J Neuropathol Exp Neurol
Vol. 00, No. 00, June 2020, pp. 1–12
doi: 10.1093/jnen/nlaa059

OXFORD

ORIGINAL ARTICLE

Molecular Correlates of Long Survival in IDH-Wildtype Glioblastoma Cohorts

Kristyn Galbraith, MD, Ashwani Kumar, MS, Kalil G. Abdullah, MD, MSc, Jamie M. Walker, MD, PhD, Steven H. Adams, BS, Timothy Prior, BS, Ryan Dimentberg, BS, Fraser C. Henderson, , MD, Kanish Mirchia, MD, Adwait Amod Sathe, PhD, Mariano S. Viapiano, PhD, Lawrence S. Chin, MD, Robert J. Corona, DO, Kimmo J. Hatanpaa, MD, PhD, Matija Snuderl, MD, Chao Xing, PhD, Steven Brem, MD, and Timothy E. Richardson, DO, PhD

Abstract

IDH-wildtype glioblastoma is a relatively common malignant brain tumor in adults. These patients generally have dismal prognoses, although outliers with long survival have been noted in the literature. Recently, it has been reported that many histologically lower-grade IDH-wildtype astrocytomas have a similar clinical outcome to grade IV tumors, suggesting they may represent early or undersampled glioblastomas. cIMPACT-NOW 3 guidelines now recommend upgrading IDH-wildtype astrocytomas with certain molecular criteria (*EGFR* amplifications, chromosome 7 gain/10 loss, and/or *TERT* promoter mutations), establishing the concept of a "molecular grade IV" astrocytoma. In this report, we apply these cIMPACT-NOW 3 criteria to 2 independent glioblastoma cohorts, totaling 393 public database and institutional glioblastoma cases: 89 cases without any of the cIMPACT-NOW 3 criteria (GBM-C0) and 304 cases with one or more criteria (GBM-C1-3). In the GBM-C0 groups, there was a trend toward longer recurrence-free survival (median 12–17 vs 6–10 months), significantly longer overall survival (median 32–41 vs 15–18 months), younger age at initial diagnosis, and lower overall mutation burden compared to the GBM-C1-3 cohorts. These data suggest that while histologic features may not be ideal indicators of patient survival in IDH-wildtype astrocytomas, these 3 molecular features may also be important prognostic factors in IDH-wildtype glioblastoma.

Key Words: Astrocytoma, GBM, Glioblastoma, IDH1/2, Long survival, Prognosis, TCGA.

From the Department of Pathology, State University of New York, Upstate Medical University, Syracuse, New York (KG, KM, RJC, TER); Eugene McDermott Center for Human Growth & Development (AK, AAS, CX); Department of Neurological Surgery (KGA), University of Texas Southwestern Medical Center, Dallas; Department of Pathology (JMW); Glenn Biggs Institute for Alzheimer's & Neurodegenerative Diseases (JMW), University of Texas Health Science Center, San Antonio, Texas; College of Medicine, State University of New York, Upstate Medical University, Syracuse, New York (SHA); Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (TP, RD, SB); Department of Neurosurgery, Medical University of South Carolina, Charleston, South Carolina (FCH); Department of Neuroscience and Physiology (MSV); Department of Neurosurgery (MSV, LSC), State University of New York, Upstate Medical University, Syracuse, New York; Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas (KJH); Department of Pathology, New York University Langone Health, New York City, New York (MS); Department of Bioinformatics and Department of Population and Data Sciences, University of Texas Southwestern Medical Center, Dallas, Texas (CX).

Send correspondence to: Timothy E. Richardson, DO, PhD, Department of Pathology, State University of New York, Upstate Medical University, 750 E. Adams St., UH 6805A, Syracuse, NY 13210; E-mail: richatim@upstate.edu

Matija Snuderl is supported in part by grants from the Friedberg Charitable Foundation and the Making Headway Foundation.

The authors have no duality or conflicts of interest to declare.

INTRODUCTION

Glioblastoma (GBM) is the third most common intracranial tumor after pituitary adenoma and meningioma (comprising 14.7% of all cases), and is the most common malignant central nervous system tumor with an annual incidence of 3.21/100 000 individuals and >11 000 new cases diagnosed each year in the United States (1, 2). Identification of *IDH1*/2 mutations in a subset of both histologically low-grade gliomas (LGGs) and GBMs (3, 4) has led to a change in the diagnosis and reporting of these tumors with an integrated histologic/molecular diagnosis focused primarily around *IDH1*/2 status (5). IDH-wildtype GBMs comprise ~90% of all GBM cases, tend to occur in older individuals (mean age at diagnosis of 62 years), and have median survival intervals of approximately 10–15 months (4, 5). Even with recent advances in treatment, the overall expected 5-year survival rate for GBM is <5% (2, 6), although rare cases with extremely long-term survival have been reported in the literature (7–9).

Longer survival among some patients diagnosed with IDH-wildtype GBM raises hope of finding additional prognostic molecular markers that may surpass traditional histologic features in predicting survival or serve as therapeutic targets. Investigation into large cohorts of IDH-wildtype GBMs demonstrated that *EGFR* alterations are one of the most common features in these patients, present in >50% of cases. Cohorts with long-term survival (>36 months) had less-frequent *EGFR* amplification (or pathogenic activating mutation), although many of these studies included both IDH-wildtype and IDH-mutant GBMs (10, 11). Other studies of the effect of *EGFR* in GBM have yielded conflicting results, with patient age being a possible confounding factor (11–17). Combined gain of whole chromosome 7 and loss of whole chromosome 10 (7+/10-) is another frequent alteration that is considered definitional for GBM and may confer poor clinical outcome within this astrocytoma subset as well as histologically lower-grade IDH-wildtype astrocytomas (18–20). *TERT* promoter (*TERT*p) mutation is similarly common in GBM and has a negative prognostic value in some glioma subsets; however, this alteration is less specific for GBM than the other 2 factors (18, 21–24). In the IDH-wildtype category, histologically LGGs (defined here as WHO grades II and III) with high-level *EGFR* amplification, 7+/10-, and/or *TERT*p mutation have been shown to have aggressive clinical outcomes indistinguishable from IDH-wildtype GBM, and thus are now considered to be "molecular grade IV" by cIMPACT-NOW update 3 criteria (18, 25–30).

Additional research suggests that groups of longer surviving patients also tend to have a lower incidence of *CDK4* amplification and homozygous *CDKN2A* deletion (10, 11). Other reports have suggested that within the IDH-wildtype GBM groups, co-gain of chromosomes 19 and 20 (19+/20+) is associated with longer overall survival (31, 32), and *MGMT* promoter methylation results in longer patient survival as it impairs the protective response to alkylating agents in tumor cells and thus confers a better response to temozolomide therapy (33–35).

In this report, we identified 299 public dataset IDH-wildtype GBM cases, including 65 cases without cIMPACT-NOW 3 factors (GBM-C0) and 234 cases with 1–3 of these factors, as well as an additional 15 LGG cases without cIMPACT-NOW factors and 51 with at least one factor from The Cancer Genome Atlas (TCGA) online repository. We analyzed the GBM and LGG groups with respect to total copy number variation (CNV), somatic mutation burden, specific mutations, and specific gene amplifications and deletions. In addition, we analyzed an institutional cohort of 24 GBM-C0 cases and 70 GBM-C1-3 cases as an independent validation cohort, using similar methods. In all 3 cohorts, the groups without cIMPACT-NOW criteria had significantly longer overall survival and younger age at initial diagnosis than those with at least one of these factors. Our results raise the possibility that these 3 molecular features may be as important in determining prognostic categories in IDH-wildtype GBMs as they are in histologically lower-grade IDH-wildtype gliomas.

MATERIALS AND METHODS

Case Selection

We performed a search of histologically confirmed GBM cases across multiple publicly available datasets available on the cBioPortal interface (www.cbioportal.org) (36, 37), TCGA database (https://portal.gdc.cancer.gov/), and other previously published publicly available databases to create a public database cohort (Cohort 1; Fig. 1) (10, 21, 38–41) (http://creativecommons.org/licenses/by/4.0/; http://creativecommons.org/publicdomain/zero/1.0/). The available histologic and molecular features were manually examined in each case; all cases with 1p/19q co-deletion, *IDH1/2* mutations, or incomplete mutational analysis precluding *IDH1/2* status evaluation were excluded. All cases were then screened for the availability of data regarding *EGFR* alterations, chromosome 7/10 status, *TERT*p status, and TERT mRNA expression levels. Notably, a number of the cases in the original TCGA datasets had only TERT mRNA expression levels without *TERT*p mutation status. In cases where both DNA and RNA sequencing were performed, the 2 measures were highly correlated; in the remainder of cases, TERT mRNA expression level was used to estimate the promoter mutation status, as previously described (10, 24). We identified a total of 65 IDH-wildtype GBMs without cIMPACT-NOW update 3 factors and an additional 234 cases with 1–3 cIMPACT-NOW 3 factors for a control group.

In addition, we searched institutional cases (2006–2017) that had sufficient molecular analysis performed for clinical purposes at the time of pathologic diagnosis on the initial resection specimen, including targeted molecular profiling and/or copy number profiling (https://www.pennmedicine.org/departments-and-centers/center-for-personalized-diagnostics/gene-panels; https://www.foundationmedicine.com/genomic-testing/foundation-one-cdx), as previously described (9, 17, 42, 43). In total, we identified 94 total IDH-wildtype GBM cases with sufficient molecular data to test our central hypothesis (24 cases without cIMPACT-NOW factors, 70 cases with 1–3 factors) (Cohort 2; Fig. 2). All molecular data are derived from tissue from the initial resection specimen. No significant differences between tumor size, extent of resection, or postsurgical treatment were found between groups in the institutional cohort. All ethical standards were followed and this retrospective study was performed with Institutional Review Board approval.

Finally, we identified 15 IDH-wildtype LGG cases without cIMPACT-NOW 3 factors (LGG-C0) and 51 LGG cases with 1–3 of these factors (LGG-C1-3) from the TGCA database (Fig. 3). All cases selected represented the first resection specimen.

Genetic and Epigenetic Analyses

The gene expression (Illumina HiSeq, RNASeq) and DNA methylation data (Illumina Human methylation 450) (Illumina, San Diego, CA) were downloaded for the selected TCGA GBM and LGG cases and analyzed with TCGAbiolinks (https://bioconductor.org/packages/release/bioc/html/TCGAbiolinks.html), Qiagen's IPA tool (www.qiagen.com/ingenuity) (Qiagen, Hilden, Germany), and R 3.4.1 (http://www.R-project.org/) (44, 45). The Affymetrix SNP 6.0 microarray data normalized to germline for copy number analysis for the same TCGA cases were downloaded from Broad GDAC Firehose (Broad Institute, Cambridge, MA). The fraction of the genome with copy number alterations was calculated from the above data as the fraction of the genome with

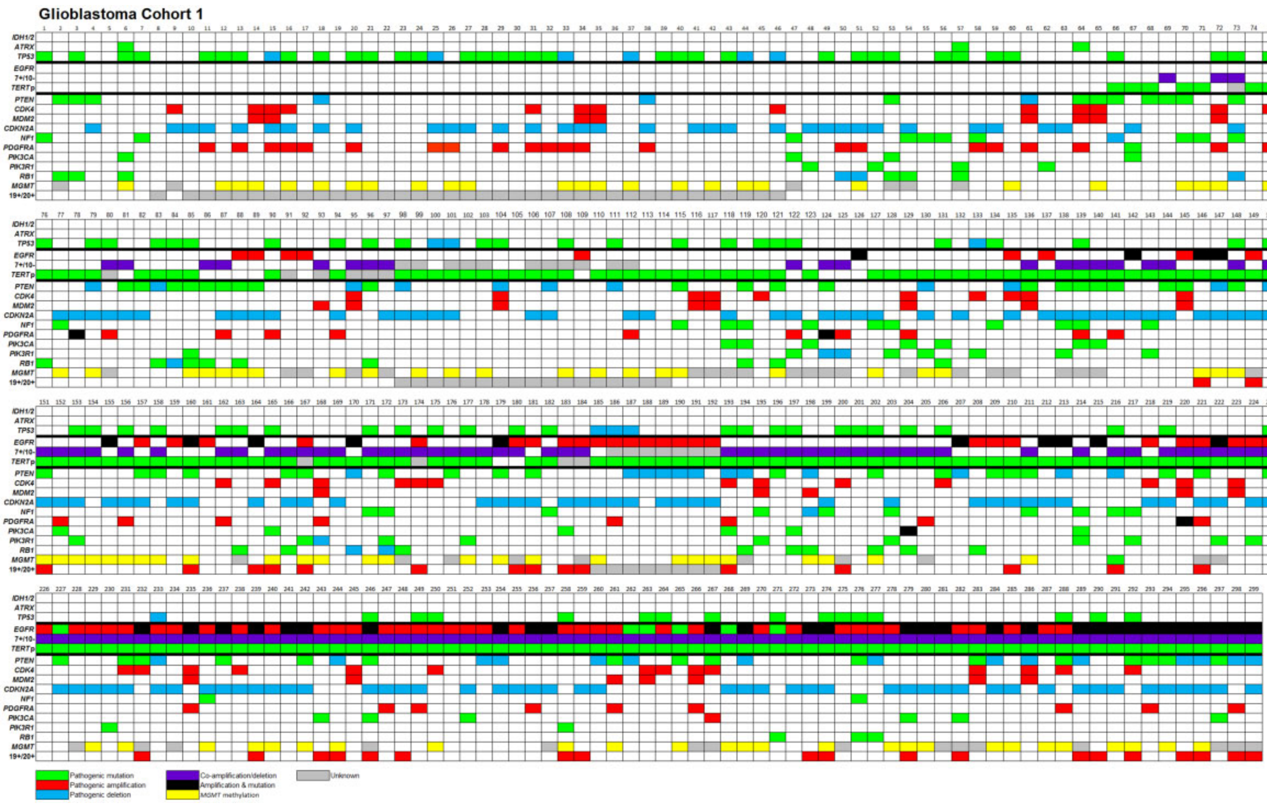

FIGURE 1. Summary chart for Cohort 1 showing key molecular alterations in the 299 assessed public dataset IDH-wildtype glioblastomas.

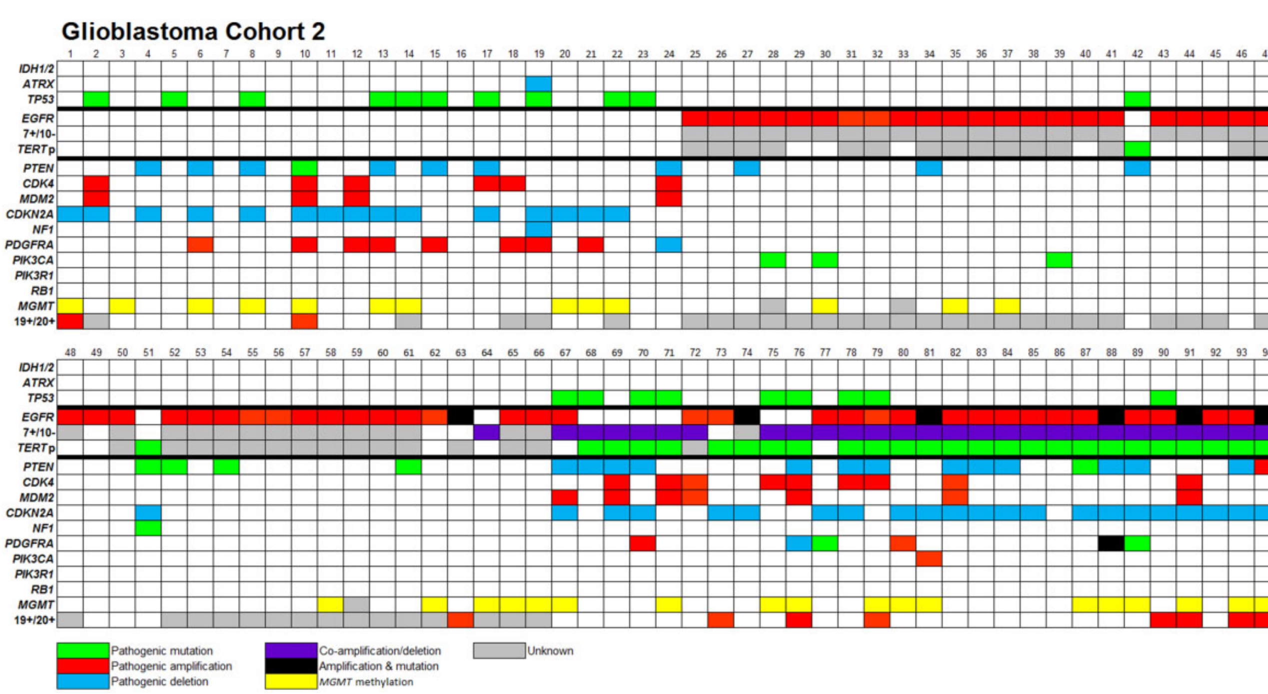

FIGURE 2. Summary chart for Cohort 2 showing key molecular alterations in the 94 assessed institutional IDH-wildtype glioblastomas.

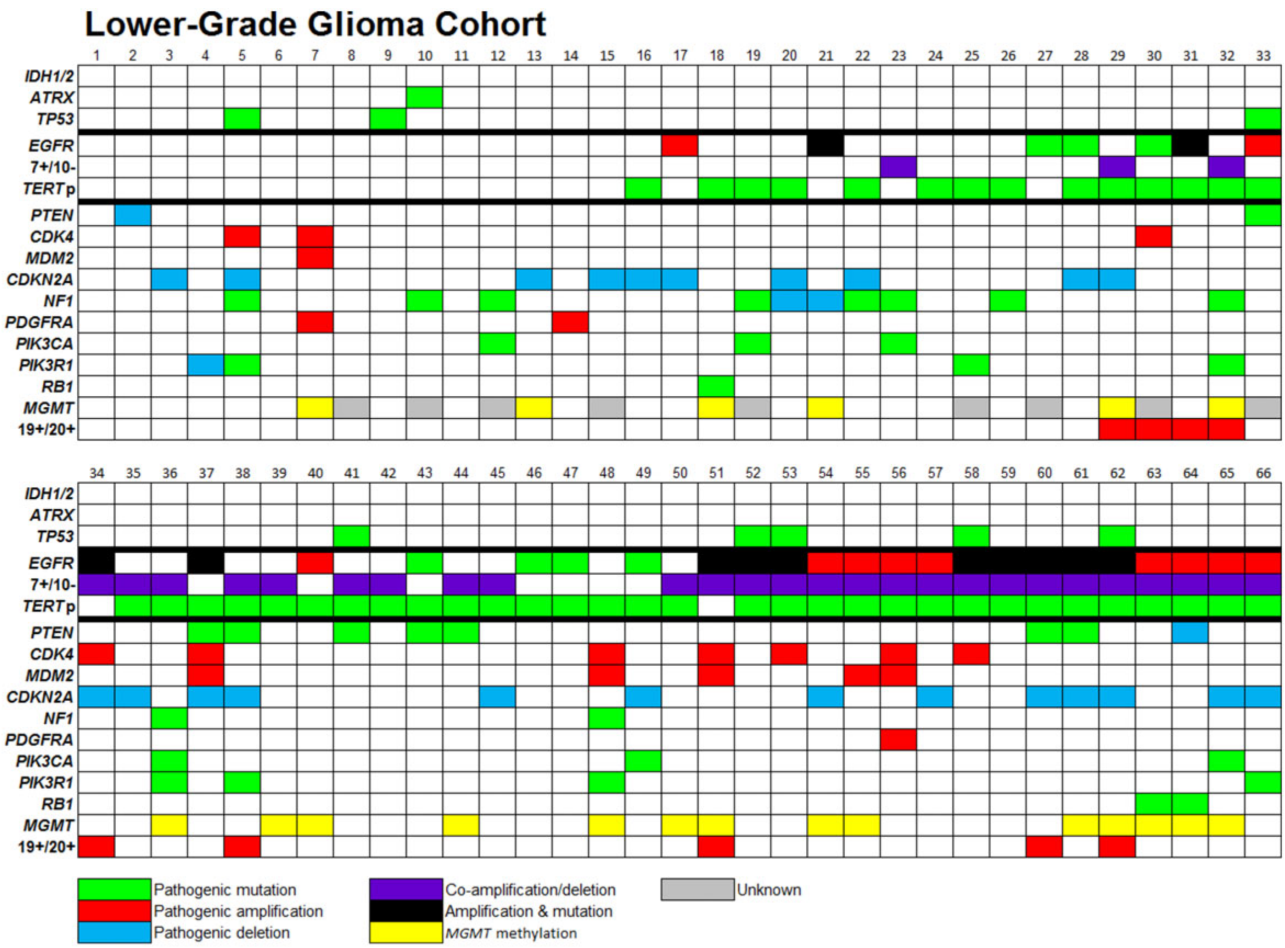

FIGURE 3. Summary chart showing key molecular alterations in the 66 assessed IDH-wildtype histologically lower-grade gliomas.

log2 of copy number >0.3 following the procedure used in cBioPortal (37).

The GISTIC 2.0 algorithm was used to identify individual regions of the genome that are significantly amplified or deleted (46). Each region with significant alteration was screened for tumor suppressor genes, oncogenes, and other genes associated with glioma and malignancy (46, 47). GISTIC 2.0 analysis was run in GenePattern (https://www.genepattern.org/) (48).

Mutation Analysis

The mutation load is the number of nonsynonymous mutations seen in a sample. Differential analysis and visualization of mutations were done using Maftools (49). *TERT*p mutation was obtained from DNA sequencing data and *TERT* mRNA expression data were correlated to *TERT*p status in the subset of cases where both measures were available, as previously described (10, 24, 50). Variant annotation was performed using COSMIC (51), dbSNP (52), ClinVar (53), CanProVar 2.0 (54), The 1000 Genomes Project (55), and FATHMM-MKL (56).

Statistical Analysis

Differences in patient age, total mutation burden, and CNV were calculated using ANOVA. Significance of survival curves was calculated using the log-rank test (Mantel-Cox test). All univariate and multivariate regression analyses and other statistical calculations were performed with MedCalc and GraphPad Prism version 8 (GraphPad, La Jolla, CA).

RESULTS

Analysis of Genomic Alterations, Clinical Characteristics, and Patient Survival in GBMs

When comparing GBM-C0, GBM-C1, GBM-C2, and GBM-C3 groups in Cohort 1 (Fig. 1), the GBM-C0 group had a nonsignificant trend toward longer recurrence-free survival ([RFS]; $p = 0.1369$) and significantly longer overall survival (OS; $p = 0.0030$) compared to the other 3 groups individually (Fig. 4A, B). Similarly, the GBM-C0 group had a nonsignificant trend toward longer median RFS (12.0 vs 6.0 months; $p = 0.0525$) and significantly longer median OS (32.2 vs 15.0 months; $p = 0.0007$) than the pooled GBM-C1-3 group (Fig. 4C, D and Table 1). Within the GBM-C1 group, no significant difference was detected between groups with single

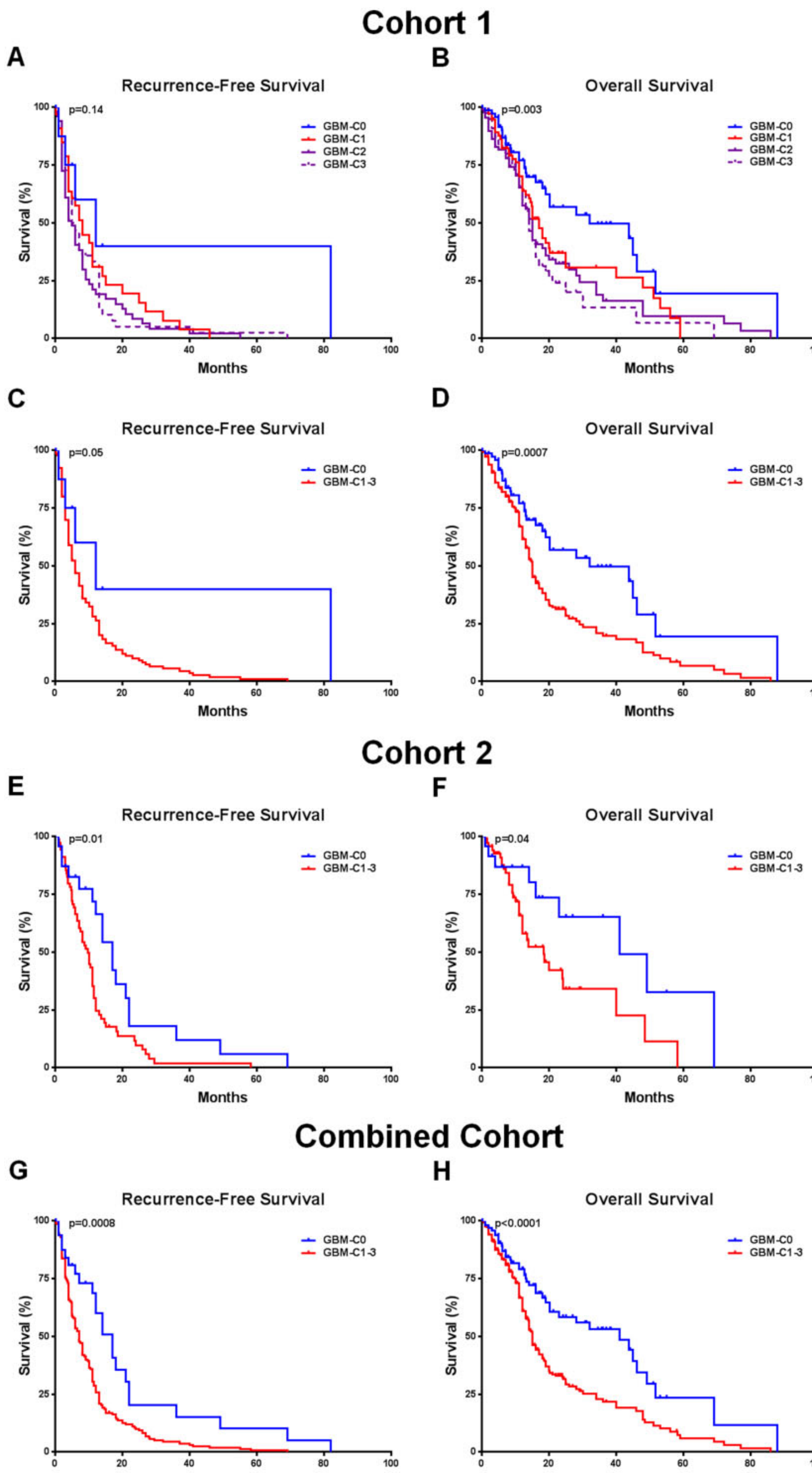

FIGURE 4. Kaplan-Meier survival curves in the Cohort 1 GBM-C0 group compared to the individual GBM-C1, GBM-C2, or GBM-C3 groups in terms of recurrence-free survival (RFS) ($p = 0.1369$) **(A)** and overall survival (OS) ($p = 0.0030$) **(B)**. Kaplan-Meier

TABLE 1. Clinical and Molecular Variables in LGG Cohort and GBM Cohorts

	LGG Cohort			GBM Cohort 1			GBM Cohort 2			Combined GBM Cohort			
	LGG-C0	LGG-C1-3	p Value	GBM-C0	GBM-C1-3	p Value	GBM-C0	GBM-C1-3	p Value	GBM-C0	GBM-C1-3	p Value	q Value
n	15	51	–	65	234	–	24	70	–	89	304	–	–
RFS (months)	37	11	0.1343	12	6	0.0525	17	9.6	**0.0125**	17	7	**0.0008**	**0.0012**
OS (months)	>39	17	**0.0222**	32.2	15	**0.0007**	41	18.4	**0.0350**	41	15	**<0.0001**	**<0.0001**
Patient age (years)	38.8±3.7	56.7±1.5	**<0.0001**	55.2±2.2	62.0±0.9	**0.0011**	52.2±2.2	61.9±1.4	**0.0006**	54.6±2.2	62.0 ± 0.8	**<0.0001**	**<0.0001**
CNV	11.3±3.2	18.7±1.8	0.0526	21.8±1.9	20.9±0.7	0.5920	–	–	–	21.8±1.9	20.9 ± 0.7	0.5920	0.3986
Mutation burden	36.1±25.9	20.5±2.7	0.2913	17.9±4.1	42.7±2.9	**<0.0001**	–	–	–	17.9±4.1	42.7 ± 2.9	**<0.0001**	**<0.0001**
PTEN	6.7%	17.6%	0.4334	13.8%	42.3%	**<0.0001**	33.3%	31.4%	1.0000	19.1%	39.8%	**0.0013**	**0.0016**
CDK4	13.3%	15.7%	1.0000	16.9%	16.7%	1.0000	25.0%	12.9%	0.1986	19.1%	15.8%	0.5164	0.3912
CDK4 + *MDM2*	6.7%	7.8%	1.0000	10.8%	7.7%	0.4490	16.7%	8.6%	0.2708	12.4%	7.9%	0.2060	0.1783
CDKN2A	26.7%	37.3%	0.5475	49.2%	56.4%	0.3262	62.5%	30.0%	**0.0071**	52.8%	50.3%	0.7181	0.4352
19+/20+	0%	17.6%	0.1055	0%	17.2%	**0.0323**	10.5%	23.5%	0.2994	4.3%	17.4%	**0.0245**	0.0248
MGMT	18.2%	39.1%	0.2958	37.3%	42.6%	0.5452	41.7%	31.3%	0.4526	38.6%	39.6%	0.8975	0.4944

TABLE 2. Hazard Ratios for Molecular Variables

	Hazard Ratio	95% Confidence Interval	Univariate p Value	Multivariate p Value
GBM-C0	–	–	–	–
GBM-C1-3	2.16	(1.61–2.90)	**<0.0001**	**<0.0001**
Age	1.67	(1.24–2.25)	**0.0012**	**0.0086**
All GBM				
CDK4 + *MDM2* amplification	1.29	(0.80–2.08)	0.2480	0.2029
PTEN mutation	1.22	(0.93–1.62)	0.1421	0.3526
Homozygous *CDKN2A* deletion	1.11	(0.85–1.45)	0.4313	0.5573
GBM-C1-3				
19+/20+	–	–	–	–
Lacking 19+/20+	2.15	(1.54–3.01)	**0.0002**	**<0.0001**
Methylated *MGMT*	–	–	–	–
Unmethylated *MGMT*	1.36	(0.96–1.91)	0.0846	0.2099

alterations in *EGFR* or 7+/10- or *TERT*p mutation in terms of RFS ($p = 0.4046$) or OS ($p = 0.3901$), suggesting that the presence of any of these 3 molecular alterations may have equivalent prognostic implications. No significant difference was found between the GBM-C1, -C2, and -C3 groups in terms of median RFS ($p = 0.4255$) or OS ($p = 0.3611$).

Similar trends were identified in Cohort 2 (Fig. 2): These institutional GBM-C0 cases had a significantly longer median RFS (17.0 months) compared to the GBM-C1-3 cases (9.6 months; $p = 0.0125$) (Fig. 4E), and a significantly longer OS (41.0 months) compared to the institutional GBM-C1-3 cases (18.4 months; $p = 0.0350$) (Fig. 4F and Table 1). In the combined cohorts, there was a significantly longer median RFS in the GBM-C0 group compared to the GBM-C1-3 group (17.0 vs 7.0 months; $p = 0.0008$) (Fig. 4G), and significantly longer median OS in the GBM-C0 group compared to the GBM-C1-3 group (41.0 vs 15.0 months; $p < 0.0001$) (Fig. 4H). The GBM-C1-3 group had a hazard ratio of 2.16 (95% confidence interval = 1.61–2.90) compared to the GBM-C0 group ($p < 0.0001$ by univariate and multivariate analyses) (Table 2).

The GBM-C0 group in Cohort 1 had a significantly younger age at initial diagnosis (55.2 ± 2.2 years) compared to tumors in the GBM-C1 (61.7 ± 1.6 years), -C2 (63.3 ± 1.3 years), or -C3 groups (60.8 ± 1.1 years; $p = 0.0122$) and pooled GBM-C1-3 group (62.0 ± 0.9 years;

FIGURE 4. Continued
survival curves in the Cohort 1 GBM-C0 group compared to combined GBM-C1-3 cases in terms of RFS ($p = 0.0525$) **(C)** and OS ($p = 0.0007$) **(D)**. Kaplan-Meier survival curves in the Cohort 2 GBM-C0 group compared to combined GBM-C1-3 cases in terms of RFS ($p = 0.0125$) **(E)** and OS ($p = 0.0350$) **(F)**. Kaplan-Meier survival curves in the combined Cohorts 1 and 2 in terms of RFS ($p = 0.0008$) **(G)** and OS ($p < 0.0001$) **(H).**

$p = 0.0011$). No significant differences in patient age are found within the GBM-C1, -C2, or -C3 groups ($p = 0.3854$). The Cohort 2 GBM-C0 cases also had a significantly younger age at initial diagnosis (52.2 ± 2.2 years) than the corresponding GBM-C1-3 (61.9 ± 1.4 years; $p = 0.0006$). These differences were significant after correcting for multiple comparisons, and with both univariate and multivariate analyses (Tables 1 and 2). There was a significantly lower level of overall mutation burden in the Cohort 1 GBM-C0 group compared to tumors with at least one cIMPACT-NOW 3 factor ($p < 0.0001$) (Table 1). Unlike IDH-mutant astrocytoma and oligodendroglioma cohorts (57–59), the overall CNV levels were not significantly different between the GBM-C0 and the GBM C1-3 groups ($p = 0.5920$) (Table 1).

In Cohort 1, there was a lower percentage of cases with *PTEN* alterations in the GBM-C0 group compared to the GBM-C1-3 group, however, this difference was not found in Cohort 2, and there was a higher percentage of GBM-C0 cases with homozygous loss of *CDKN2A* in Cohort 2 but not in Cohort 1. After correcting for multiple comparisons, only the RFS and OS, patient age, mutation burden, and frequency of *PTEN* alterations were significantly different between the GBM-C0 and GBM-C1 cohorts (Table 1). Notably, there was no difference in frequency of 19+/20+ or *MGMT* promoter methylation between the GBM-C0 and GBM-C1-3 groups after correcting for multiple comparisons (Table 1).

Analysis of Genomic Alterations, Clinical Characteristics, and Patient Survival in Histologically LGGs with Comparison to GBMs

IDH-wildtype LGGs were divided into cohorts based on the number of cIMPACT-NOW update 3 factors in each case (Fig. 3). As previously demonstrated (18, 25, 28), there was a significant difference between LGGs without cIMPACT-NOW 3 factors (LGG-C0) and those with at least one of these factors (LGG-C1-3) in terms of OS (median survival >39.0 months and 17.0 months, respectively; $p = 0.0222$), although we only found a nonsignificant trend toward longer RFS in LGG-C0 compared to LGG-C1-3 (median survival 37.0 and 11.0 months, respectively; $p = 0.1343$). No significant difference was found between LGG-C0 cases and GBM-C0 cases in terms of RFS ($p = 0.9165$) or OS ($p = 0.1827$), and there was no significant difference between the LGG-C1-3 cohort compared to the GBM-C1-3 cohort in terms of RFS ($p = 0.1536$) or OS ($p = 0.9816$) (Fig. 5A, B and Table 1). The LGG-C0 cohort had a significantly younger age at initial diagnosis compared to the LGG-C1-3 cohort ($p < 0.0001$), but no additional differences in CNV, overall mutation burden, frequency of alterations in *PTEN*, *CDK4*, *CDK4/MDM2*, *CDKN2A*, 19+/20+, or *MGMT* was identified between these groups (Table 1).

Additionally, we pooled all LGG and GBM cases without cIMPACT-NOW 3 factors into a single group (all C0 cases) and all LGG and GBM cases with at least one cIMPACT-NOW 3 factor into a single group (all C1–3 cases). There were significant differences between these groups in terms of RFS (median survival 17.0 vs 7.0 months; $p = 0.0004$) (Fig. 5C), OS (median survival of 43.7 vs 15.0 months; $p < 0.0001$) (Fig. 5D), and age at initial diagnosis (52.3 ± 1.7 vs 61.6 ± 0.7 years; $p < 0.0001$).

Analysis of *MGMT* Promoter Methylation Status As an Additional Prognostic Factor in Gliomas With cIMPACT-NOW 3 Criteria

No significant difference in frequency of *MGMT* methylation was found between the GBM-C0, -C1, -C2, or -C3 groups, or between the GBM-C0 and pooled GBM-C1-3 groups in univariate or multivariate analysis (Tables 1 and 2). In addition, no significant difference was found between tumors with and without *MGMT* promoter methylation within the GBM-C0 cohorts. Within the GBM-C1-3 groups, no significant difference was found in terms of RFS between cases with methylated and unmethylated *MGMT* in Cohort 1 ($p = 0.6077$) (Fig. 6A) or Cohort 2 ($p = 0.2932$) (Fig. 6C). In terms of OS, there was a significant difference in cases with methylated versus unmethylated *MGMT* in Cohort 1 ($p = 0.0492$) (Fig. 6B) and Cohort 2 ($p = 0.0006$) (Fig. 6D).

Analysis of 19+/20+ Status as an Additional Prognostic Factor in Gliomas With cIMPACT-NOW 3 Criteria

In Cohort 1, there was a higher frequency of 19+/20+ in GBM-C1-3 cases compared to GBM-C0 cases ($p = 0.0183$), however, no difference in 19+/20+ frequency was identified in Cohort 2 ($p = 0.2994$) or after correcting for multiple comparisons (Table 1). No significant differences were identified between GBM-C1-3 cases with 19+/20+ compared to those without this co-gain in terms of RFS in Cohort 1 ($p = 0.4159$) (Fig. 7A) or Cohort 2 ($p = 0.0961$) (Fig. 7C). There was, however, significantly longer overall survival in GBM-C1-3 cases with 19+/20+ compared to those without in both Cohort 1 ($p = 0.0013$) (Fig. 7B) and Cohort 2 ($p = 0.0073$) (Fig. 7D).The hazard ratio for cases lacking chromosome 19/20 co-gain in the GBM-C1-3 subgroup is 2.15 (95% confidence interval 1.54–3.01), which was significant in both univariate and multivariate analyses (Table 2).

DISCUSSION

Since the introduction of the 2016 WHO Classification of Tumours of the Central Nervous System dividing GBM and other adult astrocytomas into IDH-wildtype and IDH-mutant groups (5), much work has been performed to better understand the underlying molecular drivers of these tumors, and to identify reliable prognostic markers and targetable genomic alterations (8, 10, 11, 13, 21, 28, 31, 32, 35, 38, 40, 57, 60, 61). The recent cIMPACT-NOW 3 update defines the minimum molecular criteria required for upgrading an IDH-wildtype astrocytoma with WHO grade II or III histologic features to IDH-wildtype astrocytoma, molecular grade IV (26). Since these factors are now considered "definitional" of grade IV within the IDH-wildtype astrocytoma class and reliably convey a worse prognosis in histologically lower-grade tumors, there is the implication that these factors may form a "molecular baseline" for higher-grade biologic behavior,

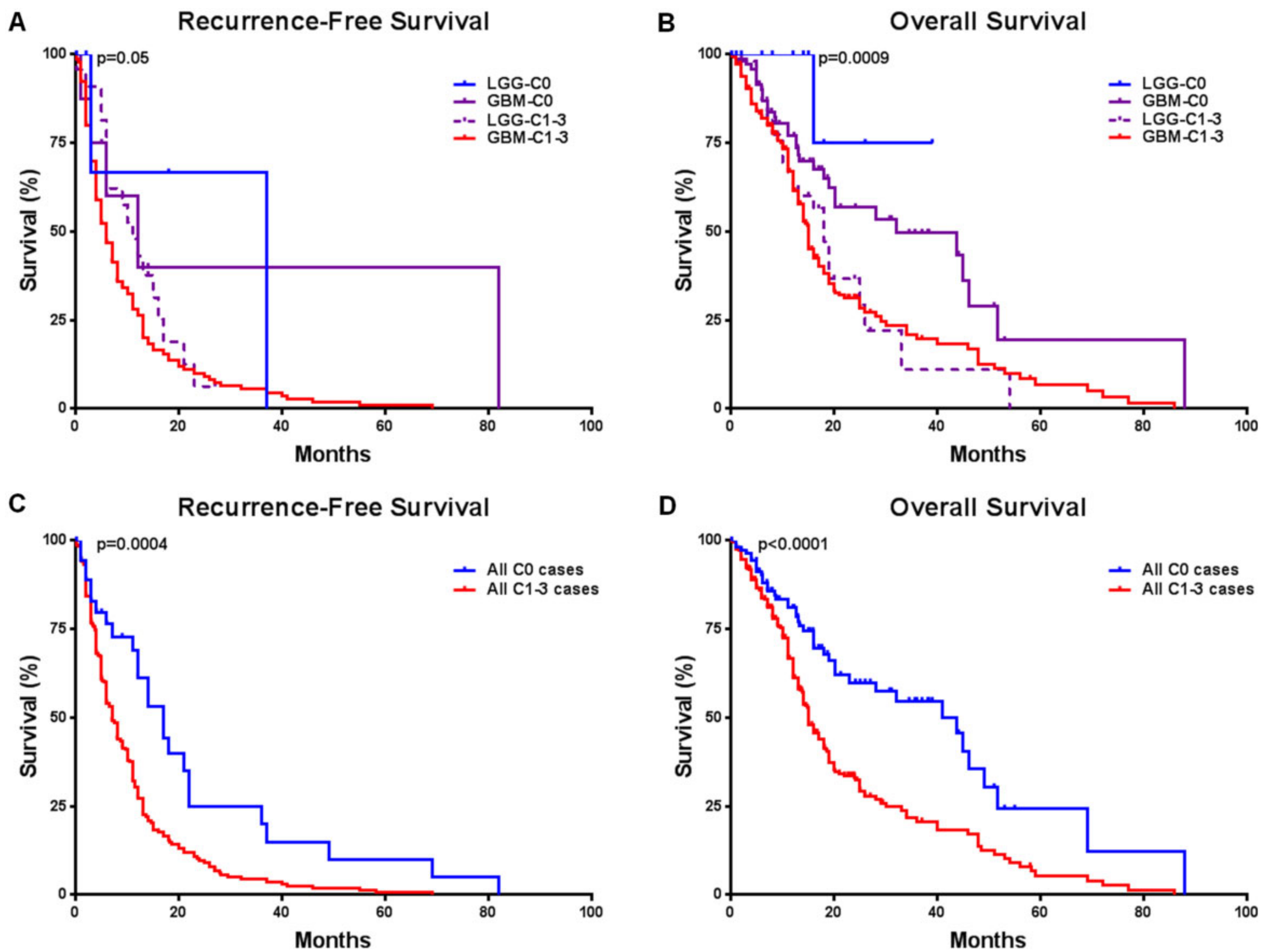

FIGURE 5. Kaplan-Meier survival curves demonstrating a nonsignificant trend toward longer survival in the LGG-C0 and GBM-C0 groups compared to LGG-C1-3 cases and GBM-C1-3 cases in terms of recurrence-free survival (RFS) ($p = 0.0506$) **(A)** and significantly longer overall survival (OS) in the LGG-C0 and GBM-C0 groups ($p = 0.0009$) **(B)**. Kaplan-Meier survival curves demonstrating longer survival in pooled LGG and GBM cases without cIMPACT-NOW 3 factors (all C0 cases) compared to pooled LGG and GBM cases with at least one cIMPACT-NOW 3 factor (all C1–3 cases) in terms of RFS ($p = 0.0004$) **(C)** and OS ($p < 0.0001$) **(D).**

although other alterations including homozygous deletion of *CDKN2A* have been considered as well (10, 11, 26).

In this context, we applied the cIMPACT-NOW 3 paradigm to IDH-wildtype GBMs to determine if there was a correlation between these factors and clinical outcomes in histologically grade IV tumors. Approximately 3% of the total GBM cases in the TCGA and cBioPortal datasets lack all 3 of these cIMPACT-NOW 3 molecular GBM criteria (GBM-C0), although they are designated as WHO grade IV tumors on the basis of histologic features (microvascular proliferation and/or tumor necrosis). It should be noted, however, that a portion of the total IDH-wildtype GBM cases do not have *TERT*p mutation status available in the TCGA and cBioPortal datasets, so this “triple-negative” GBM-C0 subgroup may be somewhat more frequent. It is also important to note that there may be an inherent selection bias in the institutional cases that were sequenced for clinical purposes and in the TCGA cases in terms of the cases initially sent from various institutions, as well as bias in sample type and molecular analysis of these cases (including batch effects) (62–65).

In this study, the GBM-C0 groups had significantly longer OS intervals compared to the GBM-C1-3 group and individual GBM-C1, -C2, and -C3 subgroups (Fig. 4). In the GBM-C1 group, there was no significant difference in RFS or OS with regard to which of the 3 criteria is present, so the presence of any of these factors appears to be sufficient to produce a worse clinical outcome. The GBM-C0 groups also presented at a younger age and had fewer overall somatic mutations (Table 1) (60). Additional analysis of LGGs revealed that the LGG-C0 group had statistically indistinguishable survival intervals with the GBM-C0 group.

Our results do not validate the previous observation that *CDK4* amplification and homozygous *CDKN2A* deletion are less frequently found in GBM cases with more favorable outcomes (10). We did, however, identify a significantly better OS in the cases with one or more cIMPACT-NOW 3 factor

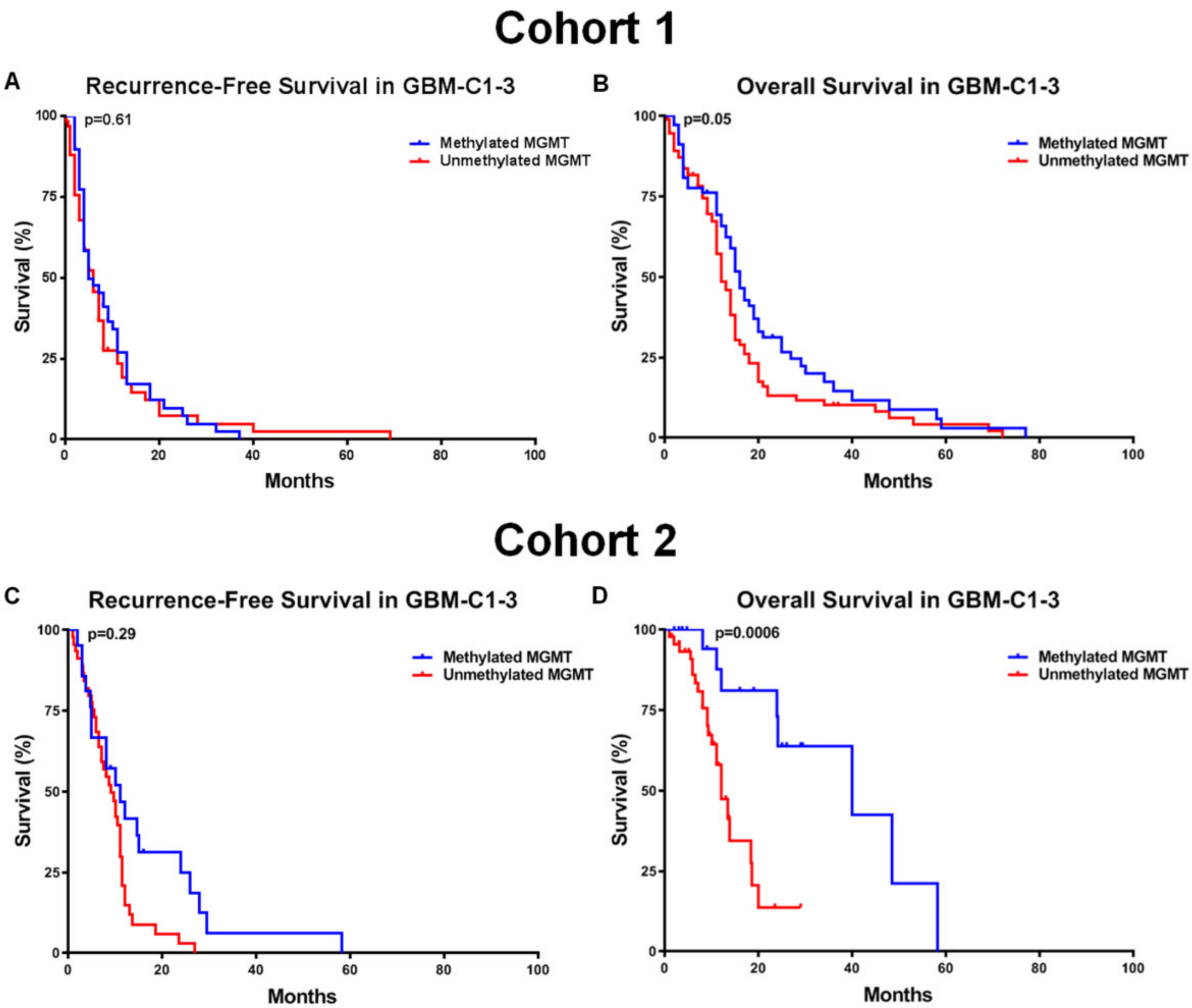

FIGURE 6. Kaplan-Meier survival curves demonstrating no significant difference in the Cohort 1 GBM-C1-3 cases with methylated *MGMT* compared to those with unmethylated *MGMT* in terms of recurrence-free survival (RFS) ($p = 0.6077$) **(A)**, however, a significant difference was observed in terms of overall survival (OS) ($p = 0.0492$) **(B)**. Kaplan-Meier survival curves demonstrating no significant difference in the Cohort 2 GBM-C1-3 cases with methylated MGMT compared to those with unmethylated MGMT in terms of RFS ($p = 0.2932$) **(C)**, however, a significant difference was observed in terms of OS ($p = 0.0006$) **(D).**

and 19+/20+ than the cases without chromosome 19/20 co-gain in both cohorts (Fig. 7 and Table 2), indicating that this may be an important additional factor to include when evaluating the prognosis in specific subsets of GBM cases. As previously reported (33, 34), GBM-C1-3 cases with *MGMT* methylation had significantly longer OS intervals than those with unmethylated *MGMT* in both Cohort 1 and Cohort 2, but no significant effect was seen in terms of RFS (Fig. 6 and Table 2).

The current report is the first to establish a statistically significant role of these combined cIMPACT-NOW update 3 factors in predicting clinical outcome in IDH-wildtype GBMs, suggesting that like histologically lower-grade astrocytomas, these molecular features may be more useful for prognostic stratification than classic histologic findings in certain subsets. While testing all IDH-wildtype GBMs for these factors may prove cost-prohibitive, our findings suggest that there may be a benefit to screening younger IDH-wildtype GBM patients for these cIMPACT-NOW 3 criteria to help refine prognosis in these cases.

AVAILABILITY OF DATA AND MATERIAL

The full dataset used for Cohort 1 in this study is freely available at www.cbioportal.org, https://portal.gdc.cancer.gov/, and (40) (http://creativecommons.org/licenses/by/4.0/; http://creativecommons.org/publicdomain/zero/1.0/).

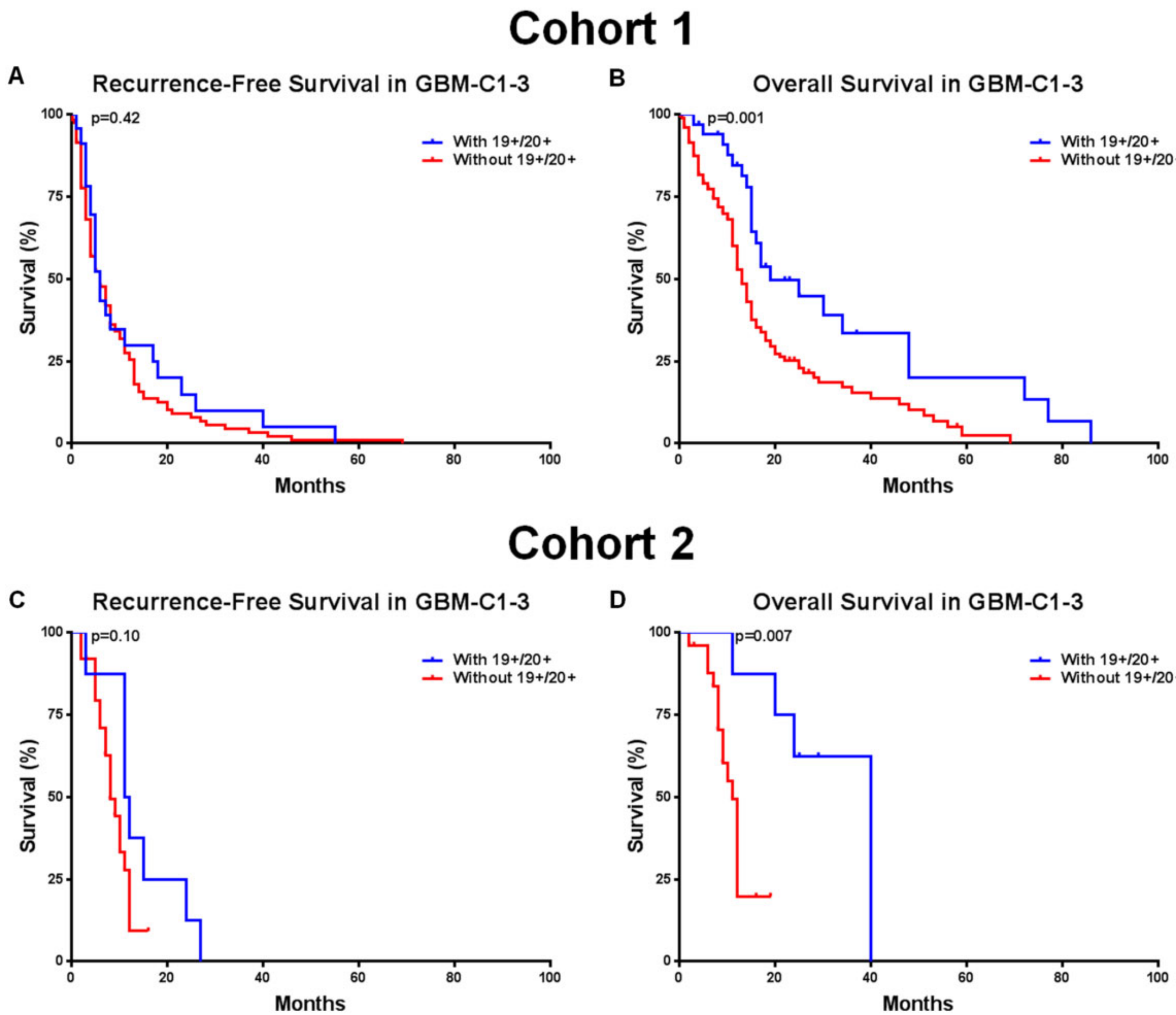

FIGURE 7. Kaplan-Meier survival curves demonstrating no significant difference in the Cohort 1 GBM-C1-3 cases with co-gain of chromosomes 19+/20+ compared to the GBM-C1-3 cases without 19+/20+ in terms of recurrence-free survival (RFS) ($p = 0.4159$) **(A)**, however, there was a significantly longer overall survival (OS) in the Cohort 1 cases with 19+/20+ ($p = 0.0013$) **(B)**. There was a trend toward longer survival in the Cohort 2 GBM-C1-3 cases with co-gain of chromosomes 19+/20+ compared to the GBM-C1-3 cases without 19+/20+ in terms of RFS, although this was not a significant effect ($p = 0.0961$) **(C)**, however, there was a significantly longer OS in the Cohort 2 cases with 19+/20+ ($p = 0.0073$) **(D).**

ACKNOWLEDGMENTS

The authors would like to thank Rena Pacheco at the University of Pennsylvania for her assistance with Institutional Review Board (IRB) and data transfer. They would also like to thank the University of Pennsylvania, Department of Neurosurgery Clinical Research Division for allowing us access to their collected glioblastoma data.

REFERENCES

1. Ostrom QT, Gittleman H, Truitt G, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. Neuro Oncol 2018;20:iv1–86
2. Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: A “state of the science”. Rev Neuro Oncol 2014;16:896–913
3. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807–12
4. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–73
5. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol 2016;131:803–20
6. Lara-Velazquez M, Al-Kharboosh R, Jeanneret S, et al. Advances in brain tumor surgery for glioblastoma in adults. Brain Sci 2017;7: 166
7. Faraz S, Pannullo S, Rosenblum M, et al. Long-term survival in a patient with glioblastoma on antipsychotic therapy for schizophrenia: A case report and literature review. Ther Adv Med Oncol 2016;8:421–8
8. Lu J, Cowperthwaite MC, Burnett MG, et al. Molecular predictors of long-term survival in glioblastoma multiforme patients. PLoS One 2016; 11:e0154313
9. Richardson TE, Patel S, Serrano J, et al. Genome-wide analysis of glioblastoma patients with unexpectedly long survival. J Neuropathol Exp Neurol 2019;78:501–7

10. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462–77
11. Bieńkowski M, Piaskowski S, Stoczyńska-Fidelus E, et al. Screening for EGFR amplifications with a novel method and their significance for the outcome of glioblastoma patients. PLoS One 2013;8:e65444
12. Kleinschmidt-DeMasters BK, Lillehei KO, Varella-Garcia M. Glioblastomas in the older old. Arch Pathol Lab Med 2005;129:624–31
13. Smith JS, Tachibana I, Passe SM, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst 2001;93:1246–56
14. Hobbs J, Nikiforova MN, Fardo DW, et al. Paradoxical relationship between the degree of EGFR amplification and outcome in glioblastomas. Am J Surg Pathol 2012;36:1186–93
15. Houillier C, Lejeune J, Benouaich-Amiel A, et al. Prognostic impact of molecular markers in a series of 220 primary glioblastomas. Cancer 2006;106:2218–23
16. Hatanpaa KJ, Burma S, Zhao D, et al. Epidermal growth factor receptor in glioma: Signal transduction, neuropathology, imaging, and radioresistance. Neoplasia 2010;12:675–84
17. Hoffman DI, Abdullah KG, McCoskey M, et al. Negative prognostic impact of epidermal growth factor receptor copy number gain in young adults with isocitrate dehydrogenase wild-type glioblastoma. J Neurooncol 2019;145:321–8
18. Stichel D, Ebrahimi A, Reuss D, et al. Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma. Acta Neuropathol 2018;136: 793–803
19. Baysan M, Woolard K, Cam MC, et al. Detailed longitudinal sampling of glioma stem cells in situ reveals Chr7 gain and Chr10 loss as repeated events in primary tumor formation and recurrence. Int J Cancer 2017; 141:2002–13
20. Inda MM, Fan X, Munoz J, et al. Chromosomal abnormalities in human glioblastomas: Gain in chromosome 7p correlating with loss in chromosome 10q. Mol Carcinog 2003;36:6–14
21. Ceccarelli M, Barthel FP, Malta TM, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell 2016;164:550–63
22. Yuan Y, Qi C, Maling G, et al. TERT mutation in glioma: frequency, prognosis and risk. J Clin Neurosci 2016;26:57–62
23. Diplas BH, He X, Brosnan-Cashman JA, et al. The genomic landscape of TERT promoter wildtype-IDH wildtype glioblastoma. Nat Commun 2018;9:2087
24. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med 2015;372:2499–508
25. Aibaidula A, Chan AK, Shi Z, et al. Adult IDH wild-type lower-grade gliomas should be further stratified. Neuro Oncol 2017;19:1327–37
26. Brat DJ, Aldape K, Colman H, et al. cIMPACT-NOW update 3: Recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". Acta Neuropathol 2018;136:805–10
27. Weller M, Weber RG, Willscher E, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. Acta Neuropathol 2015;129:679–93
28. Brat DJ, Verhaak RGW, Aldape KD, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 2015; 372:2481–98
29. Reuss DE, Kratz A, Sahm F, et al. Adult IDH wild type astrocytomas biologically and clinically resolve into other tumor entities. Acta Neuropathol 2015;130:407–17
30. Wijnenga MMJ, Dubbink HJ, French PJ, et al. Molecular and clinical heterogeneity of adult diffuse low-grade IDH wild-type gliomas: Assessment of TERT promoter mutation and chromosome 7 and 10 copy number status allows superior prognostic stratification. Acta Neuropathol 2017;134:957–9
31. Geisenberger C, Mock A, Warta R, et al. Molecular profiling of long-term survivors identifies a subgroup of glioblastoma characterized by chromosome 19/20 co-gain. Acta Neuropathol 2015;130:419–34
32. Cimino PJ, Zager M, McFerrin L, et al. Multidimensional scaling of diffuse gliomas: Application to the 2016 World Health Organization classification system with prognostically relevant molecular subtype discovery. Acta Neuropathol Commun 2017;5:39
33. Weller M, Stupp R, Reifenberger G, et al. MGMT promoter methylation in malignant gliomas: Ready for personalized medicine? Nat Rev Neurol 2010;6:39–51
34. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997–1003
35. Hartmann C, Hentschel B, Simon M, et al. Long-term survival in primary glioblastoma with versus without isocitrate dehydrogenase mutations. Clin Cancer Res 2013;19:5146–57
36. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4
37. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1
38. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8
39. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703–13
40. Umehara T, Arita H, Yoshioka E, et al. Distribution differences in prognostic copy number alteration profiles in IDH-wild-type glioblastoma cause survival discrepancies across cohorts. Acta Neuropathol Commun 2019;7:15
41. Jonsson P, Lin AL, Young RJ, et al. Genomic correlates of disease progression and treatment response in prospectively characterized gliomas. Clin Cancer Res 2019;25:5537–47
42. Richardson TE, Snuderl M, Serrano J, et al. Rapid progression to glioblastoma in a subset of IDH-mutated astrocytomas: A genome-wide analysis. J Neurooncol 2017;133:183–92
43. Richardson TE, Tang K, Vasudevaraja V, et al. GOPC-ROS1 fusion due to microdeletion at 6q22 is an oncogenic driver in a subset of pediatric gliomas and glioneuronal tumors. J Neuropathol Exp Neurol 2019;78: 1089–99
44. Silva TC, Colaprico A, Olsen C, et al. TCGA Workflow: analyze cancer genomics and epigenomics data using Bioconductor packages. F1000Res 2016;5:1542
45. Colaprico A, Silva TC, Olsen C, et al. TCGAbiolinks: An R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res 2016;44:e71
46. Mermel CH, Schumacher SE, Hill B, et al. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. Genome Biol 2011;12:R41
47. Beroukhim R, Getz G, Nghiemphu L, et al. Assessing the significance of chromosomal aberrations in cancer: Methodology and application to glioma. Proc Natl Acad Sci U S A 2007;104:20007–12
48. Reich M, Liefeld T, Gould J, et al. GenePattern 2.0. Nat Genet 2006;38: 500–1
49. Mayakonda A, Lin DC, Assenov Y, et al. Maftools: Efficient and comprehensive analysis of somatic variants in cancer. Genome Res 2018;28: 1747–56
50. Li B, Dewey CN. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 2011; 12:323
51. Forbes SA, Bindal N, Bamford S, et al. COSMIC: Mining complete cancer genomes in the catalogue of somatic mutations in cancer. Nucleic Acids Res 2011;39:D945–50
52. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: The NCBI database of genetic variation. Nucleic Acids Res 2001;29:308–11
53. Landrum MJ, Lee JM, Benson M, et al. ClinVar: Public archive of interpretations of clinically relevant variants. Nucleic Acids Res 2016;44: D862–8
54. Kurzwelly D, Glas M, Roth P, et al. Primary CNS lymphoma in the elderly: Temozolomide therapy and MGMT status. J Neurooncol 2010;97: 389–92
55. Genomes Project C, Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56–65
56. Shihab HA, Rogers MF, Gough J, et al. An integrative approach to predicting the functional effects of non-coding and coding sequence variation. Bioinformatics 2015;31:1536–43

57. Mirchia K, Sathe AA, Walker JM, et al. Total copy number variation as a prognostic factor in adult astrocytoma subtypes. Acta Neuropathol Commun 2019;7:92
58. Richardson TE, Sathe AA, Kanchwala M, et al. Genetic and epigenetic features of rapidly progressing IDH-mutant astrocytomas. J Neuropathol Exp Neurol 2018;77:542–8
59. Richardson TE, Williams M, Galbraith K, et al. Total copy number variation, somatic mutation burden, and histologic grade correlate with clinical outcome in oligodendroglioma. Clin Neuropathol 2020. Available at: https://www.dustri.com/nc/article-response-page.html?artId=186281&doi=10.5414%2FNP301260.
60. Hwang T, Mathios D, McDonald KL, et al. Integrative analysis of DNA methylation suggests down-regulation of oncogenic pathways and reduced somatic mutation rates in survival outliers of glioblastoma. Acta Neuropathol Commun 2019;7:5
61. Mirchia K, Snuderl M, Galbraith K, et al. Establishing a prognostic threshold for total copy number variation within adult IDH-mutant grade II/III astrocytomas. Acta Neuropathol Commun 2019;7:121
62. Solomon DA, Kim JS, Ressom HW, et al. Sample type bias in the analysis of cancer genomes. Cancer Res 2009;69:5630–3
63. Rasnic R, Brandes N, Zuk O, et al. Substantial batch effects in TCGA exome sequences undermine pan-cancer analysis of germline variants. BMC Cancer 2019;19:783
64. Wang VG, Kim H, Chuang JH. Whole-exome sequencing capture kit biases yield false negative mutation calls in TCGA cohorts. PLoS One 2018;13:e0204912
65. Colli LM, Machiela MJ, Myers TA, et al. Burden of nonsynonymous mutations among TCGA cancers and candidate immune checkpoint inhibitor responses. Cancer Res 2016;76:3767–72