Identification of subsets of \textit{IDH}-mutant glioblastomas with distinct epigenetic and copy number alterations and stratified clinical risks

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Abstract

Background. \textit{IDH}-mutant glioblastoma is classified by the 2016 CNS WHO as a group with good prognosis. However, the actual number of cases examined in the literature is relatively small. We hypothesize that \textit{IDH}-mutant glioblastoma is not a uniform group and should be further stratified.

Methods. We conducted methylation profiles and estimated copy number variations of 57 \textit{IDH}-mutant glioblastomas.

Results. Our results showed that 59.6\% and 40.4\% of tumors belonged to glioma-CpG island methylator phenotype (G-CIMP)-high and G-CIMP-low methylation subgroups, respectively. G-CIMP-low subgroup was associated with significantly worse overall survival (OS) as compared to G-CIMP-high (P = .005). \textit{CDKN2A} deletion (42.1\%) was the most common gene copy number variation, and was significantly associated with G-CIMP-low subgroup (P = .004). Other frequent copy number changes included mesenchymal–epithelial transition (\textit{MET}) (5.3\%), \textit{CCND2} (19.3\%), \textit{PDGFR} (14.0\%), \textit{CDK4} (12.3\%), and \textit{EGFR} (12.3\%) amplification. Both \textit{CDKN2A} deletion (P = .036) and \textit{MET} amplification (P < .001) were associated with poor OS in \textit{IDH}-mutant glioblastomas. Combined epigenetic signature and gene copy number variations separated \textit{IDH}-mutant glioblastomas into Group 1 (G-CIMP-high), Group 2 (G-CIMP-low without \textit{CDKN2A} nor \textit{MET} alteration), and Group 3 (G-CIMP-low with \textit{CDKN2A} and/or \textit{MET} alteration). Survival analysis revealed Groups 1 and 2 exhibited a favorable OS (median survival: 619 d [20.6 mo] and 655 d [21.8 mo], respectively). Group 3 exhibited a significant shorter OS (median survival: 252 d [8.4 mo]). Multivariable analysis confirmed the independent prognostic significance of our Groups.

Conclusions. \textit{IDH}-mutant glioblastomas should be stratified for risk with combined epigenetic signature and \textit{CDKN2A/MET} status and some cases have poor outcome.
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**Key points**
1. Not all IDH-mutant glioblastomas have good prognosis.
2. Combined DNA methylation subgroups and CDKN2A/mesenchymal–epithelial transition (MET) status identified a subset of IDH-mutant glioblastomas with poor outcome.
3. Glioma-CpG island methylator phenotype-low, CDKN2A deletion, and MET amplification are negative prognostic markers in IDH-mutant glioblastomas.

**Importance of the Study**
The WHO 2016 Classification of Tumors of the Central Nervous System has classified glioblastoma into IDH-wildtype and IDH-mutant, with the latter being described to have a better prognosis and to be more often found in secondary glioblastoma. However, only a small number of cases were actually examined in the literature. We hypothesize that IDH-mutant glioblastoma is not a uniform group and should be stratified further for risk to provide more precise prognostication. By profiling DNA methylation of 57 IDH-mutant glioblastomas and by mining the epigenetic data for copy number variations, we identified a subset of glioma-CpG island methylator phenotype-low, IDH-mutant glioblastomas carrying CDKN2A or mesenchymal–epithelial transition alteration and these tumors have poor survivals in spite of their being IDH mutant.

The WHO 2016 Classification of Tumors of the Central Nervous System (CNS) has classified glioblastoma into IDH-wildtype and IDH-mutant, with the latter being described to have a better prognosis and to be more often found in secondary glioblastoma. IDH-mutant glioblastoma shows different genetic, epigenetic, and clinical features compared to IDH-wildtype counterpart. Recurrent mutations in IDH genes in glioblastomas were first described in 2008. Afterwards, Yan et al. showed that 11/13 (84.6%) of secondary glioblastomas carried IDH mutations whereas such alterations were only observed in 6/123 (4.9%) of primary glioblastomas. Yan et al. also showed that some cases of IDH-mutant glioblastomas harbored 1p19q codeletion and CDKN2A deletion. Overall, 17 cases of IDH-mutant glioblastomas were actually genetically examined in that study. The TCGA database focused on primary glioblastoma and only contained 35 patients diagnosed with IDH-mutant glioblastoma. Global mRNA expression analysis revealed that IDH-mutant glioblastomas were enriched for the proneural subtypes. Overall, the number of IDH-mutant glioblastomas having been evaluated with follow-up data was small at the time of WHO 2016. A very recent paper examined 97 IDH-mutant glioblastomas and showed that CDKN2A deletion was associated with a poor prognosis. Taken together, these data suggest that IDH-mutant glioblastoma is a heterogeneous group that can be further stratified.

At the epigenetic level, researchers including our team have shown that gliomas overall can be divided by the status of glioma-CpG island methylator phenotype (G-CIMP) into G-CIMP positive and G-CIMP negative. G-CIMP positive tumors display extensive DNA hypermethylation at specific loci and are associated with IDH mutation. G-CIMP positive gliomas have an improved survival over G-CIMP negative gliomas. Recently, we further showed by unsupervised clustering analysis of methylation profiling that IDH-mutant gliomas overall could separate into three methylation subgroups, the Codel, G-CIMP-high, and G-CIMP-low subgroups. However, the number of IDH-mutant glioblastoma cases, in contrast to low-grade gliomas, was only 35 in this study, and 7 of 35 cases had available DNA methylation data spanning approximately 450,000 CpG sites. Therefore, the importance of the different DNA methylation subgroups among IDH-mutant glioblastomas is not well characterized.

On the basis of the literature, we hypothesize that IDH-mutant glioblastoma is a not a uniform group and should be further stratified for more precise prognostication and bedside management. In this study, we examined the genome-wide methylation profiles of 57 IDH-mutant glioblastomas and determined gene copy number variations (CNVs) from DNA methylation array. We were able to integrate epigenetic signature and CNVs into a stratification scheme for prognostication.

**Materials and Methods**

**Samples**
Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from the archives of the Pathology departments at Prince of Wales Hospital (Hong Kong) and Hua Shan Hospital (Shanghai, China). Local ethical approvals were obtained from The Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee and Ethics Committees of Hua Shan Hospital, Shanghai. The cohort contains 57 samples recruited from 2008 to 2017. All patients were ≥ 18 years
at the time of diagnosis. Histological diagnoses were reviewed by three pathologists (H.K.N., H.C., A.K.C.). Tumor location was determined by neuroradiological examination and intraoperative information. Data on patient demographics and therapeutic treatment were obtained from institutional paper and electronic records. Most of the patients who had undergone adjuvant chemotherapy had temozolomide (TMZ), and a few patients received nimustine (ACNU) as adjuvant chemotherapy. Survival data were ascertained from follow-up visits to clinics or by direct contact with patients or close relatives via telephone.

**IDH1/2 and TERT promoter mutation analysis**

*IDH* (*IDH1* and *IDH2*) and *TERT* promoter mutations were detected by direct sequencing as described and cases with *IDH1*- or *IDH2*-mutation were included in this study. All mutations were confirmed by independent PCR amplification and sequencing analyses.

**Illumina Infinium MethylationEPIC BeadChip Array**

FFPE sections were sent to Macrogen, Shenzhen, China (Shenzhen Millennium Spirit Technology Co, Ltd), where the DNA was extracted and subjected to DNA methylation profiling by EPIC Illumina Infinium Human Array (850,000 CpG sites) following manufacturer's protocol (Illumina). The raw data of methylation array can be found at http://www.cuhk.edu.hk/med/acp/acp/staff/hkng.html.

**Identification of Methylation Subgroups**

Background correction, global dye-bias normalization, and calculation of DNA methylation level are parts of Illumina EPIC Illumina Infinium Human Array (850,000 Cpg sites) following manufacture's protocol (Illumina). The raw data of methylation array can be found at http://www.cuhk.edu.hk/med/acp/acp/staff/hkng.html.

**Determination of Copy Number Variations With EPIC Illumina DNA Methylation Array**

Probe-level signal intensities obtained from the IDAT files were first subjected to background correction and dye-bias normalization (shifting of the 5% percentile of negative control probe intensities to 0, and scaling of the mean of normalization control probe intensities to 10,000). Probes were excluded if they targeted the sex chromosomes, contained single-nucleotide polymorphisms, or mapped to multiple locations in the human genome. One hundred nineteen control samples from the study by Capper et al. (GSE109381) were used for normalization. As the control samples were profiled through the 450k array platform, probes present in the EPIC array but not in the 450K array were also removed. CNV analysis was then performed from the methylation data using the “conumee” package in R, as previously described.

**Statistical Analysis**

Statistical analysis was performed on IBM SPSS software v22 and R software. Overall survival (OS) was defined as the period of time between surgery and death or the last follow-up. Student's t-test was used to compare mean age between two populations. Chi-squared or Fisher's test was used to determine relationships between molecular alterations and clinical parameters. Survival curves were evaluated by the Kaplan–Meier method, and survival difference between different groups was determined by the log-rank test. Multivariable analysis was performed by Cox proportional hazards model. *P* < .05 (two-sided) was considered statistically significant.

**Results**

**Samples and Clinical Features**

A summary of clinical features of the cohort in this study is shown in Table 1 and Fig. 1. The mean and median ages of this cohort were 39.8 and 38 years old, respectively. Consistent with the literature, patients with *IDH*-mutant glioblastoma were younger at diagnosis compared to those with *IDH*-wildtype glioblastoma. Male to female ratio was 1:0.73. Primary glioblastoma, which developed de novo without previous clinical or histologic evidence of a low-grade glioma, was found in 33/57 (57.9%) of our cohort, and secondary glioblastoma arising from a previous histologically confirmed low-grade lesion accounted for 24/57 (42.1%) of our samples. Histological review of the pre-existing Grade II or Grade III astrocytoma was available in 10 of these cases in our own laboratories. For the rest, such documentation is available in the medical records but histological review was not possible as the patients were treated in other hospitals. Most of the patients in this study cohort (46/57; 80.7%) had total resection (Table 1). Chemotherapy alone and radiotherapy alone were given to 6 (10.5%) and 1 (1.8%) patients, respectively (Table 1). A total of 35 (61.4%) patients were treated with both chemotherapy and radiotherapy. 43 and 52 cases had follow-up data for progression-free survival (PFS) and OS, respectively. The average and median follow-up periods were 22.9 and 13.9 months, respectively (range 1.0–85.4 mo). Univariate survival analysis was then performed in the cohort according to the clinical variables. The results revealed that age at diagnosis, gender, tumor location, operation, chemotherapies, and radiotherapy were not associated with clinical outcomes (Supplementary Table 1).

**Classification of IDH-mutant glioblastomas based on genome-wide DNA Methylation Profiling**

*IDH*-mutant glioblastomas (*N* = 57) were analyzed by Illumina MethylationEPIC (850k) arrays. We applied Random Forest (machine learning algorithm) with a two-step process and divided our 57 samples into one of the two *IDH*-mutant methylation-based gliomas subgroups (G-CIMP-high and G-CIMP-low) according to the previous publication. The results revealed that the majority of the...
samples belonged to G-CIMP-high (34/57; 59.6%), and that G-CIMP-low was present in 23/57 (40.4%) of our cohort (Fig. 1 and Table 1). The prevalence of these glioma subtypes is consistent with previous findings.10

We then investigated the association between methylation subgroups and clinical parameters. We found G-CIMP-high tumors were markedly associated with primary glioblastomas ($P = .029$; Table 1). Methylation-based subgroups were not associated with other clinical parameters including age, gender, tumor location, operation, and adjuvant therapy (Table 1).

In agreement with a previous report10, G-CIMP-low tumors exhibited a significantly shorter OS compared to G-CIMP-high tumors (median: 407 d [13.6 mo] vs 619 d [20.6 mo], $P = .005$; Fig. 2A). Methylation-based subgroups were not associated with PFS in our cohort (Fig. 2B).

### TERT Promoter Mutation in IDH-Mutant Glioblastomas

By Sanger sequencing, TERT promoter mutation was identified in 3/57 (5.3%) of IDH-mutant glioblastomas. Out of these three samples, two cases had the C250T mutation, and one case had the C228T mutation (Fig. 1). TERT promoter mutation appeared in both primary ($N = 2$) and secondary ($N = 1$) glioblastomas. An association between TERT promoter mutation and clinical parameters (age, gender, and tumor location) was not formed. All TERT promoter mutations were found in G-CIMP-low tumors (Supplementary Table 2). TERT promoter mutation was not associated with PFS or OS.

### Clinical Significance of Gene CNVs in IDH-Mutant Glioblastomas

CNVs have been recognized as a useful prognostic tool in glioblastomas.18 Therefore, we derived copy number status from EPIC 850k array data according to previous publications.14,15 We then looked at genes with established relevance in gliomas for amplification or deletion.14 These included CCND1, CCND2, CDK4, CDK6, CDKN2A, EGFR, MDM4, MET, MYC, MYCN, NF1, NF2, PDGFRA, PPM1D, PTEN, RB1, and SMARCB1. We used the cutoff established in Shirahata et al. study to determine amplification and deletion.18 We found CDKN2A deletion in 24/57 (42.1%) IDH-mutant glioblastomas, and this was the most common alteration among the gene list (Fig. 1, Supplementary Table 3).

We then evaluated the association between CNVs and methylation subgroups. We found that CDKN2A deletion was markedly associated with the G-CIMP-low subgroup.

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**Table 1. Clinical characteristics of G-CIMP-high and G-CIMP-low glioblastomas**

|                          | All tumors ($N = 57$) | G-CIMP-high ($N = 34$) | G-CIMP-low ($N = 23$) | $P$ value |
|--------------------------|-----------------------|------------------------|-----------------------|-----------|
| Age                      | mean/median/range     | 39.8/38/21–68          | 38.9/36/24–64         | 40.9/40/21–68 | .508 |
| Tumor location           |                       |                        |                       |           |
| Frontal                  | 36                    | 25                     | 11                    | .207      |
| Temporal                 | 15                    | 7                      | 8                     |           |
| Occipital                | 2                     | 1                      | 1                     |           |
| Non-hemisphere           | 4                     | 1                      | 3                     |           |
| Primary or secondary GBM |                       |                        |                       |           |
| Primary                  | 33                    | 24                     | 9                     | .029      |
| Secondary                | 24                    | 10                     | 14                    |           |
| Operation                |                       |                        |                       |           |
| Total                    | 46                    | 28                     | 18                    | .592      |
| Non-total                | 7                     | 3                      | 4                     |           |
| Not available            | 4                     | 3                      | 1                     |           |
| Adjuvant therapy         |                       |                        |                       |           |
| No therapy               | 9                     | 4                      | 5                     | .441      |
| Chemotherapy only        | 6                     | 2                      | 4                     |           |
| Radiotherapy only        | 1                     | 1                      | 0                     |           |
| Chemotherapy and radiotherapy | 35                   | 22                     | 13                    |           |
| Not available            | 6                     | 5                      | 1                     |           |

G-CIMP, glioma-CpG island methylator phenotype.
(P = .004; Supplementary Table 4). Examining the association between CNVs and clinical parameters revealed that CDKN2A deletion was enriched in secondary glioblastoma (P = .034; Supplementary Table 5). Other frequent copy number changes included CCND2 amplification (11/57; 19.3%), PDGFRα amplification (8/57; 14.0%), MYC amplification (8/57; 14.0%), CDK4 amplification (7/57; 12.3%), and EGF amplification (7/57; 12.3%). Mesenchymal–epithelial transition (MET) amplification was identified in 5/57 (5.3%) cases. Analysis for PFS was not conducted given that only two cases had MET-amplified cases with PFS data. Clinical significance was not detected for other gene CNVs (Supplementary Table 3). The results indicated that CDKN2A deletion and MET amplification are prognostic markers in IDH-mutant glioblastomas.

We then asked if combined CDKN2A and MET status could improve prognostication. We separated the cohort into (1) CDKN2A deletion + MET amplification; (2) CDKN2A deletion; and (3) Neither CDKN2A deletion nor MET amplification. Survival analysis revealed that CDKN2A deletion + MET amplification predicted poor survival (P < .001; Fig. 3D). Pair-wise comparison indicated that CDKN2A-deleted + MET-amplified tumors had a shorter OS compared to CDKN2A-deleted tumors (P = .008; Fig. 3D). Survival analysis for PFS was not conducted given that only two cases of CDKN2A deletion + MET amplification had the PFS data.

**Stratification of IDH-Mutant Glioblastomas With DNA Methylation Subgroup and CDKN2A/MET Status**

We then investigated the prognostic values of combined DNA methylation subgroups and CNVs in our cohort. Given that DNA methylation subgroup, CDKN2A deletion, and MET amplification all showed prognostic value on their own, we used these three factors in the analysis. The cohort was separated into three molecular groups: Group 1 (G-CIMP-high), Group 2 (G-CIMP without CDKN2A nor MET alterations), and Group 3 (G-CIMP-CDKN2A and/or MET alterations). A log-rank test demonstrated that groups based on combined methylation subgroups and
patients performed significantly worse than Group 2 patients \((P = .035; \text{Fig. 4A})\), suggesting that \textit{CDKN2A/MET} status would further stratify G-CIMP-low patients. As shown in Fig. 4B, groups based on combined methylation subgroups and \textit{CDKN2A/MET} status also correlated with PFS \((P = .044)\). Group 3 tumors showed a shorter PFS compared to Groups 1 and 2. Similar to the OS, \textit{CDKN2A/MET} status predicted a shorter PFS among G-CIMP-low tumors (Groups 2 and 3; \(P = .040; \text{Fig. 4B}\)), highlighting the values of \textit{CDKN2A/MET} status in stratification of G-CIMP-low tumors.

Multivariable analysis was conducted to examine the independent prognostic value of the combined methylation subgroups and \textit{CDKN2A/MET} status by adjusting for age, gender, operation, radiotherapy, chemotherapy, and clinical diagnosis (Table 2). Although there was a significant association between the combined methylation subgroups and clinical diagnosis \((P = .016; \text{Supplementary Table 6})\), interaction between these two factors was not significant in the multivariable analysis. Multivariable analysis showed that combined methylation subgroups and \textit{CDKN2A/MET} status was an independent prognostic factor in \textit{IDH}-mutant glioblastomas (Table 2).

![Figure 2](image.png)

\textbf{Fig. 2.} Kaplan–Meier survival analysis of methylation subgroups. (A) G-CIMP-low subgroup was strongly associated with a shorter OS \((P = .005)\) in \textit{IDH}-mutant glioblastomas. (B) Methylation subgroup was not associated with PFS. G-CIMP, glioma-CpG island methylator phenotype; OS, overall survival.

\textit{CDKN2A/MET} status differed significantly with respect to their OS and PFS \((P = .001 \text{ and } P = .044, \text{ respectively}; \text{Fig. 4A and B})\). As illustrated in Fig. 4A, Groups 1 and 2 exhibited prolonged survival with a median survival of 619 (242.3–995.7) days, and 655 (218 mo) days, respectively. Group 3 exhibited a poor outcome with a median survival of 252 days (8.4 mo). Although both Groups 2 and 3 were of G-CIMP-low tumors, pair-wise comparison indicated that Group 3

\textbf{Discussion}

Even with intensive treatment, the prognosis of glioblastoma is poor with a median OS of less than 15 months. However, a minority of glioblastoma patients survives longer than 2–3 years. The WHO 2016 classification of CNS tumors has defined many entities by both histology and molecular features, and majority of glioblastomas are classified as \textit{IDH}-wildtype or \textit{IDH}-mutant. \textit{IDH}-wildtype glioblastoma accounts for over 90% of primary glioblastoma and has been well studied. \textit{IDH}-mutant glioblastoma constitutes a small proportion of primary glioblastoma and around 60%–70% of secondary glioblastoma,\textsuperscript{12,28} Yan et al. showed that the median OS of \textit{IDH}-mutant glioblastomas was about two times longer than that of \textit{IDH}-wildtype glioblastomas; however, the number of \textit{IDH}-mutant glioblastomas with follow-up data in that study was small \((N = 14)\) and similarly only 35 \textit{IDH}-mutant glioblastomas are currently listed in TCGA database among which only 7 had available DNA methylation data for 450,000 CpG sites.

Genome-wide DNA methylation analysis revealed that \textit{IDH}-mutant glioblastomas (both primary and secondary) formed a group distinct from other \textit{IDH}-mutant gliomas (Grades II-III).\textsuperscript{8} Shirahata et al. demonstrated that \textit{IDH}-mutant astrocytic tumors (Grades II-IV) is not uniform in terms of histological and genetic parameters. It was suggested that the 2016 CNS WHO grading of \textit{IDH}-mutant astrocytic tumors is not as prognostically useful as needed for this group and a novel grading algorithm correlated better to prognosis for \textit{IDH}-mutant astrocytic tumors overall.\textsuperscript{14} We too speculate that \textit{IDH}-mutant glioblastoma is a heterogeneous group characterized by tumors with differing in methylation signature, copy number changes, and clinical outcomes. We also speculate that not all \textit{IDH}-mutant glioblastomas have good prognosis and it is necessary to
provide a better stratification for risk. We showed that a combination of methylation subgroups and copy number changes provided good prognostication of IDH-mutant glioblastomas.

CpG island methylator phenotype (CIMP) is defined by genome-wide hypermethylation of CpG islands and later was defined to include other non-CpG islands. CIMP alterations has been shown to lead to an inactivation of
IDH-mutant glioblastomas can be further stratified

Tumor suppressor genes or other tumor-related genes.\(^{29}\)

We previously described CIMP in adult low-grade gliomas and glioblastomas\(^{9}\) and confirmed the findings in a larger cohort.\(^{7,10}\)

Gliomas can be separated into CIMP positive (CIMP\(^+\)) and CIMP negative (CIMP\(^−\)), and the name glioma-CIMP (G-CIMP) was designated for a subgroup of gliomas with CIMP to distinguish from other non-glioma CIMP tumors.\(^{9}\)

Integrative analysis of DNA methylation data and transcriptome profiling revealed G-CIMP\(^+\) subgroup was highly enriched for proneural subtype, which is one of the four genetic types described in glioblastomas.\(^{18}\)

G-CIMP\(^+\) tumors were associated with younger age and IDH\(^+\) mutation compared to the G-CIMP\(^−\) tumors.

Recently, an integrative analysis of 1,122 adult low- and high-grade gliomas revealed that they can be divided into six methylation subgroups that are closely associated with IDH mutation status.\(^{10}\)

IDH\(^+\)-wildtype tumors could be separated into three methylation subgroups and the same applied to IDH\(^−\)-mutant tumors. The three discrete methylation subgroups among the IDH\(^+\)-mutant gliomas were Codel, G-CIMP-high and G-CIMP-low. The Codel subgroup was mainly made up of low-grade gliomas with 1p19q codeletion. G-CIMP-high and G-CIMP-low tumors were subgroups of G-CIMP\(^+\) and presented with high and low degrees of DNA methylation, respectively. This study also showed that G-CIMP-low gliomas resembled IDH\(^+\)-wildtype gliomas and had the worst OS among the three methylation subgroups of IDH\(^−\)-mutant gliomas. As >90% of the IDH\(^−\)-mutant gliomas in this study were low-grade gliomas,\(^{10}\) the clinical impact of methylation subgroup in IDH\(^−\)-mutant glioblastoma remained unknown. In addition, the clinical significance of gene copy number was not investigated in depth.

In this study, we examined genome-wide DNA methylation profiling of 57 IDH\(^−\)-mutant glioblastomas. We showed G-CIMP-high and G-CIMP-low in 59.6% and 40.4% of our cohort, respectively. The prevalence of DNA methylation subgroups in the current study is similar to the reported literature.\(^{10}\)

We demonstrated that DNA methylation subgroups correlated with survival, and G-CIMP\(^+\)-low tumors showed a poorer survival compared to G-CIMP\(^+\)-high tumors, indicating that DNA methylation subgroup is clinically relevant in IDH\(^−\)-mutant glioblastomas.

We then uploaded the raw data of methylation array to German Cancer Research Center (DKFZ) classifier (molecularneuropathology.org). Thirty-six cases were classified by the DKFZ classifier as high-grade gliomas. Twenty-one cases were classified as “not defined” (N = 19) or “no matching methylation classes with calibrated score” (N = 2). The histology of some “not defined” cases was put up in Supplementary Figure 2. It is not clear from the published literature how well IDH\(^−\)-mutant glioblastomas are represented in the methylation classifier. IDH\(^−\)-mutant glioblastomas may well be under “un-defined” by the classifier and we hope our contribution to the literature and the classifier will help clarify the issue.

CDKN2A is located on chromosome 9p21, and it encodes for two different proteins, p16INK4a and p14ARF.\(^{30}\)

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**Table 1.**

| p value | Median survival days (95% CI) | Group 1 | Group 2 | Group 3 |
|---------|-------------------------------|---------|---------|---------|
| Group 1 | 619.0 (242.3–995.7)           |         |         |         |
| Group 2 | 655.0 (513.6–796.4)           | 0.339    |         |         |
| Group 3 | 252.0 (111.1–392.9)           | <0.001   | 0.035   |         |

**Fig. 4.** Combined methylation subgroups and CDKN2A/MET status in stratification of IDH\(^−\)-mutant glioblastomas. Kaplan–Meier survival curves of (A) OS and (B) PFS according to Groups (P < .001). Group 1 (blue line) belongs to tumors of G-CIMP-high. Group 2 (green line) belongs to G-CIMP-low tumors without CDKN2A nor MET alteration. Group 3 (red line) belongs to G-CIMP-low tumors with CDKN2A and/or MET alterations. G-CIMP, glioma-CpG island methylator phenotype; MET, mesenchymal–epithelial transition; OS, overall survival.
CDKN2A negatively controls cell cycle, and CDKN2A abnormality leads to cellular proliferation and dysregulation of proapoptotic pathways.\(^{31}\) CDKN2A deletion has been described in adult and pediatric low-grade and high-grade gliomas, with frequencies ranging from 20% to 57%.\(^{32,33}\) Loss of CDKN2A is associated with poor outcomes in pediatric and adult low-grade and malignant gliomas.\(^{14,34,35}\) Recently, Korshunov et al. identified CDKN2A/B deletion was associated with shorter survival in IDH-mutant glioblastomas.\(^{8}\) A review of TCGA database revealed 35 IDH-mutant glioblastomas with CDKN2A deletion status and limited clinical follow-up. CDKN2A deletion was found in 14.3% (5/35) of the samples. Survival analysis of the TCGA cases did not reveal a close association between CDKN2A deletion and survivals, probably due to a limited number of CDKN2A deletion cases. The median OS for deleted and non-deleted samples was 24 and 34 months, respectively.

In this study, we showed that CDKN2A deletion is a common event in IDH-mutant glioblastomas (42.1%), and it is more often detected in G-CIMP-low tumors (15/23; 65.2%) whereas such alteration is present in about one-quarter of G-CIMP-high tumors. Importantly, CDKN2A deletion was a poor prognostic factor for OS in our cohort. CDKN2A deletion also exhibited negative clinical impact in a subgroup of tumors with a G-CIMP-low signature (Supplementary Figures 1A and B). Taken together, CDKN2A deletion is a poor prognostic marker in IDH-mutant glioblastomas, and it can further stratify G-CIMP-low tumors for prognostication.

MET is located on chromosome 7q21–31, and it encodes a receptor for hepatocyte growth factor. Upon binding to its ligands, MET undergoes dimerization and phosphorylation, resulting in recruitment of signal transduction molecules and induction of downstream signaling pathways such as the PI3-K/AKT and RAS/MAPK pathways.\(^{36}\) MET activation results in cell proliferation, G1/S cell-cycle progression, angiogenesis and resistant to chemotherapy in gliomas.\(^{37,38}\) In gliomas, MET is dysregulated by several mechanisms. MET amplification has been described in <10% of glioblastomas.\(^{39,40}\) Mutation of MET leading to a truncated, constitutively active protein has been reported in a small proportion of glioblastomas.\(^{7,41}\) Recurrent PTPRZ1-MET fusion transcript resulting in an increase in migratory activity has also been described in 15% of secondary glioblastomas.\(^{28,42}\) Overexpression of MET is a frequent event, and the expression is significantly higher in high-grade gliomas compared to the low-grade counterpart.\(^{43,44}\)

In this study, we showed that MET amplification is present in a small proportion of IDH-mutant glioblastomas, and can be found in both primary and secondary glioblastomas. Interestingly, all MET-amplified tumors belonged to G-CIMP-low subgroup and exhibited CDKN2A mutation. In TCGA database where the vast majority of glioblastomas are IDH-wildtype and MET amplification is found at a low frequency. Yet, none of the MET-amplified tumors in TCGA carries IDH mutation. Thus, this is the first report of the co-occurrence of IDH

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**Table 2. Multivariable analysis of IDH-mutant glioblastomas**

| Variables | Hazard ratio (HR) (95% CI) | \(P\) |
|-----------|---------------------------|-----|
| Age       | 1.03 (0.99–1.08)          | .135|
| Gender    |                          |     |
| Male      | 1                        |     |
| Female    | 0.79 (0.36–1.76)          | .567|
| Operation |                          |     |
| Non-total resection | 1             |     |
| Total resection | 1.08 (0.25–4.71) | .922|
| Radiotherapy |                      |     |
| No        |                          |     |
| Yes       | 1.12 (0.29–4.32)          | .873|
| Chemotherapy |                      |     |
| No        |                          |     |
| Yes       | 0.6 (0.13–2.73)           | .507|
| Clinical diagnosis |                  |     |
| Primary glioblastoma | 1             |     |
| Secondary glioblastoma | 0.32 (0.05–1.99) | .221|
| Combined methylation subgroup and CDKN2A/MET status |         |     |
| Group 3 (G-CIMP-low with CDKN2A and/or MET alterations) | 1         | .009|
| Group 1 (G-CIMP-high) | 0.07 (0.01–0.38) | .002|
| Group 2 (G-CIMP-low without CDKN2A nor MET alterations) | 0.08 (0.01–0.7) | .022|
| Combined methylation subgroups and CDKN2A/MET status by clinical diagnosis interaction |         | .11|

CI, confidence interval; G-CIMP, glioma-CpG island methylator phenotype; MET, mesenchymal–epithelial transition.
mutation, CDKN2A deletion, and MET amplification in glioblastomas. Our survival analysis revealed that MET amplification was associated with a short OS among all IDH-mutant glioblastomas ($P < .001$, Fig. 3C) and also among G-CIMP-low, IDH-mutant glioblastomas ($P = .017$; data not shown). Furthermore, patients with both CDKN2A deletion and MET amplification did more poorly compared to patients with only CDKN2A deletion or patients without these alterations (Fig. 3D). These data suggest MET amplification is a poor prognostic marker, and co-occurrence of CDKN2A deletion and MET amplification further enhance the aggressiveness of glioblastoma. Thus, CDKN2A deletion and MET amplification may represent a prognostically unfavorable subset of IDH-mutant glioblastomas.

Overall in this study, by integrated methylation signature and gene copy number data, we categorized three molecular subgroups (Groups 1–3) of IDH-mutant glioblastomas with different clinical behavior. The prognostic value of such molecular subgroups was also demonstrated in multivariable analysis. In particular, Group 3 (G-CIMP-low with CDKN2A and/or MET alterations) showed the worst outcomes with a median OS of 252 days (8.4 mo) and a median PFS of 207 days (6.9 mo) so these IDH-mutant glioblastomas should not be classified as glioblastomas with good prognosis as they could have been under the current WHO scheme. Our findings suggest that combination of methylation signature and gene CNVs should be used to stratify IDH-mutant glioblastomas into prognostic groups, and thus have implications for bedside management.

**Supplementary Material**

Supplementary material is available at *Neuro-Oncology* Advances online.

**Keywords**

CDKN2A deletion | DNA methylation profiling | glioblastomas | IDH mutation | MET amplification.

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