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Copy number profiling across glioblastoma populations has implications for clinical trial design

Cimino, Patrick J ; McFerrin, Lisa ; Wirsching, Hans-Georg ; Arora, Sonali ; Bolouri, Hamid ; Rabadan, Raul ; Weller, Michael ; Holland, Eric C

Abstract: Background Copy number alterations form prognostic molecular subtypes of glioblastoma with clear differences in median overall survival. In this study, we leverage molecular data from several glioblastoma cohorts to define the distribution of copy number subtypes across random cohorts as well as cohorts with selection biases for patients with inherently better outcome. Methods Copy number subtype frequency was established for 4 glioblastoma patient cohorts. Two randomly selected cohorts include The Cancer Genome Atlas (TCGA) and the German Glioma Network (GGN). Two more selective cohorts include the phase II trial ARTE in elderly patients with newly diagnosed glioblastoma and a multi-institutional cohort focused on paired resected initial/recurrent glioblastoma. The paired initial/recurrent cohort also had exome data available, which allowed for evaluation of multidimensional scaling analysis. Results Smaller selective glioblastoma cohorts are enriched for copy number subtypes that are associated with better survival, reflecting the selection of patients who do well enough to enter a clinical trial or who are deemed well enough to undergo resection at recurrence. Adding exome data to copy number data provides additional data reflective of outcome. Conclusions The overall outcome for diffuse glioma patients is predicted by DNA structure at initial tumor resection. Molecular signature shifts across glioblastoma populations reflect the inherent bias of patient selection toward longer survival in clinical trials. Therefore it may be important to include molecular profiling, including copy number, when enrolling patients for clinical trials in order to balance arms and extrapolate relevance to the general glioblastoma population.

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Full Title: Copy number profiling across glioblastoma populations has implications for clinical trial design

Authors: Patrick J. Cimino^{1,2}, Lisa McFerrin², Hans-Georg Wirsching^{2,3}, Hamid Bolouri², Raul Rabadan⁴, Michael Weller³, Eric C. Holland²

Kommentiert [HW1]: I think NO requires no numbers, but institutions with initials in parenthesis below

Affiliations:

1. Department of Pathology, Division of Neuropathology, University of Washington School of Medicine, Seattle, WA, United States
2. Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, United States
3. Brain Tumor Center and Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland
4. Department of Biomedical Informatics and Department of Systems Biology, Columbia University, New York, New York, United States

Corresponding author information:

Patrick J. Cimino, M.D., Ph.D.
Department of Pathology, Division of Neuropathology
University of Washington
325 9th Avenue, Box 359791, Seattle, WA, 98104
E-mail: pjcc@uw.edu; Phone: (206) 744-2942; Fax: (206) 897-4688

Eric C. Holland, M.D., Ph.D.
Division of Human Biology, and Solid Tumor and Translational Research
Fred Hutchinson Cancer Research Center
1100 Fairview Ave N., Mailstop C3-168, Seattle, WA, 98109
E-mail: eholland@fredhutch.org; Phone: (206) 667-4259; Fax: (206) 667-6022

Running Title: Glioblastoma molecular subtype distributions

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Authorship: PJC and ECH designed the study. Data collection was performed by PJC, RR, and MW. PJC, LM, HGW, HB, and ECH analyzed and interpreted data. All authors participated in drafting, revising, and approval of the manuscript.

Manuscript: Word count (1685) – 6000 word max, Figures (3) – 6 displays max

ABSTRACT (250 words max)

Background: Copy number alterations form prognostic molecular subtypes of glioblastoma with clear differences in median survival. In this study, we leverage molecular data from several glioblastoma cohorts to define the distribution of copy number subtypes across the general population as well as cohorts with selection biases for patients with inherently better outcome.

Methods: Copy number subtype frequency was established for four glioblastoma groups. Two large general glioblastoma populations include The Cancer Genome Atlas (TCGA) and German Glioma Network (GGN). Two smaller selective prospective cohorts include the ARTE (NCT01443676) clinical trial for elderly patients with newly diagnosed glioblastoma and a multi-institutional cohort focused on paired resected initial/recurrence glioblastoma. The paired initial/recurrence cohort also had exome data available, which allowed for evaluation of multidimensional scaling analysis.

Results: Smaller selective glioblastoma cohorts are enriched for copy number subtypes that associated with better survival, reflecting the selection of patients who do well-enough to enter a clinical trial or who are deemed well-enough to have a resection at recurrence. Adding exome data to copy number data provides additional data reflective of outcome. The distribution shift of molecular profiles is not related to clinical features such as age, sex, or KPS.

Conclusions: Molecular signature shifts across glioblastoma populations reflect the inherent bias of patient selection for better survival in clinical trials. Therefore it may be important to include molecular profiling, including copy number, when enrolling patients for clinical trials in order to balance arms and extrapolate relevance to the general glioblastoma population.

KEY WORDS:

Glioblastoma; Clinical trials; Molecular profiling; Biomarkers

IMPORTANCE OF THE STUDY (150 words max):

Glioblastoma is the most common primary malignant neoplasm of the central nervous system. Patient outcome is generally poor, but can be predicted by copy number alterations. Copy number profiling across cohorts reflects the inherent bias of clinical trial selection towards patients with better outcome. Understanding the distribution of molecular signatures across cohorts suggests a role for molecular profiling to be incorporated up front in clinical trials and studies focused upon tumor recurrence.

INTRODUCTION

In 2016, the World Health Organization (WHO) introduced a classification of central nervous tumors that incorporate molecular signatures with traditional histopathology to arrive at 'integrated' diagnostic diffuse glioma entities.¹⁻⁴ The molecular components of this classification scheme largely involve knowing the mutational status of isocitrate dehydrogenase (IDH) and co-deletion of whole chromosome arms 1p and 19q. Integrating these limited molecular alterations into the current classification system of diffuse glioma is a better predictor of clinical outcome than histopathology alone. However, there is still an issue with the current classification system in that WHO grading of diffuse gliomas is still determined solely by histopathologic features (mitotic activity, necrosis, microvascular proliferation) without taking molecular features into account. Recent attempts have been made to define molecular alterations (Beyond IDH and 1p19q) which may provide prognostic information beyond histological grading. Our group⁵ and others^{6,7} have shown that diffuse glioma survival can be predicted, to a large degree, by whole chromosome and gene level copy number alterations. Copy number alterations predict patient survival in diffuse gliomas, and subtypes with clear differences in median survival can be derived from copy number profiling.⁵ We have previously shown that using data from The Cancer Genome Atlas (TCGA) and German Glioma Network (GGN), IDH-wildtype glioblastoma, WHO grade IV, can be further stratified into three distinct prognostic DNA copy number subtypes W1 (worst survival), W2 (intermediate survival), and W3 (best survival) (Figure 1).⁵ These copy number subtypes are determined by relatively few foci: gain of whole chromosome 1, gain of whole chromosome 19, and *CDK4/MDM2* co-amplification (Figure 1). These prognostic copy number subtypes do not overlap with other

described molecular transcriptional or methylation subtypes.⁸⁻¹⁰ Given the implications for prognosis, we sought to determine copy number subtype distributions across glioblastoma populations while using the TCGA and GGN datasets as reflective of the general population. We identified two additional prospective cohorts that had an inherent bias towards including patients with better functional status and survival, which is not reflective of the general population. The first cohort is from the randomized phase II Swiss, ARTE trial focused on newly diagnosed glioblastoma in the elderly population.¹¹ The second cohort is that of paired initial and recurrent glioblastoma, where inclusion required that the patient survived long enough, and was deemed appropriate, to have a second surgery.¹² The paired initial/recurrent glioblastoma cohort also had exome data available in addition to copy number analysis, which allows for further insights into risk-stratification by molecular profiling.

MATERIALS AND METHODS

Copy Number Data from Glioblastoma Cohorts

Four separate cohorts of IDH-wildtype glioblastoma were analyzed for gain of whole chromosome 1, gain of whole chromosome 19, and *CDK4/MDM2* co-amplification. Copy number data via GISTIC 2.0 scores¹³ for TCGA glioblastomas (n=256) was downloaded from the University of California Santa Cruz cancer browser (<https://genome-cancer.ucsc.edu/>). Clinical data for the TCA glioblastoma dataset were obtained from the Genomic Data Commons (GDC) Data Portal from the National Institutes of Health (NIH).¹⁴ Glioblastoma copy number data from the German Glioma Network (n=243) (www.gliomnetzwerk.de) and ARTE (NCT01443676) clinical trial for elderly patients with newly diagnosed glioblastoma¹¹ (n=59) were derived from

450k methylation array data as previously described using the R package 'conumee' (<http://bioconductor.org/packages/conumee>) applying an adapted algorithm for baseline-correction.^{5,9} Whole chromosomal gains and *CDK4/MDM2* co-amplifications were determined by log₂-scale thresholds of 0.1 and 0.6, respectively. Copy number data from a multi-institutional (The MD Anderson Cancer Center, The University of California San Francisco, Istituto Neurologica C. Besta, Kyoto University, and Samsung Medical Center) paired initial/recurrence glioblastoma cohort¹² was determined from whole exome sequencing using the EXCAVATOR bioinformatic pipeline.¹⁵ For all human glioblastoma cohorts, molecular data was ascertained in accordance with the World Medical Association Declaration of Helsinki: Research involving human subjects.

Combined Copy Number and Single Nucleotide Data Visualization

Classic multidimensional scaling (MDS) of TCGA glioma data (single nucleotide point mutation and copy number) produced two-dimensional scatterplots and was performed using R software (Version 3.5.0, RProject for Statistical Computing, <http://www.r-project.org/>) as previously described.^{5,16} Copy number data and single nucleotide variant data (determined by SAVI2¹⁷) for the paired initial/recurrence glioblastoma cohort¹² were combined using MutComFocal¹⁸ and mapped onto the MDS map using the TCGA data as a reference set.

Statistics

Statistical analyses were performed using GraphPad Prism software (Version 7.02, <https://www.graphpad.com/scientific-software/prism>). Kaplan-Meier analysis for overall

survival was performed with *P*-values determined by Cox proportional hazards regression. Other data comparisons were performed using Chi-square test, Mann-Whitney U test, and Fisher's exact test as indicated.

RESULTS

The distribution of copy number subtypes is similar across two large data sets of initial glioblastoma across North America (The Cancer Genome Atlas [TCGA]) and in Europe (German Glioma Network [GGN]), suggesting that this is the natural distribution in the population (Figure 2). Selection bias exists towards a longer-lived glioblastoma subgroup when enrolling patients healthy enough to enter clinical trials or when deciding that patients are well enough for a resection at recurrence. Given the better overall survival of these types of pre-selected patients, we wondered whether there was evidence of a skew in the population with respect to distribution of the copy number subtypes defined above. In fact, there were two such cohorts for analysis. The first cohort is the phase II ARTE trial of hypofractionated radiotherapy with or without bevacizumab in elderly patients with newly diagnosed glioblastoma.¹¹ When compared to the elderly TCGA and GGN general glioblastoma populations, there was a distribution skew toward the better prognostic W3 copy number subtype (Figure 1A). The second prospective cohort is a multi-institutional (The MD Anderson Cancer Center, The University of California San Francisco, Istituto Neurologica C. Besta, Kyoto University, and Samsung Medical Center) paired initial/recurrence glioma group,¹² which investigated glioblastoma patients of all ages that were healthy enough to have a resection at first recurrence. This paired initial/recurrence glioma cohort also showed a skew towards the better prognostic W3 copy number subtype (Figure 2).

A more granular method to characterize DNA alterations is through multidimensional scaling (MDS) analysis by combining copy number with whole exome sequencing.^{5,16} MDS defines distinct glioma groups with differences in survival as well. Of the two selective prospective cohorts, the paired initial/recurrent glioblastoma cohort additionally has DNA exome sequencing available in addition to copy number status and allows for MDS analysis of these patients. To investigate a potential distribution skew with respect to MDS molecular signatures, we overlaid the paired initial/recurrent glioblastoma data cohort¹² onto the TCGA reference MDS map. As shown in Figure 1B, 51% of glioblastomas from TCGA exhibit a similar DNA structure that does not correspond to those appropriate for surgical resection at recurrence. This MDS group of IDH-wildtype glioblastoma largely includes, but is not limited to, glioblastomas that have the poorest survival among MDS regions and is characterized by relatively few regions of chromosomal alterations with the exception of whole chromosome 7 gain, whole chromosome 10 loss, and loss of chromosome 9p.⁵ Essentially all patients who do well-enough to have a second surgical resection at recurrence arise from the other half of glioblastomas, which tend to have one or more of the following alterations: whole chromosome 1 gain, whole chromosome 19 gain, and/or mutations in *TP53*.

The data suggest that analysis of recurrent glioblastoma samples is missing the tumors with the most common DNA structure found in the natural population. Further, it suggests that this global DNA pattern is associated with clinical characteristics that lead surgeons across North America and Asia to not operate on these first recurrent glioblastoma patients. As a point of

diagnostic concern, this MDS 'poor survival' region cannot be approximated by a small panel of genetic markers or differentially methylated probes (Supplementary Figure 1), and requires exome sequencing in addition to copy number status to determine the DNA structure. Although differentially methylated genes are not of a diagnostic utility and do not discriminate between MDS regions in our context, top hits in biology pathway enrichment analysis demonstrates that the 'poor survival' group has differentially methylated genes related mostly to cell cycle, and to a lesser extent, immune cell interaction (Supplementary Table 1), consistent with dysregulation of these pathways being associated in more aggressive forms of glioma.^{5,8,19-}

²⁶ Clinical characteristics corresponding to this DNA pattern are unclear, but there appears to be no association with median age ($p=0.15$; Mann-Whitney U test), male versus female ($p=0.052$; Fisher's exact test), or KPS ≥ 80 versus < 80 ($p=0.42$; Fisher's exact test). It also seems likely that patients with this global DNA pattern are underrepresented in clinical trials, particularly in those focused on first recurrence of glioblastoma.

DISCUSSION

Pattern shifts in the distribution of molecular subtypes across glioblastoma cohorts has overall implications for clinical trial design. In the paired glioblastoma and ARTE trial cohorts, there is a selection bias for long-term survivors reflected in the molecular marker status shown. As such, comparison of any clinical trial treatment strategy, or institutional outcomes, may be very difficult to interpret in the absence of knowing molecular subtype distributions of their patient populations. Molecular profiling to inform clinical trial structure is of increasing importance,¹⁹ and it would be ideal to have copy number subgroups evenly distributed across arms of trial up

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front. Failure to do so may contribute to occasional discordance between phase II and phase III clinical trial outcomes. Historical controls that do not account for molecular signatures are insufficient for comparators for current and future clinical trial enrollment. If molecular subtypes are not addressed up front in clinical trials, it is likely that treatments that may appear effective overall in early phase II trials, may fail in phase III trials due to unaddressed shifting molecular distributions, not reflective of general population. This can lead to very costly trials that are pre-selected to fail. For those poorest molecular survival groups, such as W1 copy number subtype, patients could be identified early and put on up front trials limited to their molecular group. Overall, informing glioblastoma clinical trials by molecular signature status, such as copy number alterations, may lead to more appropriate cohort distributions and therapeutic strategies applicable to the general population.

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References:

1. 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(6):803-820.
2. Louis DN OH, Wiestler OD, Cavenee WK. *WHO Classification of Tumours of the Central Nervous System.* 4th ed. Lyon, France: International Agency for Research on Cancer; 2016.
3. Reuss DE, Sahm F, Schrimpf D, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol.* 2015; 129(1):133-146.
4. Louis DN, Perry A, Burger P, et al. International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol.* 2014; 24(5):429-435.
5. 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(1):39.
6. Lee CH, Alpert BO, Sankaranarayanan P, Alter O. GSVD comparison of patient-matched normal and tumor aCGH profiles reveals global copy-number alterations predicting glioblastoma multiforme survival. *PLoS One.* 2012; 7(1):e30098.
7. Shirahata M, Ono T, Stichel D, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. *Acta Neuropathol.* 2018.
8. Ceccarelli M, Barthel FP, Malta TM, et al. Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell.* 2016; 164(3):550-563.
9. Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell.* 2012; 22(4):425-437.
10. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 2010; 17(1):98-110.
11. Wirsching HG, Tabatabai G, Roelcke U, et al. Bevacizumab plus hypofractionated radiotherapy versus radiotherapy alone in elderly patients with glioblastoma: the randomized, open-label, phase II ARTE trial. *Ann Oncol.* 2018.
12. Wang J, Cazzato E, Ladewig E, et al. Clonal evolution of glioblastoma under therapy. *Nat Genet.* 2016; 48(7):768-776.
13. Roszik J, Wu CJ, Siroy AE, et al. Somatic Copy Number Alterations at Oncogenic Loci Show Diverse Correlations with Gene Expression. *Sci Rep.* 2016; 6:19649.
14. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer Genomic Data. *N Engl J Med.* 2016; 375(12):1109-1112.
15. Magi A, Tattini L, Cifola I, et al. EXCAVATOR: detecting copy number variants from whole-exome sequencing data. *Genome Biol.* 2013; 14(10):R120.
16. Bolouri H, Zhao LP, Holland EC. Big data visualization identifies the multidimensional molecular landscape of human gliomas. *Proc Natl Acad Sci U S A.* 2016; 113(19):5394-5399.
17. Trifonov V, Pasqualucci L, Tiacci E, Falini B, Rabadan R. SAVI: a statistical algorithm for variant frequency identification. *BMC Syst Biol.* 2013; 7 Suppl 2:S2.
18. Trifonov V, Pasqualucci L, Dalla Favera R, Rabadan R. MutComFocal: an integrative approach to identifying recurrent and focal genomic alterations in tumor samples. *BMC Syst Biol.* 2013; 7:25.
19. Tanguturi SK, Trippa L, Ramkissoon SH, et al. Leveraging molecular datasets for biomarker-based clinical trial design in glioblastoma. *Neuro Oncol.* 2017; 19(7):908-917.

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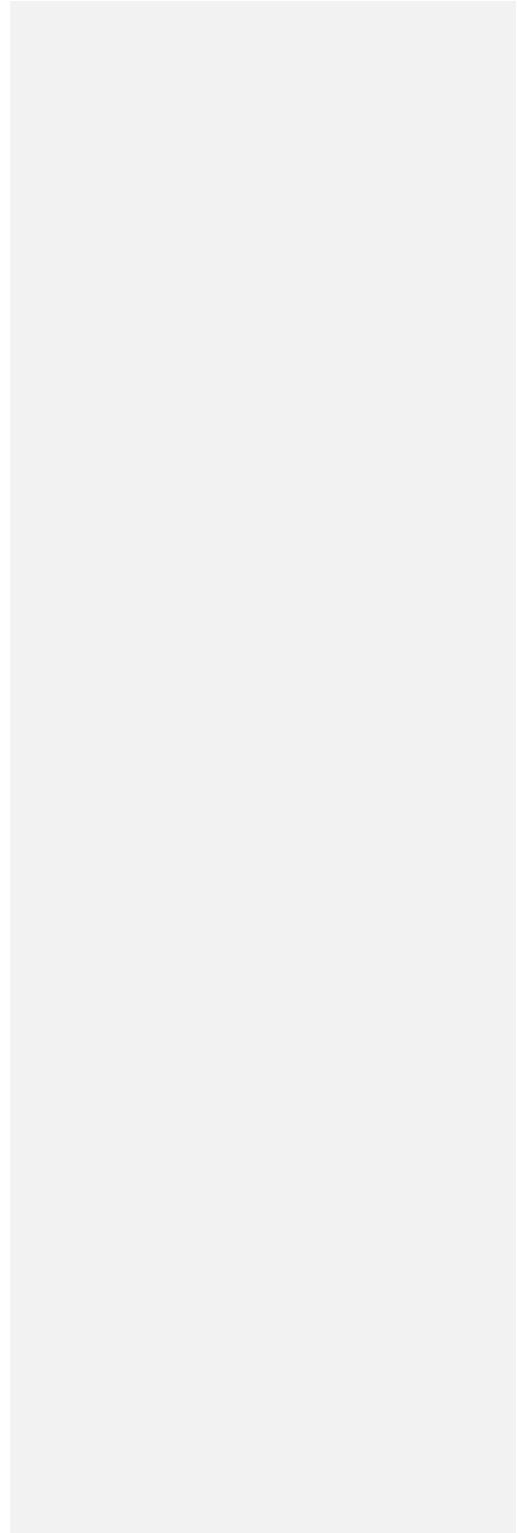


FIGURE LEGENDS

Figure 1. Copy number subtypes for IDH-wildtype glioblastoma. (A) Algorithm for copy number subtype derivation. Copy number subtypes defined by four genetic loci: gain of whole chromosome 1 (gChr1), gain of whole chromosome 19 (gChr19), and co-amplification of *CDK4/MDM2* (*caCDK4/MDM2*): W1 = [No gChr1 + No gChr19 + *caCDK4/MDM2*] or [gChr1 + *caCDK4/MDM2*]; W2 = [No gChr1 + No gChr19 + No *caCDK4/MDM2*]; W3 = [No gChr1 + gChr19] or [gChr1 + No *caCDK4/MDM2*]. (B) Overall survival of glioblastoma, copy number subtypes in TCGA dataset. TCGA copy number subtype patient numbers: W1 (n=12); W2 (n=157), and W3 (n=88). P value determined using Cox proportional hazard regression.

Figure 2. Distribution shift of copy number subtypes across glioblastoma cohorts. Distribution plots show increasing percentage of better-performing copy number subtypes in paired initial/recurrent glioblastoma and elderly clinical trial cohorts, when compared to TCGA and GGN datasets.

Figure 3. Exome sequencing added to copy number highlights molecular signature of additional poor prognostic group. (A) Multidimensional scaling (MDS) analysis based on whole exome sequencing and copy number alterations shows that mapping of the paired initial/recurrent glioblastoma cohort has uneven spatial distribution when overlaid on the TCGA reference dataset (TCGA cohort = gray; Paired initial glioma = blue; Paired recurrent glioma = red). (B) Green edges show the connections of individual paired initial and recurrent gliomas.

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Orange colored region highlights tightly clustered area of which 51% of TCGA glioblastomas exist, but only one initial glioma from the paired initial/recurrent glioblastoma dataset.

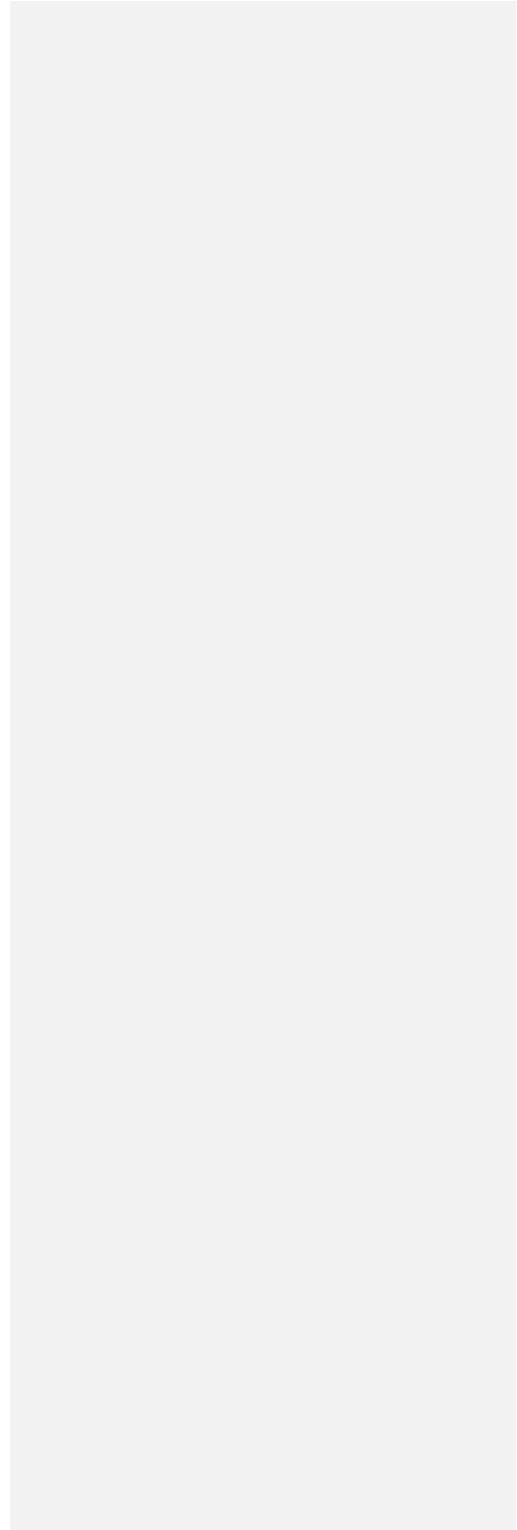


Figure 1.

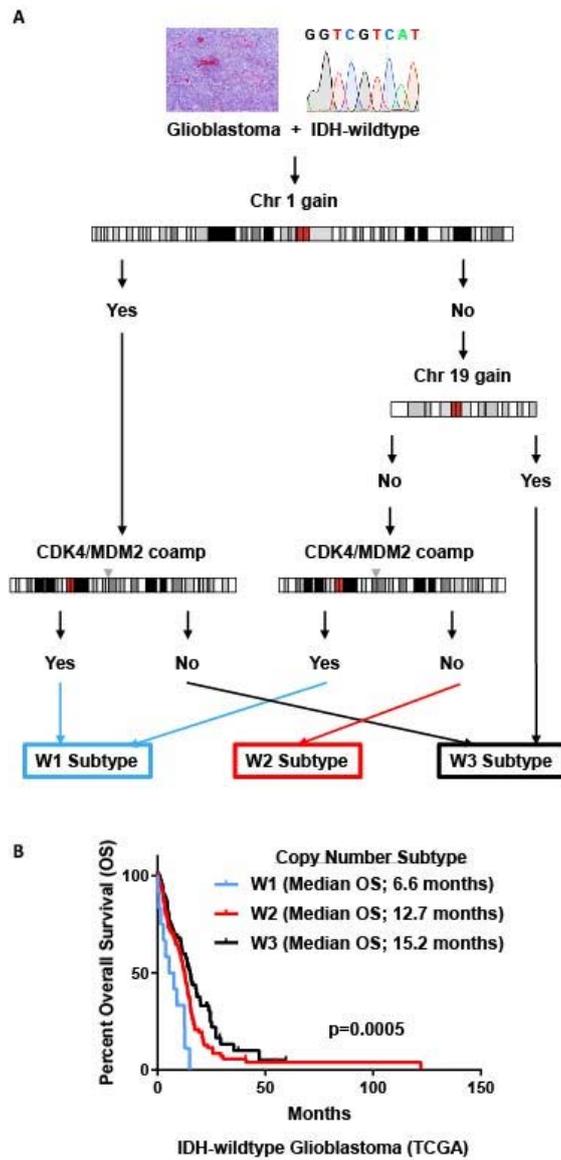


Figure 2.

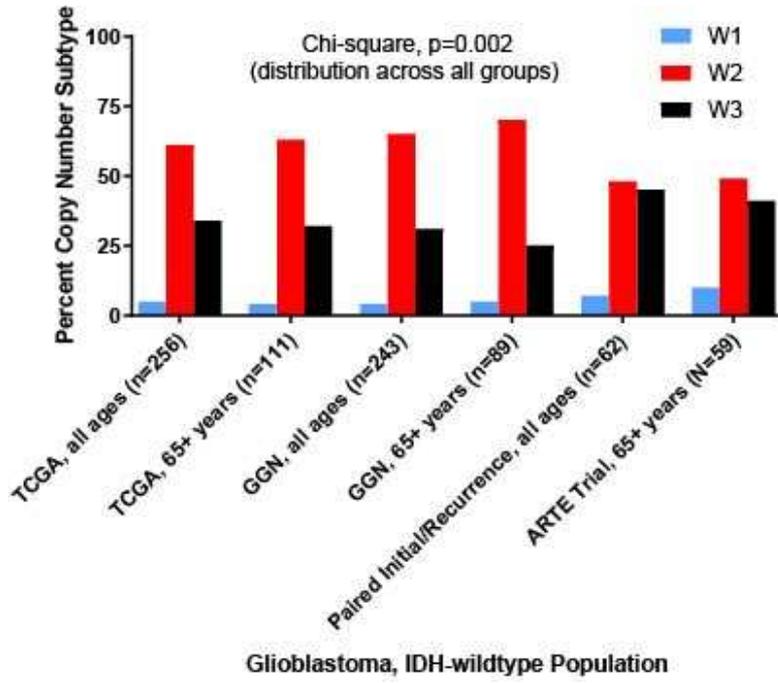
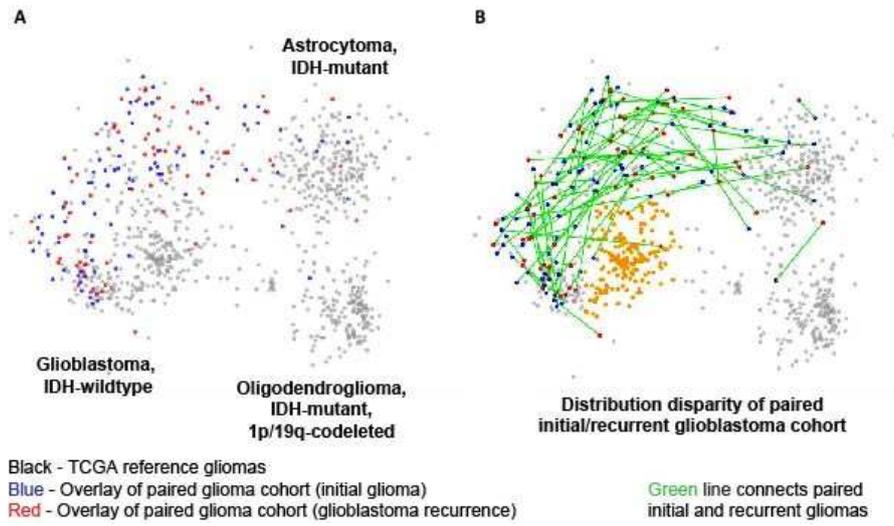


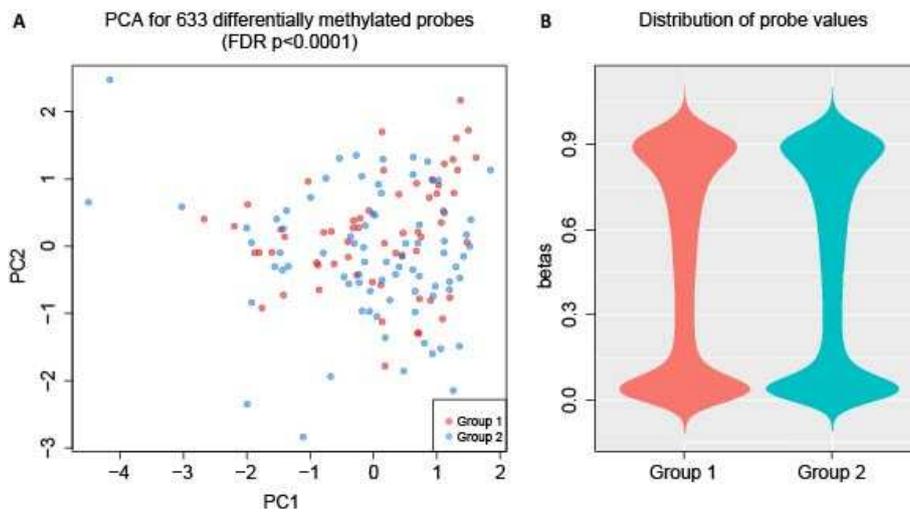
Figure 3.



SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIALS AND METHODS

TCGA methylation data for glioblastomas were downloaded from the University of California Santa Cruz cancer browser (<https://genome-cancer.ucsc.edu/>) and analyzed in using R software (Version 3.5.0, RProject for Statistical Computing, <http://www.r-project.org/>) applying the 'IlluminaHumanMethylation450kanno.ilmn12.hg19' package (<http://bioconductor.org/packages/IlluminaHumanMethylation450kanno.ilmn12.hg19>). Differentially methylated genes were plotted with principal component analysis and violin plots. Pathway analysis of differentially methylated genes was performed using TargetMine (<http://targetmine.mizuguchilab.org/>).



Supplementary Figure 1. Differentially methylated probes are not of diagnostic utility to predict poor survival group of paired initial/recurrent glioblastoma. A) Principle component analysis of highest ranked differentially methylated genes of TCGA dataset comparing regions of relatively poor (Group 1) and better (Group 2) survival. B) Violin plots showing overlapping distribution of probe values for Group 1 versus Group 2.

Pathway	B-H adj. p-value	Genes	Pathway ID
Cell Cycle	0.006628077	ATM, CCNA1, CCND2, CDK6, CDKN1C, FKBPL, HIST1H3I, HIST1H4L, HSPA2, MDC1, NUP98, PSMB8, PSMB9, RAB1B, TUBB	R-HSA-1640170
p53 signaling pathway	0.006628077	APAF1, ATM, CCND2, CDK6, IGFBP3, PTEN	hsa04115
Antigen processing and presentation	0.006628077	HSPA1A, HSPA1L, HSPA2, TAP1, TAP2, TAPBP	hsa04612
Legionellosis	0.013984079	APAF1, HSPA1A, HSPA1L, HSPA2, RAB1B	hsa05134
Cellular responses to stress	0.022219649	ATM, CCNA1, CDK6, HIST1H3I, HIST1H4L, HSPA1A, HSPA1L, HSPA2, NUP98, PSMB8, PSMB9	R-HSA-2262752
Regulation of HSF1-mediated heat shock response	0.02632611	ATM, HSPA1A, HSPA1L, HSPA2, NUP98	R-HSA-3371453
Attenuation phase	0.02632611	HSPA1A, HSPA1L, HSPA2	R-HSA-3371568
Cell Cycle, Mitotic	0.02632611	CCNA1, CCND2, CDK6, CDKN1C, FKBPL, HIST1H3I, HIST1H4L, NUP98, PSMB8, PSMB9, RAB1B, TUBB	R-HSA-69278
Transcriptional regulation by RUNX1	0.031795388	CCND2, CDK6, ESR1, GATA3, HIST1H3I, HIST1H4L, PSMB8, PSMB9	R-HSA-8878171
ER-Phagosome pathway	0.038987293	PSMB8, PSMB9, TAP1, TAP2, TAPBP	R-HSA-1236974
Generic Transcription Pathway	0.038987293	APAF1, ATM, BRD2, CCNA1, CCND2, CDK6, ESR1, GATA3, HIST1H3I, HIST1H4L, IGFBP3, MDC1, NOTCH4, PSMB8, PSMB9, PTEN, THRB, ZNF311	R-HSA-212436
Cellular response to heat stress	0.040462015	ATM, HSPA1A, HSPA1L, HSPA2, NUP98	R-HSA-3371556
Cellular responses to external stimuli	0.040462015	ATM, CCNA1, CDK6, HIST1H3I, HIST1H4L, HSPA1A, HSPA1L, HSPA2, NUP98, PSMB8, PSMB9	R-HSA-8953897
Direct p53 effectors	0.040462015	APAF1, BCL2, CAV1, IGFBP3, PTEN, TAP1	p53downstream pathway
Hepatitis B	0.044881948	APAF1, ATF6B, BCL2, CCNA1, CDK6, PTEN	hsa05161
Mitotic G1-G1/S phases	0.047027426	CCNA1, CCND2, CDK6, CDKN1C, PSMB8, PSMB9	R-HSA-453279

Supplementary Table 1. Significantly impacted biologic pathways as determined by differentially methylated genes comparing regions of poor versus better survival groups.