Corticosteroid use     | 1.099 | 0.742   | 1.627        | 0.639 |
| Methylation subtype   | 2.650 | 1.828   | 3.842        | 0.000 |
| A\(_{\text{IDH HG}}\) vs. A\(_{\text{IDH}}\) | 0.362 | 0.083   | 1.584        | 0.177 |
| Other vs. A\(_{\text{IDH}}\) | 10.763 | 3.410 | 33.970  | 0.000 |

\( \text{HR} = 95\% \text{ CI} \)
Gene expression analysis of 1p/19q codeleted gliomas present in the TCGA dataset identified 148 differentially expressed genes (expression fold change > 1 or < −1 and $p<0.01$). Similar to observed in astrocytic tumours, the majority of identified genes (123/148, 83%) were upregulated in IDH1$^{R132H}$ mutated tumours (Supplementary Table 5, online resource). Moreover, there was a relatively large degree of concordance in differential expression between the two analyses (Fig. 5d) and sixteen genes were identified in both analyses.

The number of samples and events of the various datasets in patients with 1p/19q codeleted gliomas was insufficient to determine mutation type-dependent survival differences. For example, there were only 14 non-IDH1$^{R132H}$ mutated 1p/19q codeleted tumours in the TCGA dataset, with only 1 event noted (in the IDH1$^{R132H}$ mutated tumours there were 14 events in 135 patients). The HR for TCGA samples was 0.59 (95% CI [0.077, 4.595], $p=0.62$, Fig. 5e). Also in the MSK-Impact [9] and the Chinese Glioma Genome Atlas (CGGA) [23] there were too few samples and events to determine survival benefit in patients harbouring non-IDH1$^{R132H}$ mutated tumours. In these datasets, the events/number in non-IDH1$^{R132H}$ vs. IDH1$^{R132H}$ mutated samples was 0/6 vs. 3/34 and 0/5 vs. 3/31 in MSK impact, and CGGA datasets respectively. We were not able to determine survival differences in AML (n = 12 with 5 events vs. n = 89, 54 events, HR 1.49, 95% CI [0.59, 3.75], $p=0.39$, Fig. 5f).

**Discussion**

Our data shows that IDH1/2mt gliomas are distinct when compared to other IDH1/2mt tumours in that they have a disproportionally high percentage of IDH1$^{R132H}$ mutations and raise the attractive clinical association between
different rarer (codon 132) mutations and outcome. Patients harbouring \( \text{IDH1}^{R132H} \) mutated tumours have lower levels of genome-wide DNA methylation, regardless of tumour type (1p/19q non-codeleted gliomas, 1p/19q codeleted gliomas, AML and chondrosarcomas). For 1p/19q non-codeleted \( \text{IDH1/2mt} \) gliomas, this difference is clinically relevant as patients harbouring non-R132H \( \text{IDH1/2} \)-mutated tumours have improved outcome. Since \( \text{IDH1}^{R132H} \) mutations are presumed to be relatively poor in D-2HG production, our data are in line with the observation that glioma patients with higher D-2HG levels have improved outcome [34]. Our data are also in line with data from a meeting abstract showing similar mutation-specific survival differences [17].

The observation that patients harbouring non-R132H \( \text{IDH1/2} \)-mutated gliomas have longer survival is of importance for clinical practice as the specific \( \text{IDH1/2} \) mutation could alter patient prognostication. In this respect diagnostic assays should be able to discriminate between the type of \( \text{IDH} \)-mutation present; non-R132H \( \text{IDH1/2} \)-mutations comprise ~ 10% of all \( \text{IDH} \)-mutations in astrocytomas. Moreover, the efficacy of treatment with alkylating agents, \( \text{IDH1/2} \)
inhibitors, or other novel treatments might vary per mutation type, and therefore may be taken into account as a stratification factor in future clinical trials.

It has been reported that individual IDH1/2 mutations differ in their ability to produce D-2HG. In fact, the most common mutation in gliomas, \( \text{IDH1}^{R132H} \), is reported to be...
relatively inefficient in producing this oncometabolite [5, 40]. The differential capacity of IDH mutations in D-2HG production is supported by observations from cell lines and clinical samples where tumours harbouring the IDH1-R132H mutation generally have lower D-2HG levels compared to those with other IDH mutations [21, 24, 25, 29, 40] (but not in all [10]) though confounding factors such as tumour purity may influence these observations. Previous reports have shown that D-2HG is a weak inhibitor of TET2 enzymes as relatively high levels of D-2HG are required to inhibit the enzyme [31, 53]. In fact, the IC50 value for TET2 inhibition (~ 5 mM) is in the same range as the intratumoral D-2HG levels [10, 21, 29, 31]. As TET2 mediates the first step in DNA demethylation, lower D-2HG levels may result in reduced inhibition of DNA-demethylation. Therefore, although we did not directly measure D-2HG levels, the partial inhibition of TET2 may explain the lower overall methylation in IDH1-R132H-mutated tumours.

The improved outcome of non-R132H IDH1/2-mutated astrocytomas may be explained by a reduced expression of genes that support tumour growth and/or induce treatment sensitivity caused by the increase in CpG methylation. Evidence supporting this hypothesis is the observation that many of the differentially expressed genes are involved in pathways associated with cancer. However, the improved outcome of non-R132H IDH1/2-mutated astrocytomas may also be related to the observation that D-2HG is toxic to cells, though only at high concentrations. For example, we have previously shown that exposure to D-2HG or expression of mutated IDH constructs reduced proliferation of cells, both in vitro and in vivo [6]. Later independent studies largely confirmed these observations and also conversely, reduction of D-2HG levels by mutant IDH inhibitors increased cell proliferation [18, 38, 40, 47, 55]. It should be noted, however, that in some preclinical model systems a growth inhibitory effect of IDH-inhibitors was observed [39, 41]. Functional experiments should confirm this hypothesis. Alternatively, differences in genetic stress and related mutational signatures may also explain the differential distribution of mutations in IDH [2, 3].

Apart from the type of IDH mutation present in the tumour, other prognostically relevant factors have also been described [43]. This includes histological tumour grade where patients with grade 2 astrocytomas have longer survival than those with grade 3 or grade 4 [36]. It should be noted, however, that we find that tumour grade is not a prognostic factor for the TCGA samples included in this study while the type of IDH-mutation is. In addition, the CATNON trial was performed on anaplastic (grade 3) tumours only.

Limitations of this study include the relatively small sample size of several datasets, especially those with a diagnosis other than the non-1p/19q codeleted gliomas. In addition, the absence of D-2HG level data limits the exploration of a direct correlation between IDH1/2 mutation type and genome-wide DNA methylation.

In short, we described the effect of IDH1/2 mutation type on patient outcome and the strong correlation between these specific mutations and genome-wide DNA methylation status. Our observation that non-R132H IDH1/2-mutated 1p/19q non-codeleted gliomas have a more favourable prognosis than their IDH1-R132H-mutated counterpart is clinically relevant and should be taken into account for patient prognostication.

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**Declarations**

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