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ABSTRACT

Purpose: Despite recent advances in the molecular characterization of gliomas, it remains unclear which patients benefit most from which second-line treatments. The TAVAREC trial was a randomized, open-label phase II trial assessing the benefit of the addition of the angiogenesis inhibitor bevacizumab to treatment with temozolomide in patients with a first enhancing recurrence of World Health Organization grade 2 or 3 glioma without 1p/19q codeletion. We evaluated the prognostic significance of genome-wide DNA methylation profiles and copy-number variations on the TAVAREC trial samples.

Experimental Design: Isocitrate dehydrogenase (IDH) mutation status was determined via Sanger sequencing and IHC. DNA methylation analysis was performed using the MethylationEPIC BeadChip (Illumina) from which 1p/19q codeletion, *MGMT* promoter methylation (MGMT-STP27), and homozygous deletion of *CDKN2A/B* were determined. DNA methylation classes were determined according to classifiers developed in Heidelberg and The Cancer Genome Atlas (TCGA; "Heidelberg" and "TCGA" classifier respectively).

Results: DNA methylation profiles of 122 samples were successfully determined. As expected, most samples were IDH-mutant (89/122) and *MGMT* promotor methylated (89/122). Methylation classes were prognostic for time to progression. However, Heidelberg methylation classes determined at time of diagnosis were no longer prognostic following enhancing recurrence of the tumor. In contrast, TCGA methylation classes of primary samples remained prognostic also following enhancing recurrence. Homozygous deletions in *CDKN2A/B* were found in 10 of 87 IDH-mutated samples and were prognostically unfavorable at recurrence.

Conclusions: DNA methylome Heidelberg classification at time of diagnosis is no longer of prognostic value at the time of enhancing recurrence. *CDKN2A/B* deletion status was predictive of survival from progression of IDH-mutated tumors.

Introduction

DNA methylation profiling was recently demonstrated to be of diagnostic value in primary brain tumors (1, 2). For isocitrate dehydrogenase (IDH)-mutant gliomas, especially those without 1p/19q codeletion, DNA methylation classes are also of important prognostic value (3–5). Although some methylation profiling studies have analyzed DNA methylation at tumor progression (6, 7), the prognostic relevance of these classes at tumor progression remains to be determined. The presence of an IDH mutation in gliomas is associated with a relatively favorable prognosis while contrast enhancement on MRI is associated with a more aggressive tumor type (8–10). However, the prognostic significance of IDH mutations together with the associated genome-wide methylation profiles in the presence of contrast enhancement at the time of progression after first-line treatment is unknown.

The TAVAREC European Organisation for Research and Treatment of Cancer (EORTC) 26091 trial was a randomized, open-label phase II trial assessing the benefit of the addition of the angiogenesis inhibitor bevacizumab to treatment with temozolomide in patients with a first enhancing recurrence of grade 2 or grade 3 glioma without 1p/19q codeletion (11). No evidence of improved overall survival (OS) was found when bevacizumab was added to temozolomide chemotherapy. Despite the negative overall results, this trial presents the unique opportunity to evaluate the predictive value of DNA methylation profiling of the primary tumors once the tumor shows contrast enhancement on MRI scans.

Here, we present the analysis of the DNA methylome of tumor specimens from patients enrolled into TAVAREC. Our data demonstrates that, expectedly, there are large differences in patient survival

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Despite recent advances in the molecular characterization of gliomas, treatment of recurrent lower grade glioma remains a clinical challenge and is subject to debate. Here, we present an integrated analysis of clinical and molecular (DNA methylome profiling) data of World Health Organization grade 2 or grade 3 glioma without 1p/19q codeletion. We firstly confirm that methylation classes at time of diagnosis are prognostic for time to progression. However, our main and clinically relevant finding is that we demonstrate that methylation classes derived from primary samples lose their prognostic value following enhancing recurrence of the tumor. However, methylation classes determined on re-resected tumors remained of prognostic value, despite the presence of radiological signs of dedifferentiated tumor progression. The limited prognostic value of methylation profiles determined at time of diagnosis following enhancing recurrence is of relevance in prognostication and determining the treatment strategy for such patients.

between those harboring IDH wild-type (WT) and IDH-mutant tumors, similar to previously reported.

DNA methylation–based tumor classifications have developed into powerful accurate diagnostic tools. Here, we classified tumor samples as defined by the The German Cancer Research Center (DKFZ) in Heidelberg (1) and as determined from The Cancer Genome Atlas (TCGA) datasets (2). Heidelberg methylation classes determined at the time of diagnosis were no longer prognostic following MRI enhancing recurrence of the tumor but TCGA methylation remained prognostic also following recurrence.

Materials and Methods

Sample processing

The TAVAREC study was an open-label, multicenter, two arm randomized controlled phase II trial assessing the activity of bevacizumab in combination with temozolomide at tumor recurrence of locally diagnosed grade 2 and grade 3 gliomas without 1p/19q codeletion, with a control arm treated with temozolomide alone. Patient accrual, randomization, treatment, sample, and clinical data collection have been described previously (11). All institutions obtained ethics approval from their institutional review boards or ethics review committees and was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent according to local, national, and international guidelines prior to study enrollment.

In view of the absence of differences in outcome between treatment arms, the study arms were aggregated for the current prognostic analysis. Formalin-fixed paraffin-embedded (FFPE) tumor samples were centrally collected (EMC Rotterdam) for most of the trial patients (139/155). FFPE (4 μm) sections were hematoxylin and eosin stained and reviewed by a central neuropathologist (J.M. Kros). Areas with the highest tumor content (>70%) were macro-dissected from 15 to 20 (10 μm) sections from which DNA and RNA was isolated using the Qiagen Allprep DNA/RNA FFPE kit according to the manufacturer's instructions with an added overnight proteinase K digestion.

Molecular profiling and data processing

DNA methylome profiling was performed using the Infinium MethylationEPIC BeadChip with 30 to 250 ng DNA input (Illumina, San Diego, California) interrogating 865,859 methylation sites. The minimum DNA input (30 ng) was determined by assessing the performance in technical replicates ($N = 3$) with lower DNA inputs of 150, 80, and 30 ng respectively. Array data (IDAT files) were processed in *R* (version 4.0.3) using the Bioconductor minfi package (version 1.36.0) to obtain the raw signal intensities (12). Quality control plots, M/Beta values densities, and control probe intensities were visualized using the shinyMethyl package (13). Samples with poor quality, i.e., in which <95% were detected with P value < 0.01, were removed from the analysis. Unprocessed IDAT array data files were uploaded to the web-based Heidelberg profiling classifier (MolecularNeuropathology.org) to obtain Heidelberg classifications, copy-number data (derived from the conumee Bioconductor package), and O^6-methylguanine DNA methyltransferase (*MGMT*) promotor methylation scores (1). *MGMT* promotor methylation was defined as a MGMT-STP27 (14, 15) score above 0.3582.

TCGA DNA methylation profile classifications were obtained through Bioconductor TCGAbiolinks 2.18.0. glioma-CpG island methylator phenotype (G-CIMP)-high samples were classified as "Risk" and "No-risk" to progression to a G-CIMP-low profile as described, based on the methylation of seven of the following CpG sites: cg09732711, cg09326832, cg24665265, cg06220958, cg10245915, cg11689625, and cg11799650.(3)

Probes mapping to the X and Y chromosomes, probes containing a SNP within 5 basepairs of the targeted CpG site, probes which did not (uniquely) map to the bisulfite-converted human reference genome (hg19) and probes with partial overlap to nonunique elements (off-target hybridization) were masked from the analysis (16). Differentially methylated region (DMR) analysis was performed using the Bioconductor MEAL package (version 1.23.0).

IDH1 and *IDH2* mutations were determined using Sanger sequencing. In case of inconclusive sequencing results (e.g., because of poor DNA quality or insufficient quantity) we performed IHC using IDH1R132H-specific antibodies (Dianova, Germany). Positive staining on IHC was scored as IDH mutation positive, whereas negative staining was scored as indeterminate IDH status because other non-R132H mutations might be present.

Statistical analysis and plotting

OS was measured from both the time of initial diagnosis (defined as the date of first surgery) and the time of randomization. All statistical analysis was performed with *R* (version 4.0.3). Survival analysis was performed with the survminer R package. Survival comparison between groups was tested for statistical significance using the logrank test. The most variable CpG probes were plotted as a heatmap using Ward's minimum variance method for hierarchical clustering (ComplexHeatmap R package). Circos plotting of Heidelberg, TCGA and World Health Organization (WHO) 2021 classifications was performed with the circlize R package. Recursive partitioning survival analysis was performed within the party R package using conditional inference trees. Analysis was exploratory, $P < 0.05$ was used to indicate statistical significance.

Data availability statement

EORTC supports data sharing and invites researchers within and outside the EORTC to access datasets according to its data sharing policy. This is explained in the data sharing policy agreement of the EORTC; https://www.eortc.org/app/uploads/2018/02/pol008.pdf; see also the EORTC website https://www.eortc.org/data-sharing/. Data is available via the European Genome-Phenome archive (EGA-archive.org) with study id EGAS00001006015 and dataset EGAD00010002289.

Results

Sufficient material was available for 125 of 155 trial samples of which 122 samples passed methylation data quality control. Most samples were derived from the primary sample at tumor diagnosis (101/122). In 21 cases, the sample was derived from the recurrent tumor. Baseline characteristics for these 122 patients are shown in **Table 1**. There were no significant differences in baseline clinical characteristics with respect to patient age, sex, clinical performance score, tumor grade, corticosteroid usage, treatment arm, and survival between the included patients and the patients without methylation profiling data of the TAVAREC trial ($N = 33$; Supplementary Table S1). Of the samples with methylation data, most were IDH-mutant ($N = 89$, 73.0%) and *MGMT* methylated ($N = 89$, 73.0%). DNA methylation profiling copy-number data revealed a codeletion of 1p19q in 3 samples. These samples were removed from the survival analysis.

We first screened for differences in methylation profiles between samples obtained at initial diagnosis and samples obtained at tumor recurrence. There were no significant DMRs in the recurrent IDH-mutant samples compared with the primary IDH-mutant samples. The recurrent samples showed a relative hypomethylation compared with the primary samples (Supplementary Fig. S1).

Prognostic value of WHO 2021 and DNA methylation classes at time of MRI enhancing recurrence

Samples were first classified according to the WHO 2021 criteria (ref. 17; including both the IDH sequencing data, the molecular data extracted from the methylation profiles and histology grading) into: astrocytoma, IDH-mutant, grade 2 ($N = 29$); astrocytoma, IDH-mutant, grade 3 ($N = 44$); astrocytoma, IDH-mutant, grade 4 ($N = 11$); glioblastoma, IDH WT ($N = 18$); and oligodendroglioma, IDH-mutant and 1p19q codeleted ($N = 3$).

The CNS tumor classifier as defined by Capper and colleagues (ref. 1; 'Heidelberg classifier') identified the following DNA methylation classes in our sample cohort: astrocytoma, IDH-mutant ($N = 58$); high-grade astrocytoma IDHmt ($N = 32$); glioblastoma, IDH WT subclasses ($N = 23$; 3 RTK I, 14 RTK II, 3 mesenchymal subclass, 2 MYCN, and 1 midline); oligodendroglioma, IDH-mutant, 1p/19q

Table 1. Baseline characteristics.

Variable	*N* = 122	%		*N* = 122	%
Age (years)	43 (34, 52)		**MGMTp methylation status**		
Age groups (years)			Methylated	89	73.0
<40	48	39.3	Unmethylated	33	27.0
40–65	62	50.8	**1p19q status**		
≥65	12	9.8	Codeleted	3	2.5
Gender			Intact	119	97.5
F	42	34.4	**WHO 2021 classification**		
M	80	65.6	Astrocytoma, IDH-mutant, grade 2	29	23.8
WHO performance status			Astrocytoma, IDH-mutant, grade 3	44	36.1
0	50	41.0	Astrocytoma, IDH-mutant, grade 4	11	9.0
1	61	50.0	Glioblastoma, IDH WT	18	14.8
2	11	9.0	Inconclusive	17	13.9
Grade at first diagnosis (local)			Oligodendroglioma, IDH mut, 1p/19q codel	3	2.5
NA	1	0.8	**Heidelberg classification**		
Grade 2	67	54.9	Anaplastic PA	1	0.8
Grade 3	54	44.3	Astrocytoma, IDH mut	58	47.5
Grade at first diagnosis (central)			Atypical teratoid, subclass SHH	1	0.8
NA	3	2.5	Control tissue, white matter	1	0.8
Grade 1	1	0.8	Glioblastoma, IDH WT, subclass midline	1	0.8
Grade 2	42	34.4	Glioblastoma, IDH WT, subclass MYCN	2	1.6
Grade 3	51	41.8	Glioblastoma, IDH WT, subclass RTK I	3	2.5
Grade 4	22	18.0	Glioblastoma, IDH WT, subclass RTK II	14	11.5
Other	3	2.5	High-grade astrocytoma, IDH mut	32	26.2
Corticosteroids usage	38	31.1	Low-grade glioma, unspecified	1	0.8
Prior radiotherapy			Not determinable	5	4.1
No	3	2.5	Oligodendroglioma, IDH mut, 1p/19q codel	3	2.5
Yes, radiotherapy	119	97.5	**TCGA classification**		
Prior chemotherapy			Classic-like	8	6.6
No	94	77.0	Codel	7	5.7
Yes, PCV	2	1.6	G-CIMP-high - High-risk	18	14.8
Yes, TMZ	26	21.3	G-CIMP-high - Low-risk	61	50.0
TAVAREC arm			G-CIMP-low	9	7.4
TMZ	61	50.0	Mesenchymal-like	13	10.7
TMZ+Bv	61	50.0	PA-like	6	4.9
IDH status			**Surgery at recurrence**	41	33.6
Mutated	89	73.0			
R132H staining negative	6	4.9			
Undetermined	1	0.8			
WT	26	21.3			

codeleted ($N = 3$); anaplastic pilocytic astrocytoma (PA; $N = 1$); low-grade glioma, unspecified ($N = 1$); H3 K27-mutant diffuse midline glioma ($N = 1$); Atypical teratoid/rhabdoid tumor, subclass SHH ($N = 1$); and finally two samples were classified as control tissue. High-grade astrocytomas (IDH-mutant glioblastoma and IDH-mutant anaplastic astrocytoma) were equally distributed between primary and recurrent samples (26/101 and 6/21 respectively; Supplementary Table S2). The classifier as defined by the TCGA (ref. 2; 'TCGA classifier') identified the following DNA methylation subclasses in our cohort: G-CIMP-high ($N = 79$), G-CIMP-low ($N = 9$), mesenchymal-like ($N = 13$), classic-like ($N = 8$), PA-like ($N = 6$), and codel ($N = 7$). G-CIMP-low status was present in 3 of 18 recurrent IDH-mutant samples and 6 of 71 primary IDH-mutant samples ($P = 0.5518$ Pearson χ^2 test). Similar to previously observed, these classification schemes split samples in prognostically different subtypes from initial diagnosis (Supplementary Fig. S2A–S2C). These DNA methylation subtypes continue to be prognostic for survival from the time of enhancing recurrence (**Fig. 1A–C**).

To determine whether profiles from the initial surgery remained prognostic at tumor recurrence, we performed survival analysis only on samples that were derived from the initial tumor ($n = 101$). We found that the methylation classes as defined by the Heidelberg classifier were no longer prognostic following enhancing recurrence. The HR for astrocytoma, IDH-mutant vs. high-grade astrocytoma, IDH-mutant was 1.35, $P = 0.2820$ (**Fig. 2A**). The distribution between Heidelberg classifications were highly similar between primary and recurrent IDH-mutant samples (Supplementary Table S3).

Within WHO 2021 IDH-mutant grade 3 astrocytomas, Heidelberg classification was prognostic at tumor recurrence: 24.9 months post-progression survival for astrocytoma IDH-mutant versus 12.9 months for high-grade astrocytoma IDH-mutant, $P = 0.01$, but not from initial diagnosis (Supplementary Fig. S3A and S3B). In contrast, within Heidelberg high-grade IDH-mutant astrocytomas, WHO grade was not prognostic both from initial diagnosis and from tumor recurrence (Supplementary Fig. S3C and S3D).

In contrast, the methylation classes as defined by the TCGA classifier remained of prognostic value. The HR for G-CIMP-low versus G-CIMP-high was 4.06, $P = 0.0032$ (**Fig. 2B**). Heidelberg methylation classes derived from recurrent samples ($N = 17$) were prognostic following enhancing recurrence (median OS from recurrence 28.5 months for astrocytoma, IDH-mutant vs. 13.2 months for high-grade astrocytoma, IDH-mutant, $P = 0.0001951$; **Fig. 2C**).

We found no survival differences in OS and post-progression survival and PFS between primary G-CIMP-high tumors at risk and not at risk to G-CIMP-low progression in the TAVAREC samples (Supplementary Fig. S4).

There was no significant difference in survival between G-CIMP-low tumors diagnosed at primary or recurrent setting and there was no

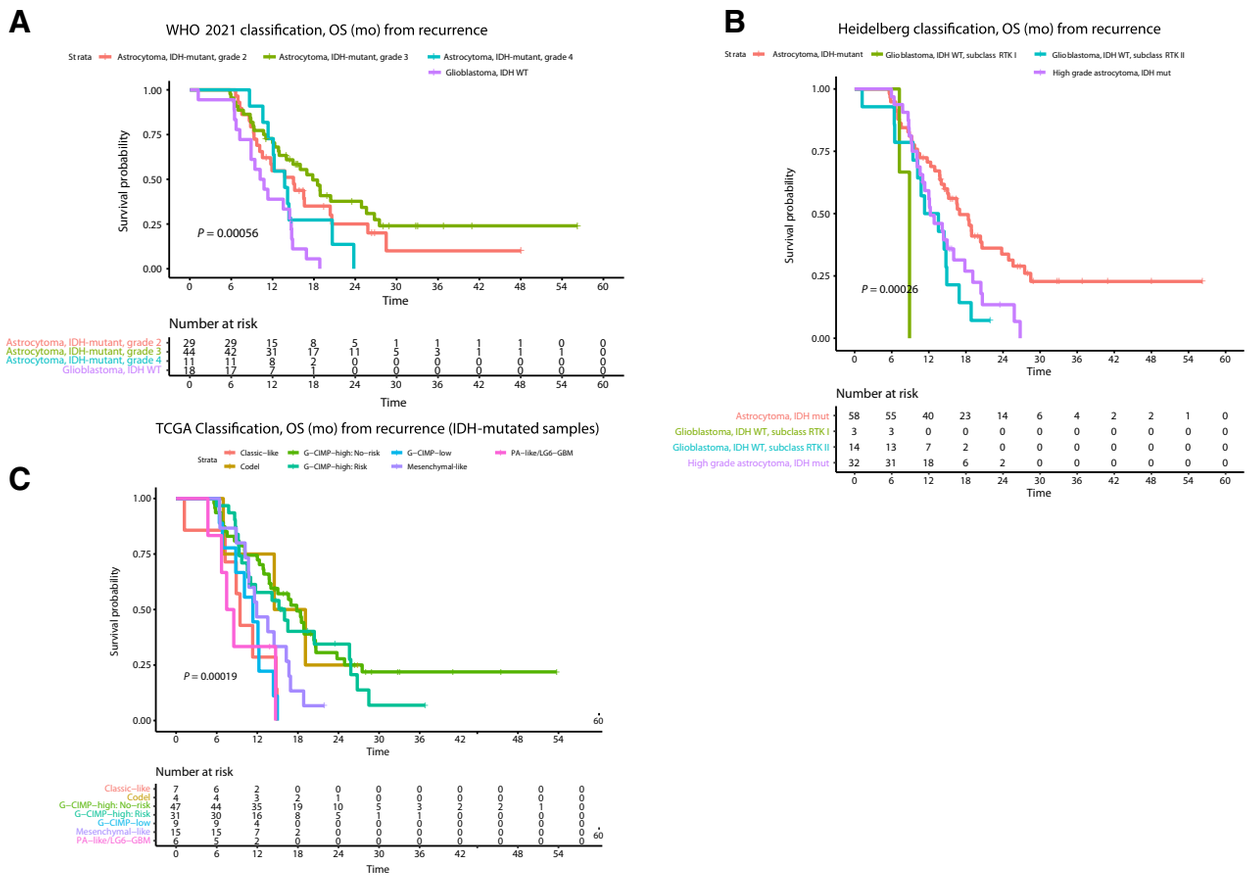

Figure 1.
Survival from recurrence in months (Kaplan–Meier) for WHO 2021 classification (**A**), Heidelberg DNA methylome classifications (**B**), and TCGA DNA methylation classifications (**C**).

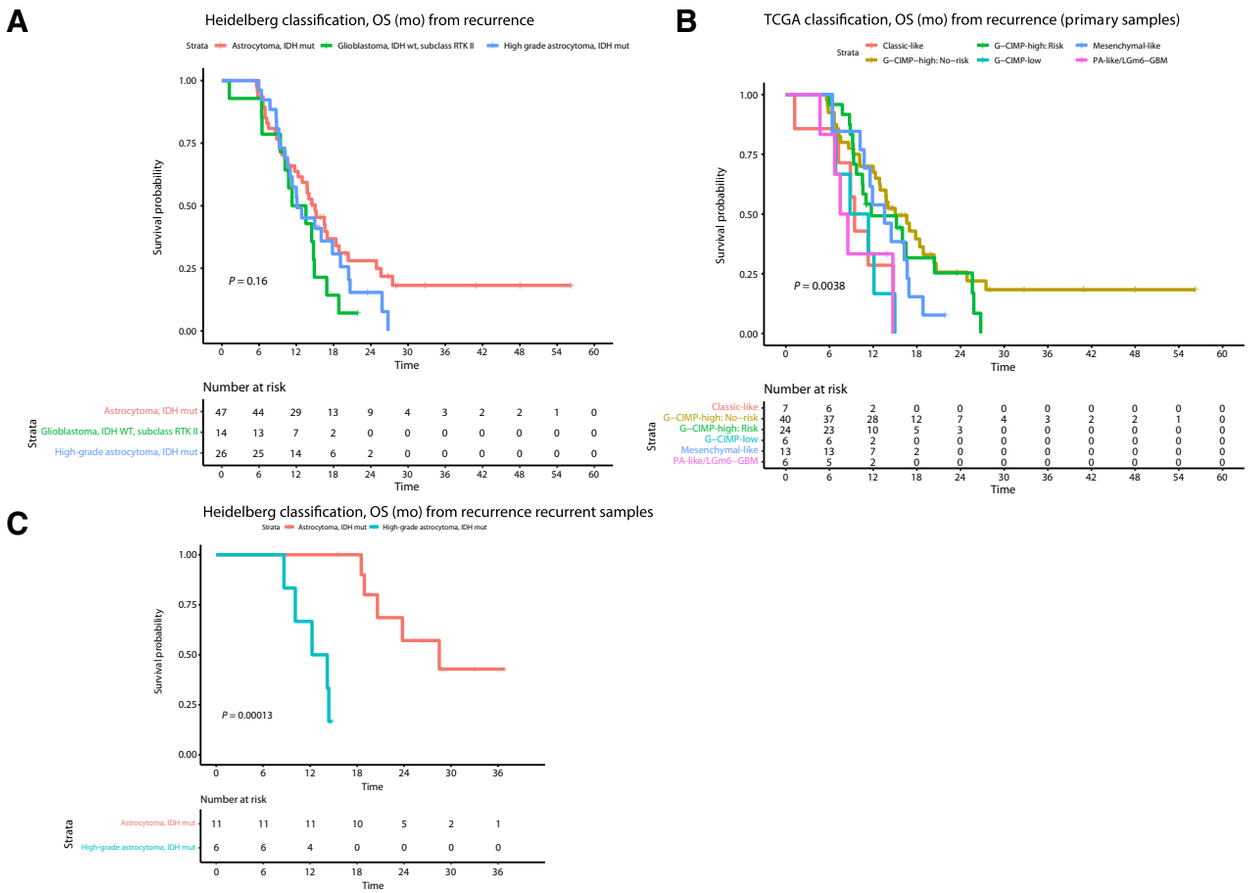

Figure 2.

Survival from recurrence in months (Kaplan–Meier) for Heidelberg DNA methylome classifications in primary samples (**A**), TCGA DNA methylation classifications in primary samples (**B**), and Heidelberg DNA methylome classifications in recurrent samples (**C**).

distinctive hypo- or hypermethylation pattern when comparing primary and recurrent G-CIMP-low samples (Supplementary Fig. S5).

DNA methylome hierarchical clustering

Figure 3 provides an integrated overview of the DNA methylome profiling (using the 1,000 most variable CpG sites across the dataset) and clinical data. Unsupervised clustering of the samples results in a clear separation of IDH WT and IDH-mutant samples and G-CIMP^{+} versus G-CIMP^{-} samples. Patients with IDH WT tumors presented at an older age (median 56.6 vs. 40.2 years; $P < 0.001$).

Figure 4A–C shows the overlap in sample classifications between the WHO 2021, Heidelberg and TCGA classifiers. None of the astrocytoma, IDH-mutant grade 4 samples was classified as glioblastoma (or subtypes thereof) in both the TCGA and Heidelberg classifications. When comparing the Heidelberg and TCGA classifications, most (55/58) Astrocytoma, IDH-mutant samples are assigned to G-CIMP-high tumors and 8 of 32 (25%) of Astrocytoma, high-grade are assigned to the G-CIMP-low subclass.

Within the IDHmt tumors, multivariable survival analysis confirmed the prognostic value of age, clinical performance status, corticosteroid use, and DNA methylation classification (Supplementary Table S4). *MGMT* methylation status was not prognostic in multivariable analysis. Within recursive partitioning analysis of survival at IDH-mutant tumor recurrence (integrating clinical and molecular features of IDH-mutant samples), corticosteroid use was the most predictive factor (Supplementary Fig. S6; HR, 2.13; 95% confidence interval, 1.19–3.81; $P = 0.011$). Tumor grade at initial diagnosis was not predictive of survival at tumor recurrence (16.5 vs. 13.4 months, $P = 0.31$).

MGMT and *CDKN2A/B* status at time of progression

As expected, most (73/87) IDH-mutant samples were *MGMT* promotor methylated (83.9%). *MGMT* promotor methylation is mostly retained in recurrent glioma (18). Within the IDH-mutated samples, *MGMT* promotor methylation was prognostic for survival from recurrence (median 16.6 months vs. 12.9 months, $P = 0.049$; **Fig. 5A**), but not from initial diagnosis (median 66.2 months vs. 70.5 months, $P = 0.11$). Thirty-six of 73 *MGMT* methylated IDH-mutated tumors (49%) showed a complete or partial objective response to second-line chemotherapy compared with 8 of 14 (57%) for the *MGMT* unmethylated tumors ($P = 0.597$). No complete response was measured in the *MGMT* unmethylated tumors (0/14) compared with 9 of 73 (12%) *MGMT* methylated tumors (**Table 2**).

Homozygous deletions of *CDKN2A/B* were more prevalent in IDH WT tumors (16/32; 50.0% vs. 10/87, 11.5%, $P < 0.001$). At initial surgery, 6 of 70 IDH-mutant samples showed a homozygous deletion

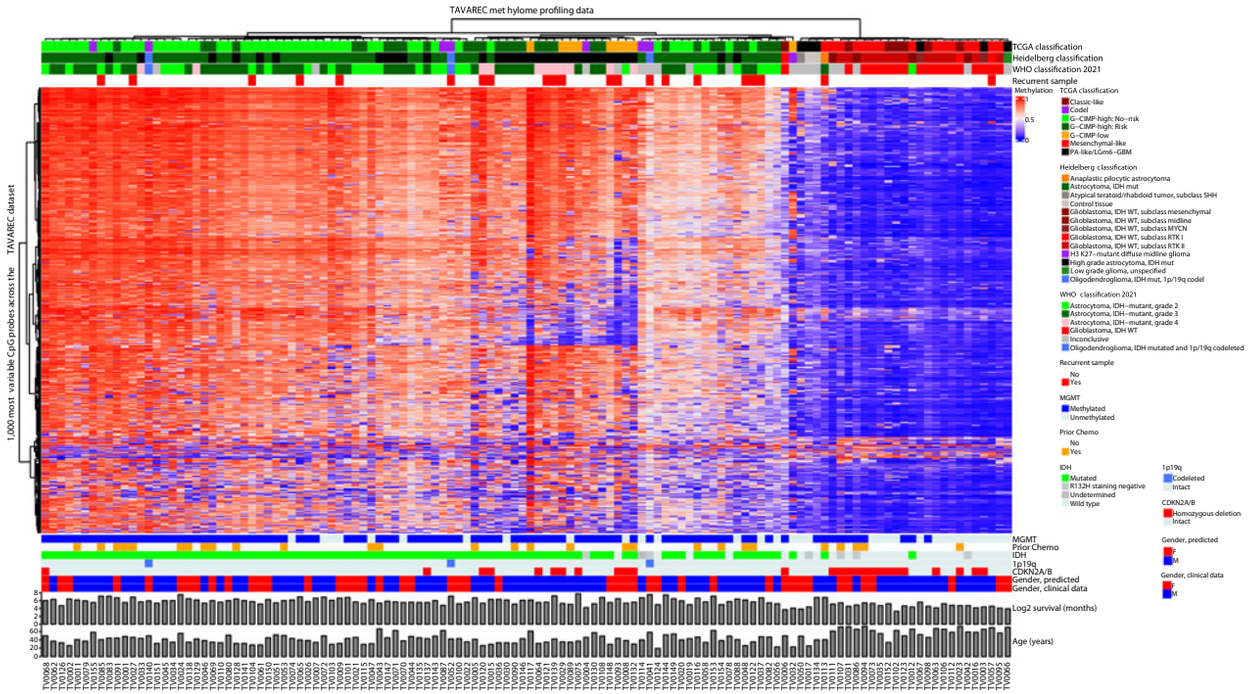

Figure 3.
Heatmap of the 1,000 most variable CpG probes across the TAVAREC dataset annotated with clinical and molecular features.

of *CDKN2A/B* (8.6%) compared with 54% (13/24) of IDH WT primary samples. At recurrent surgery, 4 of 17 (23.5%) of IDH-mutant samples showed a homozygous deletion of *CDKN2A/B* compared with 1 of the 2 IDH WT samples. Overall, for IDH-mutant tumors *CDKN2A/B* status was not predictive of survival at initial diagnosis (Supplementary Fig. S7A). There was also no difference in progression-free survival for IDH-mutated samples with or without a homozygous *CDKN2A/B* deletion (3.21 vs. 3.50 years, $P = 0.47$; Supplementary Fig. S7B), though sample size is too small to draw firm conclusions. However, at IDH-mutant tumor progression, *CDKN2A/B* homozygous deletion was predictive of worse OS (9.9 vs. 15.0 months, $P = 0.024$; **Figure 5B**). *CDKN2A/B* status was not prognostic for IDH WT tumors (10.8 vs. 10.5 months, $P = 0.88$; Supplementary Fig. S7C).

Discussion

Standard of care for WHO grade 2 or 3 IDH-mutant astrocytoma consists of a maximal safe resection followed by either wait-and-scan or radio- or chemotherapy or combinations thereof. However, all gliomas inevitably relapse. At tumor recurrence, treatment options depend on general and neurological status, progression pattern and previous first-line therapies. Current EANO guidelines propose to

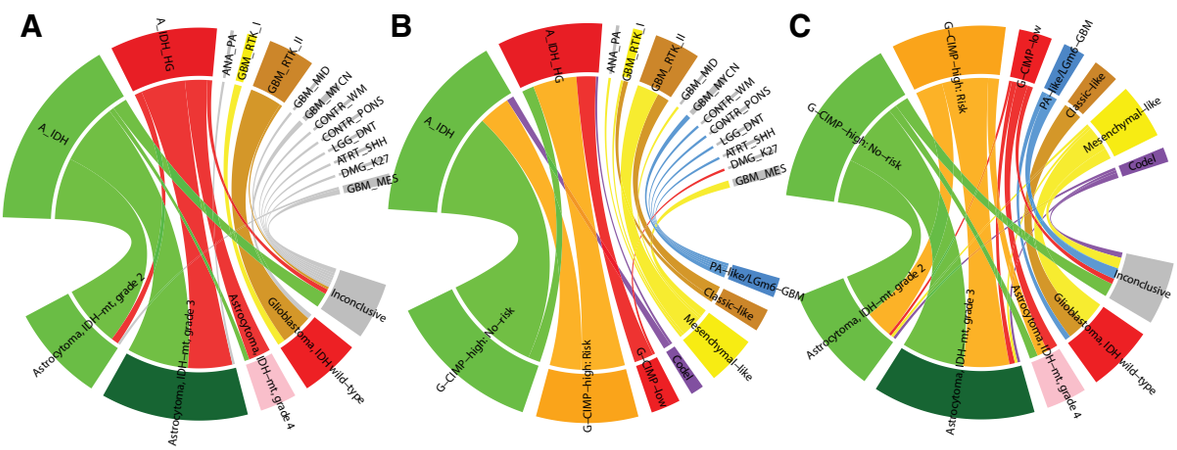

Figure 4.
Circos plot comparing the classification schemes of WHO 2021 (**A**) and Heidelberg, Heidelberg and TCGA (**B**), and TCGA and WHO 2021 (**C**).

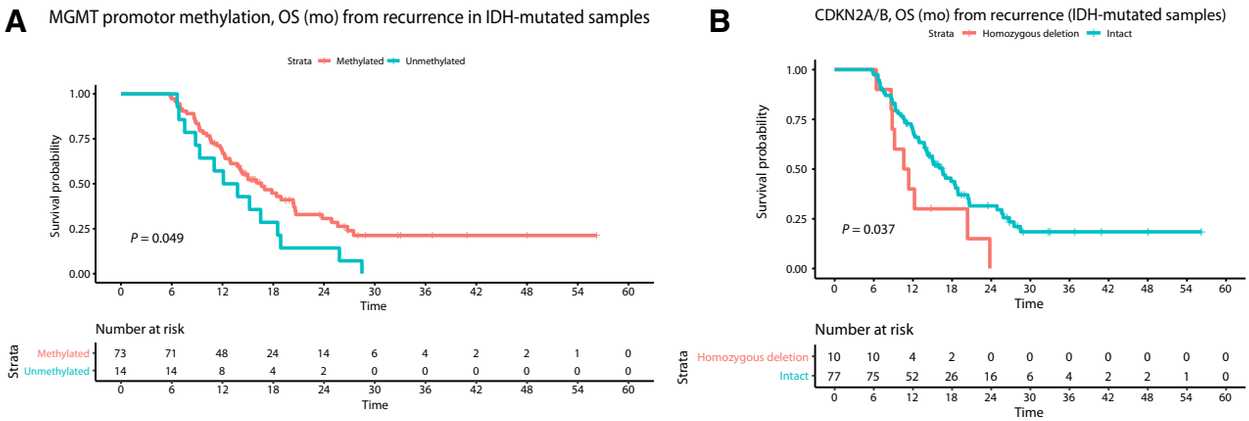

Figure 5.
Survival in months (Kaplan–Meier) from recurrence in IDH-mutant primary samples *MGMT* promoter status (**A**) and *CDKN2A/B* status (**B**).

always consider a second surgery as second-line treatment (19). Despite recent advances in molecular characterization of gliomas (20, 21), debate remains which patients benefit most from which second-line treatments. In this study, we present the methylome profiling data for 122 grade 2 and 3 tumors with a first and enhancing recurrence treated in the TAVAREC trial. Methylome classification performed at tumor diagnosis is of limited prognostic value at IDH-mutant tumor recurrence. Furthermore, we demonstrate that *CDKN2A/B* status was predictive of survival at IDH-mutant tumor recurrence while tumor grade was not.

The TAVAREC trial was based on the WHO 2007 classification, which was revised in 2016 and again in 2021. Since 2016, the classification distinguishes between non-1p/19q codeleted astrocytoma IDH WT from IDH-mutant. Our data demonstrates the molecular heterogeneity of 1p/19q non-codeleted astrocytomas, with up to 1 of 3 of tumors being IDH WT, of which >50% (*TERT* promoter mutational status was unknown) would currently be diagnosed as glioblastoma (21). Similar percentages have also been observed in the CATNON trial. Also similar to CATNON was the presence of a small percentage of samples harboring 1p19q codeletion despite local molecular testing (3/122; ref. 5).

An interesting addition to the TCGA classifier was the identification of patients at risk of dedifferentiated progression: G-CIMP-high patients that are at risk of progression to G-CIMP-low have poorer survival than those not at risk to G-CIMP-low progression (3, 5). However, we found no survival differences in OS and post-progression survival and PFS between primary G-CIMP-high tumors at risk and not at risk to G-CIMP-low progression in the TAVAREC samples.

Table 2. Best response in *MGMT* methylated and unmethylated IDH-mutated samples.

Characteristics	Methylated, $N = 73$[a]	Unmethylated, $N = 14$[a]	P[b]
Best response			0.2
CR	9 (12%)	0 (0%)	
Missing	7 (9.6%)	0 (0%)	
PD	10 (14%)	4 (29%)	
PR	27 (37%)	8 (57%)	
SD	20 (27%)	2 (14%)	

[a]*n* (%).
[b]Fisher exact test.

MGMT promotor methylation status is a predictive marker for response to alkylating chemotherapy in IDH WT gliomas and was repeatedly shown to be of no prognostic value in IDH-mutant gliomas (22–24). Within our cohort, the *MGMT* was not prognostic of survival at tumor recurrence for IDH-mutant astrocytoma in multivariable analysis. A homozygous deletion of *CDKN2A/B* is a prognostic marker for poor survival in IDH-mutant gliomas (25–27). In our cohort, the *CDN2A/B* status was predictive of a poor outcome at tumor recurrence in univariable analysis. Despite the relatively small sample size, we observed a relative higher percentage of *CDKN2A/B* deletions in recurrent samples. It is possible that more tumors from which we only received material from the recurrent tumor have developed loss at this locus at recurrence. Tumor grade at initial diagnosis (WHO grade 2 vs. 3) was not predictive of survival at tumor recurrence. Thirteen of 26 IDH WT tumors (50%) would be classified as glioblastoma according to the current guidelines depending on the presence of a combined gain of chromosome 7 and loss of chromosome 10 and/or *EGFR* amplification (*TERT* mutation status remained unknown; ref. 21). Tumor grade at recurrence in the 34 patients with an IDH-mutant tumor was also not significantly associated with post-progression survival within this cohort.

IDH-mutant gliomas have been established as a molecular and clinical separate entity from IDH WT gliomas. We therefore limited the survival analysis to the IDH-mutant samples which reduced the power of our analysis with only samples from 87 patients with IDH-mutant tumors. Of note, this trial included patients with enhancing first recurrences, which will have resulted in a selection based on molecular features as many astrocytoma IDH-mutant relapse initially with a non-enhancing progression. Also, for most samples we only had tumor specimens available at initial diagnosis. We were therefore unable to correct for molecular changes at tumor recurrence (e.g., more frequent CDKN2A/B homozygous deletions at tumor recurrence). It is possible that the loss of CDKN2A/B locus affects tumor classification in the Heidelberg classifier more often than the TCGA classifier, which could be an explanation to why the Heidelberg classification was not prognostic at tumor recurrence.

In conclusion, we presented an integrated analysis of clinical and molecular (DNA methylome profiling) data of 122 patients with WHO grade 2 and 3 non-1p/19q codeleted glioma patients with a first enhancing recurrence. We demonstrate the limited prognostic value of DNA methylome tumor classification of primary samples after enhancing tumor recurrence. *CDKN2A/B* status was prognostic at IDH-mutant tumor recurrence.

Authors' Disclosures

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Disclaimer

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Authors' Contributions

K. Draaisma: Formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **C.M.S. Tesileanu:** Formal analysis, writing–review and editing. **I. de Heer:** Data curation, project administration, writing–review and editing. **M. Klein:** Writing–review and editing. **M. Smits:** Writing–review and editing. **J.C. Reijneveld:** Writing–review and editing. **P.M. Clement:** Writing–review and editing. **F.Y.F. de Vos:** Writing–review and editing. **A. Wick:** Writing–review and editing. **P.J. Mulholland:** Writing–review and editing. **M.J.B. Taphoorn:** Writing–review and editing. **M. Weller:** Writing–review and editing. **O.L. Chinot:** Writing–review and editing. **J.M. Kros:** Investigation, writing–review and editing. **T. Verschuere:** Writing–review and editing. **C. Coens:** Writing–review and editing. **V. Golfinopoulos:** Writing–review and editing. **T. Gorlia:** Writing–review and editing. **A. Idbaih:** Resources, writing–review and editing. **P.A. Robe:** Writing–review and editing. **M.J. van den Bent:** Conceptualization, resources, supervision, funding acquisition, investigation, writing–original draft, writing–review and editing. **P.J. French:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, writing–review and editing.

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