Genomic predictors of patterns of progression in glioblastoma and possible influences on radiation field design

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Abstract

We present a retrospective investigation of the role of genomics in the prediction of central versus marginal disease progression patterns for glioblastoma (GBM). Between August 2000 and May 2010, 41 patients with GBM and gene expression and methylation data available were treated with radiotherapy with or without concurrent temozolomide. Location of disease progression was categorized as within the high dose (60 Gy) or low dose (46 Gy) volume. Samples were grouped into previously described TCGA genomic groupings: Mesenchymal (m), classical (c), proneural (pn), and neural (n); and were also classified by MGMT-Methylation status and G-Cimp methylation phenotype. Genomic groupings and methylation status were investigated as a possible predictor of disease progression in the high dose region, progression in the low dose region, and

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Compliance with ethical standards

Conflicts of interest None.

time to progression. Based on TCGA category there was no difference in OS (p = 0.26), 60 Gy progression (PN: 71 %, N: 60 %, M: 89 %, C: 83 %, p = 0.19), 46 Gy progression (PN: 57 %, N: 40 %, M: 61 %, C: 50 %, p = 0.8) or time to progression (PN: 9 months, N:15 months, M: 9 months, C: 7 months, p = 0.58). MGMT methylation predicted for improved OS (median 25 vs. 13 months, p = 0.01), improved DFS (median 13 vs. 8 months, p = 0.007) and decreased 60 Gy (p = 0.003) and 46 Gy (p = 0.006) progression. There was a cohort of MGMT methylated patients with late marginal disease progression (4/22 patients, 18 %). TCGA groups demonstrated no difference in survival or progression patterns. MGMT methylation predicted for a statistically significant decrease in in-field and marginal disease progression. There was a cohort of MGMT methylated patients with late marginal progression. Validations of these findings would have implications that could affect radiation field size.

Keywords

Glioblastoma; Genomics; Patterns of progression

Introduction

Recent advances in the understanding of glioblastoma (GBM) have revealed several genomic classes with distinct genetic expression patterns [1]. Clinical correlations demonstrated that only one of these classes, the proneural subtype with *IDH1* mutation has prognostic significance. Much less is known, however, of how genomic subtypes of GBM may affect the clinical behavior of these tumors. Recent data has suggested patients with the proneural GBM subtype have tumors that are more resectable [2, 3].

Genetic variability can affect a number of clinical variables in patients with GBM including responsiveness to therapy, likelihood of pseudoprogression, and the ability to form multiple distinct foci. Traditionally, GBM has demonstrated a fairly consistent progression pattern, with the great majority of tumors recurring within the highest dose radiation volume, and within 2 cm of the index tumor [4]. Since the advent of temozolomide, multiple studies have identified a cohort of longer-term survivors that have been found to progress outside the highest dose radiation region [5, 6]. The variation from the traditional progression pattern in these prolonged survivors suggests that the initial radiotherapy volume may have insufficiently treated occult disease in certain patients. If these patients could be identified in the upfront setting prior to the radiation treatment planning stage, patients who are destined to progress marginally could be treated preemptively with larger treatment volumes to potentially better encompass microscopic disease and thereby delay recurrence.

To this end, a single institution retrospective review was performed comparing the genetic expression patterns of tumors with different patterns of progression. The goal of the analysis was to determine if there were specific genetic profiles including TCGA classification, glioma-CpG island methylator phenotype (G-cimp) or hypermethylation of the O6-methylguanine-DNA-methyltransferase (MGMT) which predicted for delayed treatment progression outside of the original high dose radiation volume.

Methods

Data acquisition

This study was approved by the Wake Forest School of Medicine Institutional Review Board. Our departmental database was searched for patients with a diagnosis of GBM who were treated with radiation therapy. Between August 2000 and May 2010, 161 patients with GBM were treated with radiotherapy using modern treatment planning techniques. Of this group, 51 patients had pre-treatment tissue available in the tumor tissue bank. Of 51 patients for which sample existed in the tumor bank, 41 patients had sufficient tissue for analysis. Patient characteristics for this population study are summarized in Table 1.

Radiotherapy

Patients were treated at the Wake Forest University Comprehensive Cancer Center using a shrinking field technique. The clinical target volume (CTV) margins used for individual patients were based upon physician discretion unless the patient was enrolled on a clinical trial, in which case the CTV margins were dictated by the trial guidelines. CTV margins for this analysis varied from 0.5 to 2.0 cm. Planned treatment volume (PTV) margins were 5 mm. Intensity modulated radiation therapy was performed on 23 (55 %) patients in this cohort, and was generally used in cases where treatment volumes were in close proximity with the optic apparatus or brainstem. Radiation dose was 46 Gy prescribed to the pre-boost clinical target volume CTV, which included peritumoral edema and CTV margin. The boost volume received 60 Gy and included residual enhancing tumor and resection cavity with a CTV margin.

Patient follow-up and response assessment

Patients were followed clinically by a multidisciplinary team and with serial MRI. Imaging was generally performed in the immediate post-operative setting, 1 month after completion of radiation therapy, and then every 2 months for the first six months after completion of radiation therapy. Subsequent imaging was performed every 3 months, unless patients developed new or progressive symptoms warranting an earlier scan. Electronic medical records and imaging review were used to retrospectively determine date of initial progression using the response assessment in neuro-oncology (RANO) criteria published by Wen et al. [4]. Treatment planning data were fused to MRI at date of progression using the Pinnacle treatment planning system (Philips Healthcare, Andover, MA). Location of treatment progression was categorized as within the 60 Gy volume, within the 46 Gy volume or as distant progression (beyond the 46 Gy volume). Patients were considered to have progression in both the 60 and 46 Gy volumes if MRI on the date of progression demonstrated new or worsening areas of enhancement that were both enclosed by the 60 Gy isodose line, and between the 60 and 46 Gy isodose lines.

Genomic analysis

Fresh-frozen tumor tissues from 51 subjects were available for genomic analyses using Affymetrix Human Exon 1.0 ST GeneChips (Affymetrix, Santa Clara, CA) and Infinium HumanMethylation (HM) 450 BeadChip from Illumina (Illumina, San Diego, CA). Tissue

banking was standardized at our institution in 2008, and tissue obtained prior to that was done so more sporadically and was based upon neurosurgeon participation. Tissue banking was generally not pursued in patients undergoing stereotactic biopsies because of insufficient quantity of tissue. Genomic DNA and total RNA were isolated from these tumor samples using a QIAGEN DNA purification kit and TRIzol Reagent from Invitrogen, respectively, according to manufacturer's instructions. The quality of genomic DNA and total RNA was evaluated as described previously [7].

We purchased all reagents and carried out transcriptome and genome-wide methylation assays following manufacturer's instructions. Raw data generated from the exon GeneChips were first analyzed and evaluated using Affymetrix Expression Console. The data sets passed quality controls and were then imported into Partek Genomics Suite 6.6 software and Quantile-normalized. Pre-background was adjusted for GC content and for probe sequence. Log 2 probe intensities were finally analyzed for association between gene expression and clinical outcomes.

These 41 tumors were clustered into clinically relevant gene expression-based molecular classifications as previously described by The Cancer Genomic Atlas (TCGA) project including the proneural (PN), mesenchymal (M), neural (N), and classical (C) subgroups [8]. Hierarchical clustering with average linkage and a panel of 840 genes with high predictive values was performed. Raw data generated from the HM450 BeadChip were obtained using GenomeStudio software (Illumina). Color balance adjustment, simple scaling normalization and data analysis were performed using the Bioconductor lumi package [9]. The b (methylation) value was calculated as described previously [10]. DNA methylation profiles for these 41 samples were evaluated for methylation of the O6-methylguanine-DNA methyltransferase (MGMT) gene, and for G-Cimp methylation phenotype as previously described [11, 12].

Statistics

Patterns of progression, time to progression and overall survival were compared based on genomic subgroups, and MGMT methylation status. G-CIMP methylation status was excluded from analysis due to very small number of patients. Kaplan–Meier analysis was used to generate survival curves for time to progression and overall survival. Log rank test was used to compare differences between survival curves. The chi square and fisher's exact tests were used to determine differences between genomic groupings and methylation states. All statistics were performed using STATA.

Results

Genomic groupings

Genomic subtyping data is summarized in Table 1. Of the 41 patients with gene expression data available, 21 (51 %) were found to be of the mesenchymal subgroup. Forty-one patients had methylation data available, and of these, 22 (54 %) were MGMT-methylated, and 5 (12 %) patients were found to have the G-Cimp methylation phenotype.

Survival

Median overall survival for the entire cohort was 17 months. There was no detectable difference in overall survival based on TCGA subtype ($p = 0.26$). Overall survival also did not differ by G-Cimp methylation phenotype ($p = 0.34$). Figure 1 depicts Kaplan-Meier plots for overall survival based on MGMT-methylation status, showing that patients with methylated MGMT had significantly improved overall survival (median 13 vs. 25 months, $p = 0.01$).

Progression pattern

Of 41 total patients, 36 (88 %) suffered an intracranial progression at a median time of 8 months. Time to progression did not differ significantly by TCGA grouping (PN: 9 months, N:15 months, M: 9 months, C: 7 months, $p = 0.58$). MGMT-methylated patients were found to have significantly longer time to progression (median 13 vs. 8 months, log rank $p = 0.007$).

Progression in the 60 Gy volume did not differ significantly by TCGA group (PN: 71 %, N: 60 %, M: 89 %, C: 83 %, $p = 0.19$). Progression in the 46 Gy volume also did not differ by TCGA group (PN: 57 %, N: 40 %, M: 61 %, C: 50 %, $p = 0.8$) (Fig. 2). MGMT-methylated patients were less likely to have 60 Gy progression (65 vs. 90 %, $p = 0.003$) and 46 Gy progression (47 vs. 63 %, $p = 0.006$). Overall MGMT-methylated patients were more likely to have late progression >12 months, and there was a cohort of MGMT methylated patients ($n = 4$, 18 %) who survived >12 months and had progression involving the 46 Gy volume.

Radiation toxicity and salvage therapy

Use of temozolamide, salvage treatment and progression volume are shown in Table 2. Overall only three patients (7 %) developed a grade 3 toxicity as a result of radiation and five patients (12 %) developed grade 1–2 fatigue. Salvage therapy was delivered to 18 patients (43 %) (median number of salvage treatments of zero), and 8 patients (19 %) received bevacizumab at any point in their salvage course.

Discussion

The genomic classification of GBM is an evolving field with several distinct genomic groupings that appear to have very different biological behaviors, and unique genomic alterations [1]; however there is a paucity of data regarding clinical relevance of these genomic groupings, specifically as they relate to patterns of progression. Our current study presents data that would suggest there is no difference in the patterns of progression based on TCGA genomic groupings, or G-Cimp methylation status. This result is somewhat surprising given that TCGA groupings and G-Cimp methylation have been showed to affect overall survival [13], yet seem to not affect patterns of progression. Whether this lack of correlation represents a biological phenomenon or a limitation of our small sample size is unknown and will require a larger patient cohort.

The proper radiation treatment volume for patients with GBM has been a controversial topic for decades. Whole brain radiotherapy (WBRT) was once the accepted radiation treatment

volume because of the local and distant infiltrative behavior of GBM cells [14]. However, multiple series have demonstrated that in spite of WBRT, tumors generally recurred within 2–3 cm of the index tumor[15, 16]. Moreover, biopsy studies have demonstrated consistent infiltration of GBM cells into regions of peritumoral edema [17] causing many to argue for targeted inclusion of the T2 signal abnormality into the GBM treatment volume. As a result of this difference in targeting philosophies, radiation treatment volumes have evolved differently between European cooperative groups, which suggested a 2–3 cm margin around the enhancing index tumor, and those in the US including the cohort described here, which additionally targeted the T2- or FLAIR-hyperintense regions [14]. Recently, studies have also demonstrated that treating smaller margins as low as a 5 mm CTV may also be safe [6, 18].

Perhaps the most intriguing observation of the present study was the cohort of MGMT methylated patients who had low dose or out-of-field progression. We found that 5 of 16 MGMT methylated patients (31 %) who had experienced progression did so in either the low dose region or distantly. Patients without MGMT methylation experienced some component of progression within the highest dose region in 16 out of 19 patients (85 %). There appears to be a group of MGMT methylated patients who respond well to initial treatment only to progress in the margin. MGMT is a DNA repair enzyme that when methylated becomes transcriptionally down-regulated. When MGMT levels are lower, DNA-damaging agents such as temozolomide and radiotherapy become more effective, because the tumor cells cannot repair the DNA damage as efficiently. It may be that MGMT-methylated tumors, because of their increased sensitivity to treatment, represent a population where increased radiation dose to the regions of minimal tumor spread may be more relevant because the doses to gross disease are more effective. As such, if the current data can be validated in larger cohort this might suggest that MGMT-methylated tumors represent a population that may benefits from larger high dose CTV margins. With any increase in margin size or dose there is likely to be an associated risk of side effects, including long term effects especially in those patients who are found to have a substantially longer survival (MGMT methylated patients) [19].

While our study is underpowered to statistically significantly suggest the possibility of individualized radiation fields based on biologic characteristics of the tumor, there are several other examples of biology affecting management in GBM. Multiple recent reports have suggested that the genetic subtype of GBM can affect the resectability of the tumor. Beiko et al performed IDH1 mutation sequencing and immunohistochemical analysis on 335 patients with high-grade glioma [3]. 93 % of tumors with IDH1 mutations underwent complete resection versus 67 % of wild-type tumors (p <0.001). It is not farfetched to consider that in the near future, the aggressiveness of surgical resection, the size of radiotherapy margins, and the choice of chemotherapeutic agents will be dictated by individual tumor biology.

Use of genomic analysis of GBM has thus far identified the population of IDH mutants with a clear survival advantage over other subtypes [13]. Other clinical behaviors that may be affected include multifocality [20], the presence of satellite nodules [21], and the likelihood of pseudoprogression [22]. A major disadvantage of genomic analyses of GBM behavior is

that they are generally underpowered studies because of the expense and availability of fresh frozen tissue in a tumor bank. Large prospective trials of GBM, however, are now mandating the banking of tissue for genetic analysis. As such, it is likely that radiation planning and treatment will continue to evolve such that in time, patterns of progression may be predicted prior to designing a radiation field, allowing the radiation oncologist to personalize the prescribed CTV margin.

Conclusion

TCGA groups demonstrated no difference in survival or progression patterns. MGMT methylation predicted for a statistically significant decrease in in-field and marginal progression. There was a cohort of MGMT methylated patients with late marginal progression. Validations of these findings would have implications that could affect radiation field size.

References

1. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G, Chin L. The somatic genomic landscape of glioblastoma. Cell. 2013; 155:462–477.10.1016/j.cell.2013.09.034 [PubMed: 24120142]

2. Qi S, Yu L, Li H, Ou Y, Qiu X, Ding Y, Han H, Zhang X. Isocitrate dehydrogenase mutation is associated with tumor location and magnetic resonance imaging characteristics in astrocytic neoplasms. Oncol Lett. 2014; 7:1895–1902.10.3892/ol.2014.2013ol-07-06-1895 [PubMed: 24932255]

3. Beiko J, Suki D, Hess KR, Fox BD, Cheung V, Cabral M, Shonka N, Gilbert MR, Sawaya R, Prabhu SS, Weinberg J, Lang FF, Aldape KD, Sulman EP, Rao G, McCutcheon IE, Cahill DP. IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. Neuro Oncol. 2014; 16:81–91.10.1093/neuonc/not159 [PubMed: 24305719]

4. Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. Int J Radiat Oncol Biol Phys. 1979; 5:1725–1731. [PubMed: 231022]

5. Brandes AA, Tosoni A, Franceschi E, Sotti G, Frezza G, Amista P, Morandi L, Spagnolli F, Ermani M. Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation With MGMT promoter methylation status. J Clin Oncol. 2009; 27:1275–1279.10.1200/JCO.2008.19.4969 [PubMed: 19188675]

6. Paulsson AK, McMullen KP, Peiffer AM, Hinson WH, Kearns WT, Johnson AJ, Lesser GJ, Ellis TL, Tatter SB, Debinski W, Shaw EG, Chan MD. Limited margins using modern radiotherapy techniques does not increase marginal failure rate of glioblastoma. Am J Clin Oncol. 2014; 37:177–181.10.1097/COC.0b013e318271ae03 [PubMed: 23211224]

7. Liu W, Lindberg J, Sui G, Luo J, Egevad L, Li T, Xie C, Wan M, Kim ST, Wang Z, Turner AR, Zhang Z, Feng J, Yan Y, Sun J, Bova GS, Ewing CM, Yan G, Gielzak M, Cramer SD, Vessella RL, Zheng SL, Gronberg H, Isaacs WB, Xu J. Identification of novel CHD1-associated collaborative alterations of genomic structure and functional assessment of CHD1 in prostate cancer. Oncogene. 2012; 31:3939–3948.10.1038/onc.2011.554 [PubMed: 22139082]

8. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W,

Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010; 17:98–110.10.1016/j.ccr. 2009.12.020 [PubMed: 20129251]

9. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microarray. Bioinformatics. 2008; 24:1547–1548.10.1093/bioinformatics/btn224 [PubMed: 18467348]

10. Kim JW, Kim ST, Turner AR, Young T, Smith S, Liu W, Lindberg J, Egevad L, Gronberg H, Isaacs WB, Xu J. Identification of new differentially methylated genes that have potential functional consequences in prostate cancer. PLoS One. 2012; 7:e48455.10.1371/journal.pone. 0048455 [PubMed: 23119026]

11. Bady P, Sciuscio D, Diserens AC, Bloch J, van den Bent MJ, Marosi C, Dietrich PY, Weller M, Mariani L, Heppner FL, McDonald DR, Lacombe D, Stupp R, Delorenzi M, Hegi ME. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol. 2012; 124:547– 560.10.1007/s00401-012-1016-2 [PubMed: 22810491]

12. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010; 17:510–522.10.1016/j.ccr.2010.03.017 [PubMed: 20399149]

13. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell. 2006; 9:157–173.10.1016/j.ccr.2006.02.019 [PubMed: 16530701]

14. Chan MD, Tatter SB, Lesser G, Shaw EG. Radiation oncology in brain tumors: current approaches and clinical trials in progress. Neuroimaging Clin N Am. 2010; 20:401–408.10.1016/j.nic. 2010.04.005 [PubMed: 20708554]

15. Hess CF, Schaaf JC, Kortmann RD, Schabet M, Bamberg M. Malignant glioma: patterns of failure following individually tailored limited volume irradiation. Radiother Oncol. 1994; 30:146–149. [PubMed: 8184112]

16. Wallner KE, Galicich JH, Krol G, Arbit E, Malkin MG. Patterns of failure following treatment for glioblastoma multiforme and anaplastic astrocytoma. Int J Radiat Oncol Biol Phys. 1989; 16:1405–1409. [PubMed: 2542195]

17. Hochberg FH, Pruitt A. Assumptions in the radiotherapy of glioblastoma. Neurology. 1980; 30:907–911. [PubMed: 6252514]

18. McDonald MW, Shu HK, Curran WJ Jr, Crocker IR. Pattern of failure after limited margin radiotherapy and temozolomide for glioblastoma. Int J Radiat Oncol Biol Phys. 2011; 79:130– 136.10.1016/j.ijrobp.2009.10.048 [PubMed: 20399036]

19. Peiffer AM, Leyrer CM, Greene-Schloesser DM, Shing E, Kearns WT, Hinson WH, Tatter SB, Ip EH, Rapp SR, Robbins ME, Shaw EG, Chan MD. Neuroanatomical target theory as a predictive model for radiation-induced cognitive decline. Neurology. 2013; 80:747–753.10.1212/WNL. 0b013e318283bb0a [PubMed: 23390169]

20. Paulsson AK, Holmes JA, Peiffer AM, Miller LD, Liu W, Xu J, Hinson WH, Lesser GJ, Laxton AW, Tatter SB, Debinski W, Chan MD. Comparison of clinical outcomes and genomic characteristics of single focus and multifocal glioblastoma. J Neurooncol. 2014; 119:429– 435.10.1007/s11060-014-1515-1 [PubMed: 24990827]

21. LaPlant Q, Pitter K, Young RJ, Yamada Y, Chan TA, Huse JT, Brennan CW, Beal K. Radiographic features, patterns of treatment failure, and clinical outcomes in 56 cases of molecularly subtyped glioblastoma multiforme patients. Int J of Radiat Oncol, Biol, Phys. 2014; 90:S281.10.1016/j.ijrobp.2014.05.959

22. Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, Bertorelle R, Bartolini S, Calbucci F, Andreoli A, Frezza G, Leonardi M, Spagnolli F, Ermani M. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol. 2008; 26:2192–2197.10.1200/JCO.2007.14.8163 [PubMed: 18445844]
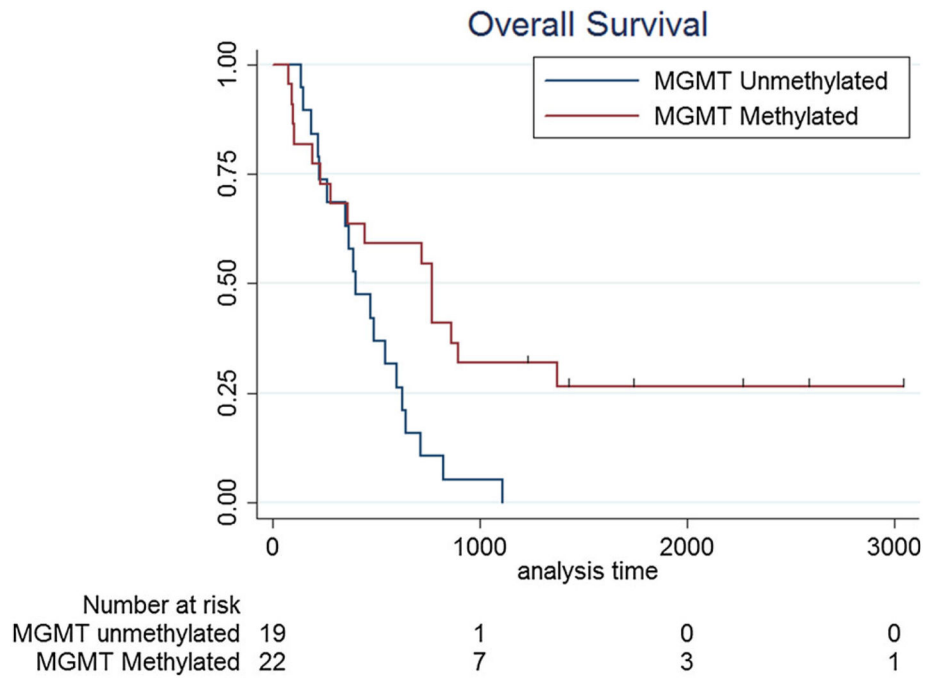
Overall Survival

Fig. 1.
Kaplan–Meier plot of overall survival based on MGMT-methylation status

Fig. 2.

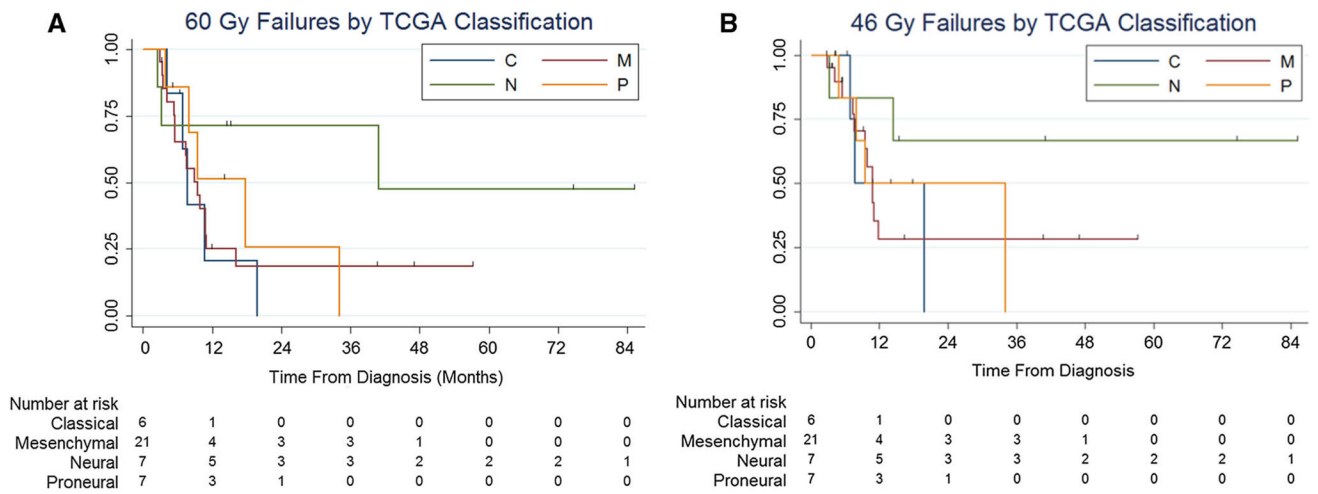**a** Kaplan–Meier plot of time to progression within the 60 Gy volume based on TCGA subtype. **b** Kaplan–Meier plot of time to progression within the 46 Gy volume based on TCGA subtype. (*C* classical, *N* neural, *M* mesenchymal, *PN* proneural)

Table 1

Patient characteristics, genomics and methylation groupings

Demographics	
Age (mean, range)	58 (33–84)
Sex	
Male	21 (50 %)
Female	21 (50 %)
Race	
White	36 (86 %)
Other	6 (14 %)
RT Modality	
3D Conformal	19 (45 %)
IMRT	23 (55 %)
Resection type	
Gross total resection (GTR)	21 (50 %)
Sub-total resection (STR)	20 (48 %)
Biopsy (bx)	1 (2 %)
Overall Survival (days)	Mean 729 Median 518 Range 77–3037
Treatment Year	2005–2010
Genomics and Methylation Groupings	
TCGA Groupings	N (%)
Mesenchymal	21 (51)
Neural	7 (17)
Proneural	7 (17)
Classical	6 (15)
MGMT Methylation	
MGMT-methylated	22 (54)
MGMT Wild Type	19 (46)
G-CIMP Methylation	
G-Cimp	5 (12)
Non-G-Cimp	36 (88)

Table 2

Concurrent temozolomide, salvage therapy and progression location by MGMT methylation status

	MGMT methylated *n* (%)	MGMT wild type *n* (%)
Total[a]	22	19
Concurrent Temozolomide	20 (91)	18 (95)
Adjuvant Temozolomide	14 (64)	14 (74)
Lines of Salvage Therapy		
0	14 (64)	9 (47)
1	4 (18)	7 (37)
2	3 (14)	2 (11)
3	1 (5)	1 (5)
Progression volume		
High dose only	5 (23)	6 (32)
Overlapping	6 (27)	10 (53)
Low dose only	2 (9)	1 (5)
Distant	3 (14)	2 (11)
No progression	6 (27)	0 (0)

[a]One patient did not have MGMT methylation status available