



Published in final edited form as:

*Clin Cancer Res.* 2014 October 1; 20(19): 5023–5031. doi:10.1158/1078-0432.CCR-14-0822.

## Phase II Study of Bevacizumab, Temozolomide and Hypofractionated Stereotactic Radiotherapy for Newly Diagnosed Glioblastoma

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### Abstract

**PURPOSE**—Bevacizumab is associated with decreased vascular permeability that allows for more aggressive radiotherapy schedules. We conducted a phase II trial in newly-diagnosed

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#### Disclosures:

Genentech provided drug and partial financial support.

The authors report the following disclosures:

Employment: L.E.A. is currently an employee of Roche.

Consulting: A.O. (Roche, Novocure, CarThera); L.M.D. (Genentech, CarThera); P.G. (Genentech); A.B.L. (Merck Sharp & Dohme/Schering Plough, BMS, Campus Bio, Cephalon, Eisai, Genentech, Roche, GSK, Imclone, Sigma Tau, Abbot, Novartis, Amgen, Kyowa Hakkō Kirin Pharma, RadMD, Stemline, Agenus).

Honoraria: A.B.L. (Merck Sharp & Dohme/Schering Plough, Omniprex)

Partial results presented at the American Society of Clinical Oncology (ASCO) Annual Meeting, 2010 (poster discussion) and Society for Neuro-Oncology Annual Meeting, 2011 (platform presentation).

**Clinical trials.gov identifier:** NCT01392209 (available at <http://clinicaltrials.gov/ct2/show/NCT01392209?term=omuro&rank=2>)

glioblastoma utilizing a novel hypofractionated stereotactic radiotherapy (HFSRT) schedule combined with temozolomide and bevacizumab.

**EXPERIMENTAL DESIGN**—Patients with tumor volume  $\leq 60$ cc were treated with HFSRT (6 $\times$ 6Gy to contrast-enhancement and 6 $\times$ 4Gy to FLAIR hyperintensity with dose painting) combined with concomitant/ adjuvant temozolomide and bevacizumab at standard doses. Primary endpoint was 1-year overall-survival (OS): Promising=70%; non-promising=50%;  $\alpha=0.1$ ;  $\beta=0.1$ .

**RESULTS**—Forty patients were enrolled (median age: 55y; methylated MGMT promoter: 23%; unmethylated: 70%). The 1-year OS was 93% (95%CI 84–100) and median OS was 19m. The median progression-free survival was 10m, with no pseudo-progression observed. The objective response rate (ORR) was 57%. Analysis of TCGA glioblastoma transcriptional subclasses (Nanostring assay) suggested patients with a proneural phenotype (26%) fared worse (ORR=14%, versus 77% for other subclasses;  $p=0.009$ ). Dynamic susceptibility-contrast perfusion MRI showed marked decreases in rCBV over time ( $p<0.0001$ ) but had no prognostic value, whereas higher baseline ADC ratios and persistent hypermetabolism at the 6-month FDG-PET predicted poor OS ( $p=0.05$  and  $0.0001$ , respectively). Quality-of-life (FACT-BR-4) and neuropsychological test scores were stable over time, although some domains displayed transient decreases following HFSRT.

**CONCLUSIONS**—This aggressive radiotherapy schedule was safe and more convenient for patients, achieving an OS that is comparable to historical controls. Analysis of advanced neuro-imaging parameters suggests ADC and FDG-PET as potentially useful biomarkers, whereas tissue correlatives uncovered the poor prognosis associated with the proneural signature in non-IDH-1 mutated glioblastoma.

### Keywords

glioblastoma; hypofractionated radiotherapy; bevacizumab; temozolomide

### Introduction

Glioblastoma, the most common and aggressive malignant glioma, is characterized not only by a short survival (1–3), but also by a particularly poor quality of life (QoL), as brain function is progressively compromised by both disease burden and effects of treatments (4,5); new therapeutic options are clearly needed.

The standard of care for newly-diagnosed glioblastoma was established in a phase III study testing focal radiotherapy (60 Gy in 2 Gy fractions over 6 weeks) with concomitant and adjuvant temozolomide versus radiotherapy alone (1). In that study, the chemoradiotherapy arm achieved a median overall survival (OS) of 15 months, compared to 12 months for radiotherapy alone. A post-hoc tissue analysis (6) suggested that tumors with promoter methylation of the DNA repair enzyme O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) had a better prognosis; in the chemotherapy arm, the median OS was 22 months for methylated versus 13 months for unmethylated tumors.

In the present study, we sought to optimize this standard first line regimen through two innovative approaches: 1) utilization of an alternative radiotherapy schedule based on

aggressive hypofractionation, made possible with the adoption of stereotactic technology and 2) addition of bevacizumab to prevent symptomatic radionecrosis and associated brain edema through decreased vascular permeability resulting from VEGF blockade (7). Exploratory correlative studies consisted of 1) advanced neuroimaging, including dynamic susceptibility contrast (DSC) MR perfusion, diffusion MRI and FDG-PET; 2) tissue correlates, including analysis of TCGA transcriptional subclasses (8,9) and expression of angiogenesis and hypoxia-related genes; and 3) evaluation of QoL and neuropsychological testing throughout treatment.

## Patients and Methods

### Patients

Main inclusion criteria (Supplementary Data 1, online) included histologically confirmed newly-diagnosed glioblastoma, tumor volume  $\leq 60$  cc (approximately 5cm maximum diameter) and age  $\geq 18$ . Exclusion criteria included multicentric disease and contra-indication to the use of bevacizumab. Therapeutic anticoagulation (e.g. for venous thromboembolism) was allowed.

### Treatment

The study treatment schema is shown in Figure 1. Starting 4–6 weeks from surgery, the hypofractionated stereotactic radiotherapy (HFSRT) was delivered in 6 treatments over two weeks, typically on a Monday, Wednesday, Friday schedule, as detailed in Supplementary Data 2 (online). Areas with contrast enhancement received  $6 \times 6$  Gy and areas with FLAIR hyperintensity received  $6 \times 4$  Gy, with dose painting for homogeneous dose distribution.

Chemotherapy concomitant with HFSRT consisted of bevacizumab 10mg/kg IV on days 1 and 15, and temozolomide 75mg/m<sup>2</sup> given orally daily throughout HFSRT, for a total of 2 weeks. Supportive anti-emetic therapy (e.g. ondansetron) and pneumocystis prophylaxis were recommended. Corticosteroids were given at the discretion of the treating physician.

Adjuvant chemotherapy started approximately 3 weeks after HFSRT, consisting of 28-day cycles of temozolomide given at conventional doses (150–200 mg/m<sup>2</sup>) on days 1–5, combined with bevacizumab 10 mg/kg on days 1 and 15. A total of 6 adjuvant cycles were mandated per protocol; treatment continuation beyond 6 cycles was left to the treating physician's discretion. Weekly CBCs, and monthly comprehensive metabolic panel and urine/ plasma creatinine (UPC) ratio were obtained.

### Advanced Neuro-Imaging Evaluation

In addition to standard MRI, all patients underwent DSC perfusion MRI at baseline, as well as post-radiotherapy (pre-adjuvant chemotherapy), and every 2 months thereafter. Supplementary Data 3 (online) details imaging processing and acquisition parameters. Commercially available software (FuncTools 2.6.9, GE Healthcare) was used to determine perfusion parameters such as relative cerebral blood volume (rCBV), relative peak height (rPH) and peak signal recovery (PSR), as well as apparent diffusion coefficient (ADC). In

addition, a dedicated brain FDG-PET scan was obtained after 6 adjuvant cycles for characterization of disease activity and correlation with outcome.

### Tissue Correlates

As detailed in Supplementary Data 4, TCGA glioblastoma transcriptional subclasses (8) (proneural, mesenchymal, classical and neural) were assessed from mRNA extracted from formalin-fixed paraffin-embedded tissue obtained at initial surgery, using a gene expression profiling assay (Nanostring nCounter, Seattle, WA) based on 81 genes selected from the initial TCGA publication (8). Genes for which expression levels are known to directly reflect copy number alterations (Supplementary Data 4) were also added to the probe set, along with VEGF target genes (*ANGPTL4*, *CA-9*, *EGLN-3*, *GLUT1* and *PDK1*).

In a separate analysis, tumor MGMT promoter methylation status was determined utilizing real-time methylation-specific PCR, as described previously (6). *IDH1 R132H* mutation status was determined by immunohistochemistry (Dianova, Hamburg, Germany; 1:30).

### Neuropsychological evaluation

Prospective neuropsychological evaluations (10–12) were performed in consenting, progression-free, patients at baseline and four, eight and twelve months. Raw test scores were compared with published normative values according to age and education, and converted into z-scores; a z-score  $\leq -1.5$  represents impairment. Evaluated domains included Executive Function (Trail Making; Brief Test of Attention; Controlled Oral Word Association), Verbal Memory (Hopkins Verbal Learning Test-Revised) and Visual Memory (Brief Visuospatial Memory Test-Revised). QoL was evaluated with the Functional Assessment of Cancer Therapy-Brain Cancer v4 (FACT-BR-4). Fatigue was evaluated with the Functional Assessment of Chronic Illness Therapy-Fatigue Subscale 4 (FACIT-FS) and depression with the Beck Depression Inventory II (BDI).

### Statistics

The primary endpoint was survival probability at one year (1y-OS). A single stage binomial design was utilized, with 1y-OS of 70% considered promising and 50% non-promising;  $\alpha=0.1$ ;  $\beta=0.1$ . Forty patients were to be enrolled; if 24 were alive at one year, the regimen would be considered worthy of further study. Secondary endpoints consisted of progression-free survival (PFS), response rate (Macdonald (13) and RANO criteria (14)) and toxicity profile (NCI-CTCAE v3). Exploratory endpoints included feasibility and preliminary evaluation of the prognostic value of advanced neuroimaging parameters and tissue-based biomarkers, as well as QoL and neuropsychological evaluation. Neuropsychological test scores were summarized using descriptive statistics, and longitudinal trajectories evaluated using linear mixed models (LMMs) controlling for age and fatigue. Exact follow-up assessment times from baseline were calculated, and both linear and quadratic terms were estimated by the LMMs.

This investigation was performed after approval by the Institutional Review Board and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Informed consent was obtained from each subject or subject's guardian.

## Results

### Patient Characteristics

Table 1 shows the characteristics of the 40 patients enrolled.

### Treatment and toxicity

Treatment was generally well tolerated and followed the established toxicity profile of each drug (Supplementary Data 5, online). One patient discontinued bevacizumab because of thrombotic microangiopathy with irreversible grade 4 renal failure. One patient developed grade 4 surgical wound infection without dehiscence but was able to resume treatment. Two patients had grade 4 pulmonary embolism and one experienced a late ischemic stroke. One patient with a history of difficult to control seizures died suddenly during sleep, while on treatment; autopsy found no tumor hemorrhage or thromboembolic event. CNS bleeding was reported in two patients, both asymptomatic: One grade 1 intra-tumoral hemorrhage and one grade 1 hemorrhage in a pre-existing cavernoma, in a patient receiving concomitant full-dose anticoagulation.

A total of 11 patients elected to continue treatment beyond the required 6 adjuvant cycles. Those patients received a median of 12 cycles in total (range: 7–23 cycles).

### Efficacy

Thirty-seven patients were alive after one year (1-yr OS: 93% [95%CI 84–100]), meeting the study's primary endpoint (Figure 2A). The median OS was 19 months (95%CI 15–23). The median follow-up of survivors was 42 months. The objective response rates (complete + partial responses) were 87% (Macdonald) and 57% (RANO) (Table 2). The median PFS was 10 months (95%CI 8–11) and 1-year PFS=28% (95%CI 14–41); the results were identical using RANO criteria, with median PFS of 10 months (95%CI: 8–11) and 1-year PFS of 28% (95%CI 14–41)(Figure 2B). Younger patients (age < 50) survived longer (median 26 months) than patients aged >50 (median 17 months,  $p=0.02$ , Figure 2C). Patients undergoing gross total resection achieved median OS of 26 months, as compared to 16 months in patients undergoing partial resection or biopsy ( $p=0.01$ ).

No patient developed worsening of symptoms or radiographic pseudoprogression within the first four months following radiotherapy. In patients free of progression, the KPS was maintained throughout treatment, and the use of corticosteroids significantly decreased over time ( $p < 0.0001$ ; Figure 3B). The mean daily dexamethasone dose at baseline was 2.8 mg; following radiotherapy: 1.5 mg; at 6 months: 0.2 mg. Three patients thought to have tumor progression manifest by increased FLAIR hyperintensity without increased contrast enhancement underwent surgical resection; on pathology, tumor progression was confirmed in one patient, and two had mostly necrotic tissue.

The pattern of radiographic progression (15) was determined in 29 patients during the trial. Among those, 25 (86%) had local progression, defined as focus of enhancing or nonenhancing tumor at, or within 3 cm of the primary site resection cavity. Four patients (14%) had distant progression, defined as new single focus of enhancement or FLAIR,

developing more than 3 cm from the primary site. No patient displayed the so called diffuse pattern of progression (15) defined as recurrence centered or extending more than 3 cm from the primary site with poorly defined margins, or a multifocal pattern.

### Exploratory Tissue Correlates

MGMT promoter methylation status could be determined in 37 patients, and was methylated in 23% and unmethylated in 70%. There was no association between MGMT status and response ( $P=1.0$ ), PFS ( $P=0.39$ ) or OS ( $P=0.56$ , Figure 2D).

The transcriptional glioblastoma subclass was determined in 31 patients (Table 3): Proneural: 26%; mesenchymal: 42%; classical: 29%; and neural: 3%. Patients with proneural tumors had lower response rate compared to other phenotypes ( $P=0.009$ ), and survived a median of 15m, as compared to 21m for other phenotypes ( $P=0.56$ ). There were no statistically significant differences in age distribution according to glioblastoma subclass or MGMT promoter methylation.

Among the 25 patients with sufficient tissue for immunohistochemistry, none displayed the IDH-1 R132H mutation, including all of the evaluated proneural tumors.

The expression levels of angiogenesis and hypoxia-related genes had no prognostic value. Similarly, we found no correlations between inferred copy number alterations based on gene expression data and patient outcome.

### Exploratory Imaging Correlates

Analysis of DSC MR perfusion and DWI parameters are shown in Figure 3A and Supplementary Data 6 (online). Initiation of treatment resulted in a progressive reduction in mean rCBV over time: Mean rCBV was 3.4 at baseline, versus 2.3 after radiotherapy ( $p<0.0001$ ), 1.5 after 2 months, and 1.4 after 4 months. However, DSC MR perfusion parameters such as baseline rCBV, change in rCBV after radiotherapy, rPH and PSR did not predict PFS or OS. Conversely, lower baseline ADC was associated with prolonged OS ( $HR=0.25$ ;  $p=0.05$ ), but not PFS (RANO  $p=0.13$ ). The association between ADC ratios and OS was maintained even after patients with a complete resection were excluded ( $p=0.03$ ). There was no association between ADC parameters and response.

The presence of hypermetabolism on FDG-PET performed at the 6-month time point was associated with poor overall survival ( $p<0.0001$ , Figure 2E). Patients with tumors displaying hypermetabolism on the 6-month FDG-PET survived a median of 7 months from the date of the exam, as compared to 14 months in patients without hypermetabolism.

### Exploratory Neurocognitive Findings

A total of  $N=37$  (93%) patients agreed to undergo neuropsychological evaluations (Supplementary Data 7, online). At baseline, cognitive test z-scores were in the low-average to average range on most tests. The LMMs showed significant linear improvement in verbal fluency over time (COWA;  $p=0.001$ ). There were no changes in grapho-motor speed (TMTA), attention (BTA) or visuospatial delayed recall (BVMT-R-DEL). After decreasing at the 4-month assessment, verbal learning (HVLT-R-TL) and verbal recognition memory

(HVL-T-R-DI) scores improved on subsequent evaluations to baseline levels or higher (quadratic time  $p=0.01$  and  $0.08$ , respectively). Cognitive flexibility scores (TMTB) decreased at the 4-month assessment (linear time  $p=0.023$ ) before somewhat stabilizing (quadratic time  $p=0.07$ ) below baseline levels at the 8 and 12-month assessments. Visuospatial learning (BVMT-R-TL) was stable at 4-months, with a possible indication of decline at subsequent follow-ups (quadratic time  $p=0.08$ ). There were no significant changes in mood, self-reported QoL or fatigue across the study period.

## Discussion

In this phase II trial, patients with newly diagnosed glioblastoma were treated with a hypofractionated radiotherapy schedule in combination with bevacizumab and temozolomide, and achieved a 1y-OS of 93%, median OS of 19 months and median PFS of 10 months. As compared to standard chemoradiotherapy, this regimen constituted a more convenient treatment option for patients, given the decreased use of corticosteroids and a notably shorter radiotherapy schedule, consisting of 6 treatments within two weeks, instead of the standard 30 treatments delivered daily over 6–7 weeks.

The value of adding bevacizumab to standard chemoradiotherapy for newly diagnosed glioblastoma is currently a matter of debate. Prospective phase II studies testing this combination reported a median PFS of 10–14 months and median OS of 19–21 months (16,17). More recently, preliminary results of two phase III trials examining standard chemoradiotherapy with or without bevacizumab, AVAGLIO (18) and RTOG 0825 (19), suggested that adding bevacizumab resulted in improvements in PFS (AVAGLIO: 11 vs 6 months; RTOG 0825: 11 vs 7 months), but not in OS (AVAGLIO: 17 months in both arms; RTOG 0825: 16 months in both arms).

While meaningful anti-tumor effects and survival benefit with anti-VEGF therapies remain to be demonstrated in glioblastoma, the effects on vascular permeability are unquestionable, as demonstrated by rapid reductions in contrast enhancement and peritumoral edema following treatment initiation (20). This “corticosteroid-like” effect made possible the hypofractionated schedule used in this trial, designed to produce radiobiological effects comparable to the standard regimen of 60 Gy/1.8-2Gy fractions. Advances in radiotherapy techniques, particularly IMRT and stereotactic technology, have allowed for remarkable improvements in spatial targeting, but the development of hypofractionated schedules has been limited (21). In addition to convenience, the biological rationale for hypofractionation is based on potentially enhanced direct cell kill and reduction in the accelerated tumor cell repopulation (22), although late-responding neural tissue injury and toxicity may increase. To date, the best studied use of hypofractionated or abbreviated radiotherapy schedules has been in poor prognosis populations, such as the elderly or poor performance status patients, who have been treated with biological doses that are equivalent to, or lower than standard schedules. Such studies have found median OS of 5–12 months (21,23–27), which in small randomized trials (23,25) did not appear inferior to standard schedules, although non-inferiority trial designs have not been used. Also under investigation is the use of hypofractionation and IMRT in escalation studies to deliver higher tumor biological effective doses, although an increased corticosteroid requirement and radionecrosis are

major deterrents to the wide application of such schedules (21,28–32). In our study, the addition of bevacizumab appears to have offset the development of symptoms and radiographic signs of radionecrosis typically associated with hypofractionation, decreasing the need for corticosteroids. Interestingly, pathology in some of our re-operated patients showed signs of significant tissue damage; it is possible that other patients thought to have non-enhancing tumor progression in fact had non-enhancing treatment effects. It must also be noted that results of our exploratory neuropsychological testing showed some transient radiotherapy effects on neurocognitive functions, which seemed less marked in comparison to standard fractionation schedules (33), although definitive conclusions are limited by our small patient number, lack of a control arm and confounding effects of bevacizumab. Analysis of RTOG 0825 (19) suggested that the addition of bevacizumab resulted in more frequent cognitive decline as compared to standard chemoradiotherapy, although interpretation of such findings is limited by the fact that progressing patients were not evaluated, and patients remained longer on study in the bevacizumab arm, which implies that the cognitive decline could represent unrecognized tumor progression, rather than direct bevacizumab effects.

The survival curve in our trial followed the typical pattern observed in bevacizumab glioblastoma studies, with a decrease in early death rates, but no improvement in later survival endpoints in comparison to contemporary phase II historical controls, which have reported median OS of 18–19 months (34). Therefore, identifying imaging and tissue-based biomarkers and subgroups of patients who benefit from, or who are harmed by, this type of treatment remains crucial (35). We examined bevacizumab effects on tumor blood perfusion as measured by DSC-MRI, and found a nearly universal decrease in blood perfusion over time, demonstrating that the intended targeting of angiogenesis was accomplished. However, for prognostic purposes, diffusion-based parameters were more helpful. Tumors with the lowest ADCs appeared to benefit most perhaps representing highly hypoxic, or densely cell-packed phenotypes, therefore constituting optimal candidates for anti-angiogenic treatments (36). Interestingly, we found the FDG-PET at the 6-month time point to be a reliable marker of treatment failure (Figure 2E), even when other imaging modalities suggested stability. Taken together, our findings support the use of imaging techniques based on cell proliferation and metabolism, rather than perfusion and vasculature imaging, to guide future development of anti-angiogenic therapies.

Finally, we evaluated a range of potential tissue-based biomarkers implicated in glioblastoma and angiogenesis. A previous study in recurrent malignant gliomas suggested that high VEGF expression as determined by immunohistochemistry was associated with increased response to bevacizumab but did not predict survival, whereas expression of CA9 was associated with poor survival outcome (35). In our study, we focused on applying the TCGA-based transcriptional classification, under the hypothesis that the marked differences in oncogenetic and angiogenic drivers across distinct expression signatures might translate into differential responses to anti-VEGF therapy. Unexpectedly, results suggested that proneural glioblastomas were associated with worse outcomes as compared to other subclasses. Proneural glioblastomas had previously been associated with dysregulated PDGF signaling (8,37,38) and a relatively favorable prognosis (37), in sharp contrast to our findings. Subsequent work, however, suggested that the majority, if not the entirety, of this



prognostic advantage derived from the enrichment of the proneural subclass with IDH-mutant tumors, a well-known predictor of prolonged survival (8,9). None of the proneural tumors in our study expressed mutant IDH-1, which may explain their poor outcome in this group. Based on our results, a post hoc analysis of tissue collected in the AVAGLIO study has been initiated, utilizing our Nanostring-based methodology. Preliminary results showed that proneural glioblastomas without IDH-1 mutation were associated with a poor prognosis in the placebo arm, confirming our finding that this molecular signature is associated with a poor prognosis, and suggesting this is not associated with a detrimental effect of bevacizumab in these patients. In fact, additional analysis suggested that adding bevacizumab may actually improve survival in these poor prognosis patients, a finding that will require further validation in larger, prospective studies. Interestingly, in our trial, patients with mesenchymal tumors benefitted from treatment and did not fare worse than other subclasses, which is consistent with their high VEGF expression. This finding contrasts with a preliminary analysis of the RTOG 0825 study, which found that some mesenchymal-associated genes conferred a worse prognosis in patients exposed to bevacizumab (39). Further studies are needed to determine whether this discrepancy is explained by our small number of patients, differing tissue study techniques, or to an effect unique to the hypofractionated radiotherapy schedule utilized in this study.

Our study has a number of limitations. Results are applicable to unifocal tumors, with a volume of 60 cc or less. However, for the most part, our population of patients had prognostic factors comparable to historical controls (34). Many trials used as historical controls have also excluded multifocal tumors (2,19) and larger tumors are also often excluded because of poor KPS. Another limitation is that a baseline FDG-PET was not obtained, and therefore it is not possible to determine whether the hypermetabolism observed at the sixth month was already present at the time of treatment start, or if developed throughout treatment. Finally, our correlative studies and neuropsychological testing are limited by small patient numbers, and few long-term survivors. Our analyses should be regarded as exploratory and hypothesis-generating; further investigations in larger, randomized studies will be necessary to validate our findings and determine whether potential biomarkers are of a predictive or prognostic value.

In summary, we describe a new use for bevacizumab in newly diagnosed glioblastoma, capitalizing on the anti-permeability effects to develop a convenient hypofractionated radiotherapy schedule. In our hands this regimen was found to be safe, associated with minimal detrimental effects on QoL, and with survival results comparable to other regimens. Our prospective correlative studies provide new insights into the biology of bevacizumab and hypofractionated radiotherapy in glioblastoma, and uncover candidate imaging and tissue-based biomarkers with either prognostic or predictive value that warrant further investigation in adequately powered randomized studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

Research funding: K.S.P. and A.S.R. (NIH P30 CA008748); A.B.L. (Abbott, Abbvie, Aeterna Zentaris, Agenus, Amgen, Angiochem, Bayer/Onyx, BMS, Celldex, Millennium, Northwest Biotherapeutics, Novartis, Novocure, NPM Pharma, DCVax, Pfizer, Plexxicon, Roche, Theorum, Merck Sharp & Dohme/ Schering Plough, Genentech, Keryx, Sigma Tau, Astra Zeneca, Medimmune, Boehringer Ingelheim, Novartis).

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**Translational relevance**

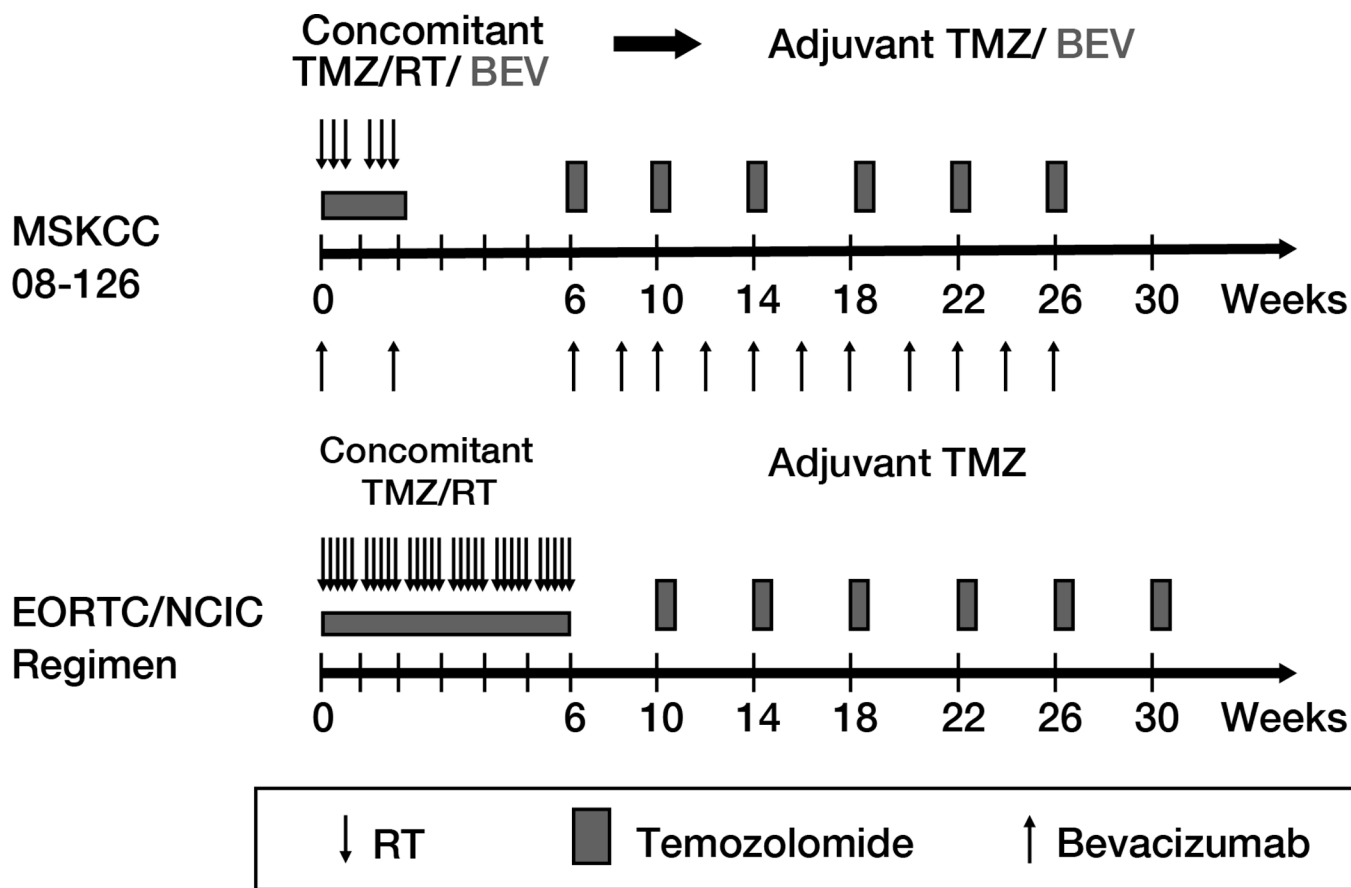
We describe a new hypofractionated radiotherapy schedule for newly-diagnosed glioblastoma, made possible through anti-permeability and corticosteroid-like effects of bevacizumab. The resulting treatment schedule (6 treatments in two weeks) is more convenient than the standard 30 treatments in 6–7 weeks, and was associated with comparable survival. Comprehensive exploratory correlative studies were performed to optimize patient selection, and provided new insights into the biology of bevacizumab and hypofractionated radiotherapy in glioblastoma. Analysis of TCGA glioblastoma transcriptional subclasses (Nanostring assay) showed patients with a proneural phenotype achieved unexpectedly poor outcomes, suggesting that in the absence of IDH-1 mutation, this phenotype is a marker of poor, and not better prognosis as previously thought. Dynamic susceptibility-contrast perfusion MRI showed marked decreases in rCBV over time, suggesting a role as a biomarker of anti-angiogenic effects. However, meaningful anti-tumor effects were better captured by imaging techniques based on cell proliferation and metabolism, such as ADC and FDG-PET, which were of prognostic value in this trial.

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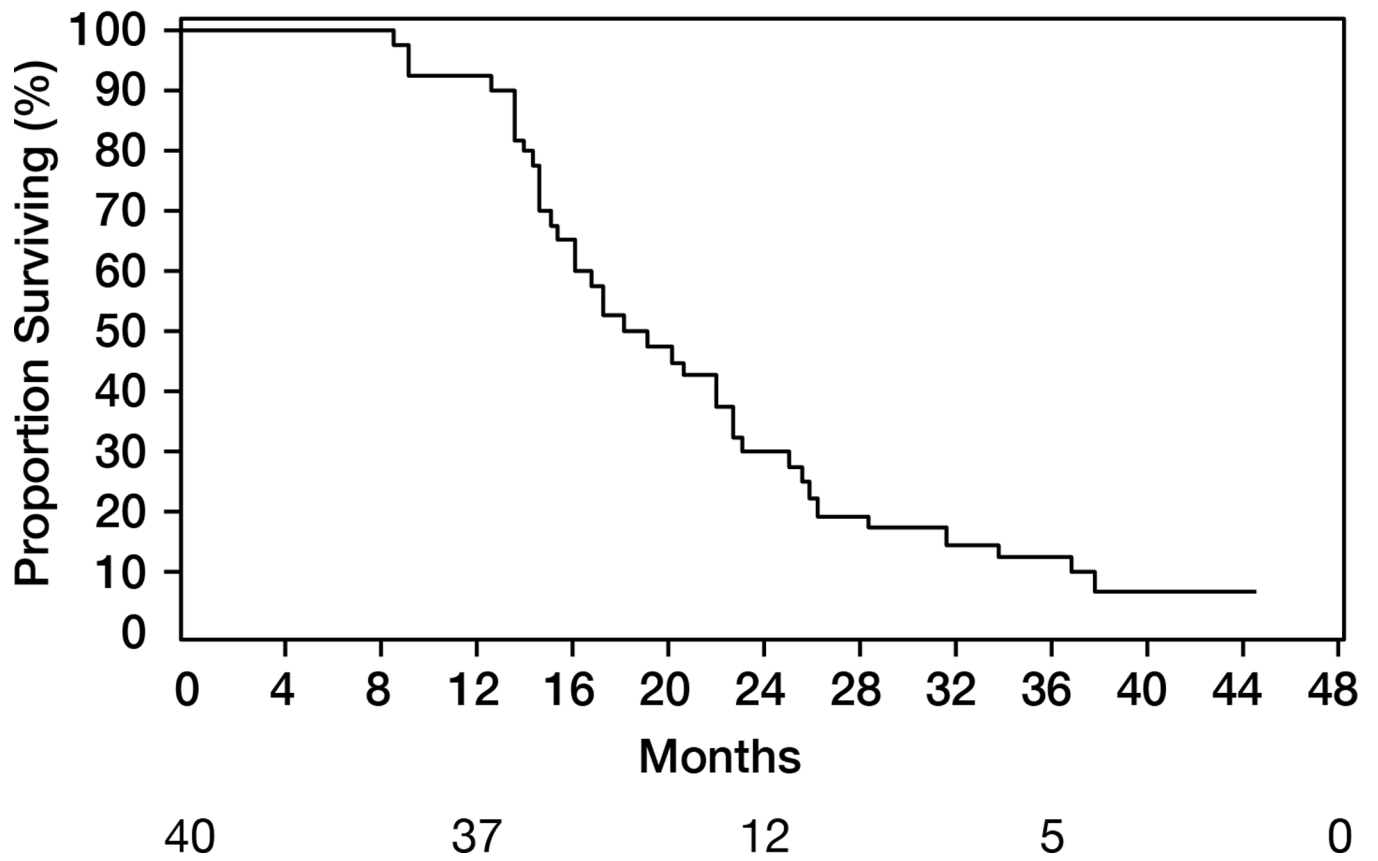
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**Figure 1.** Study treatment schema and comparison with the EORTC/ NCIC standard glioblastoma regimen (1).

A

### Overall Survival



40

37

12

5

0

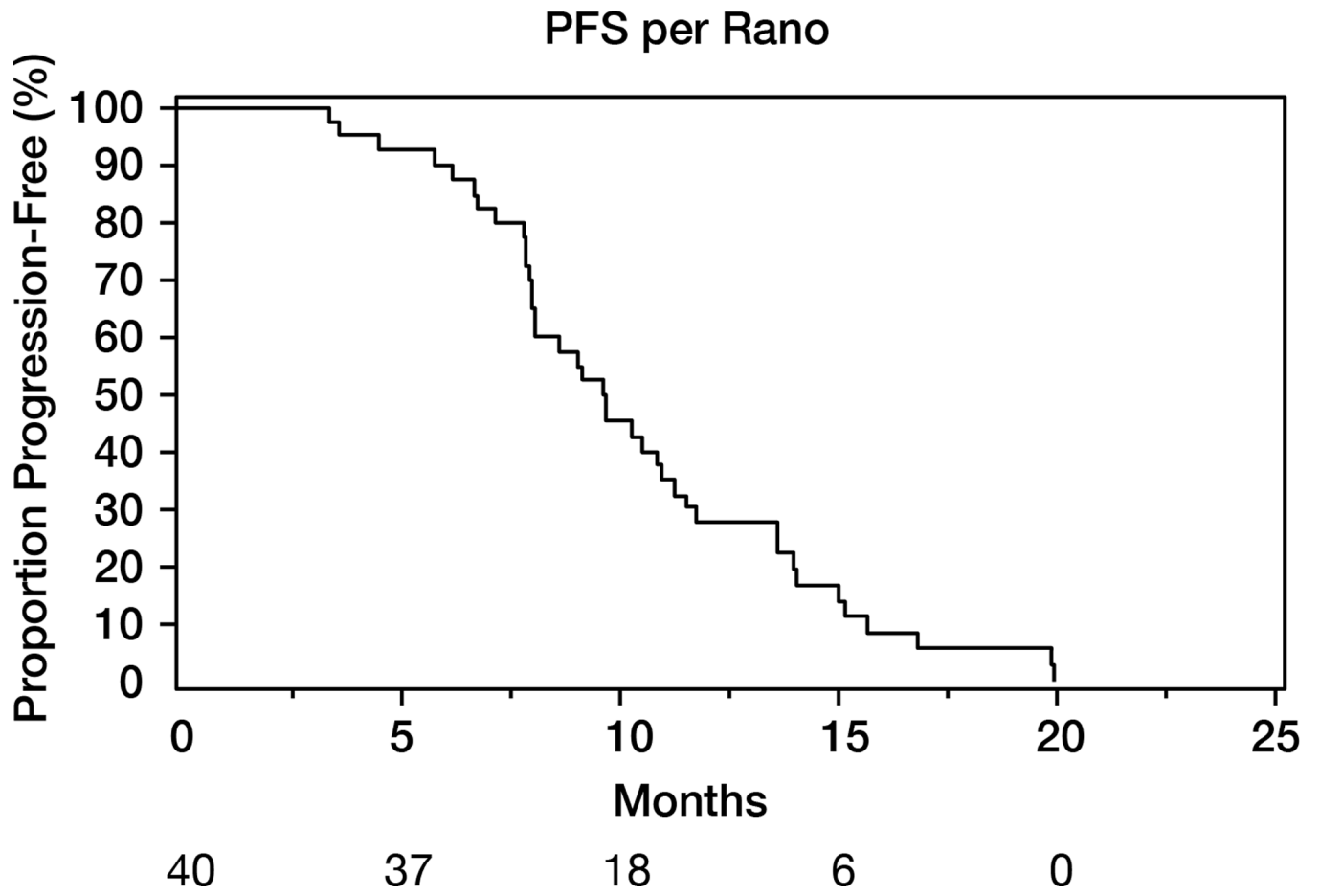
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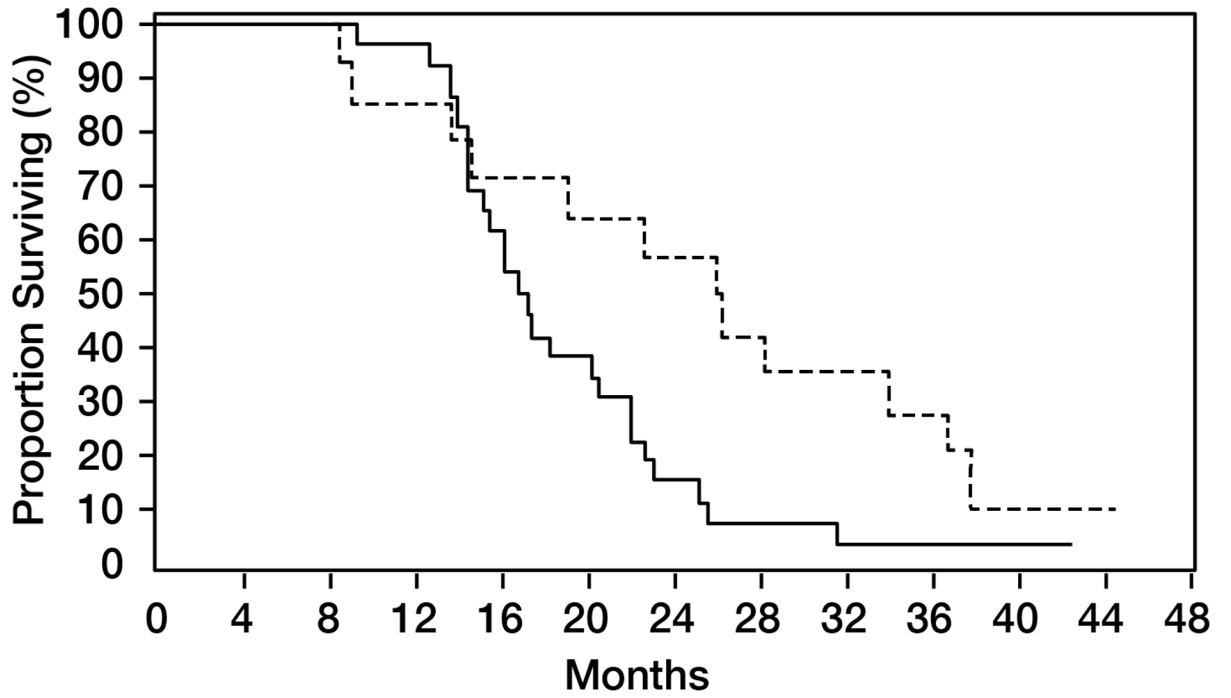
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C

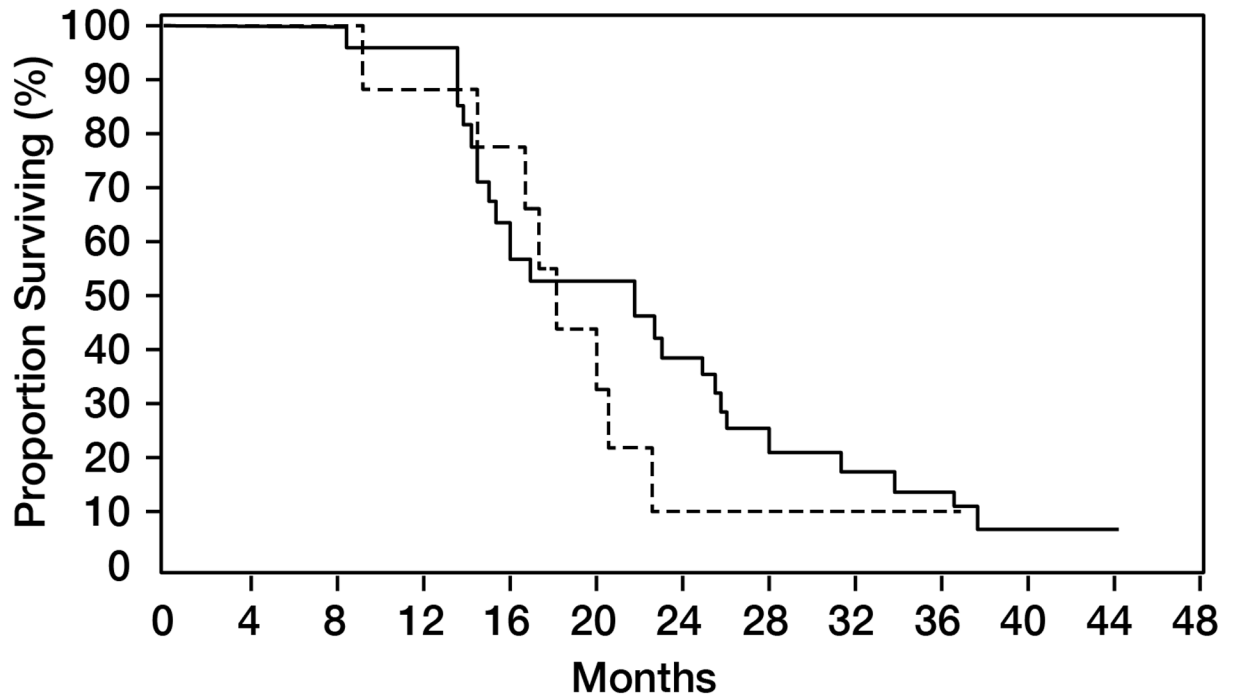
### Overall Survival by Age



Older than 50	26	26	4	1	0
50 and younger	14	12	8	4	0
	—— Older than 50		----- 50 and younger		

D

### Overall Survival by MGMT

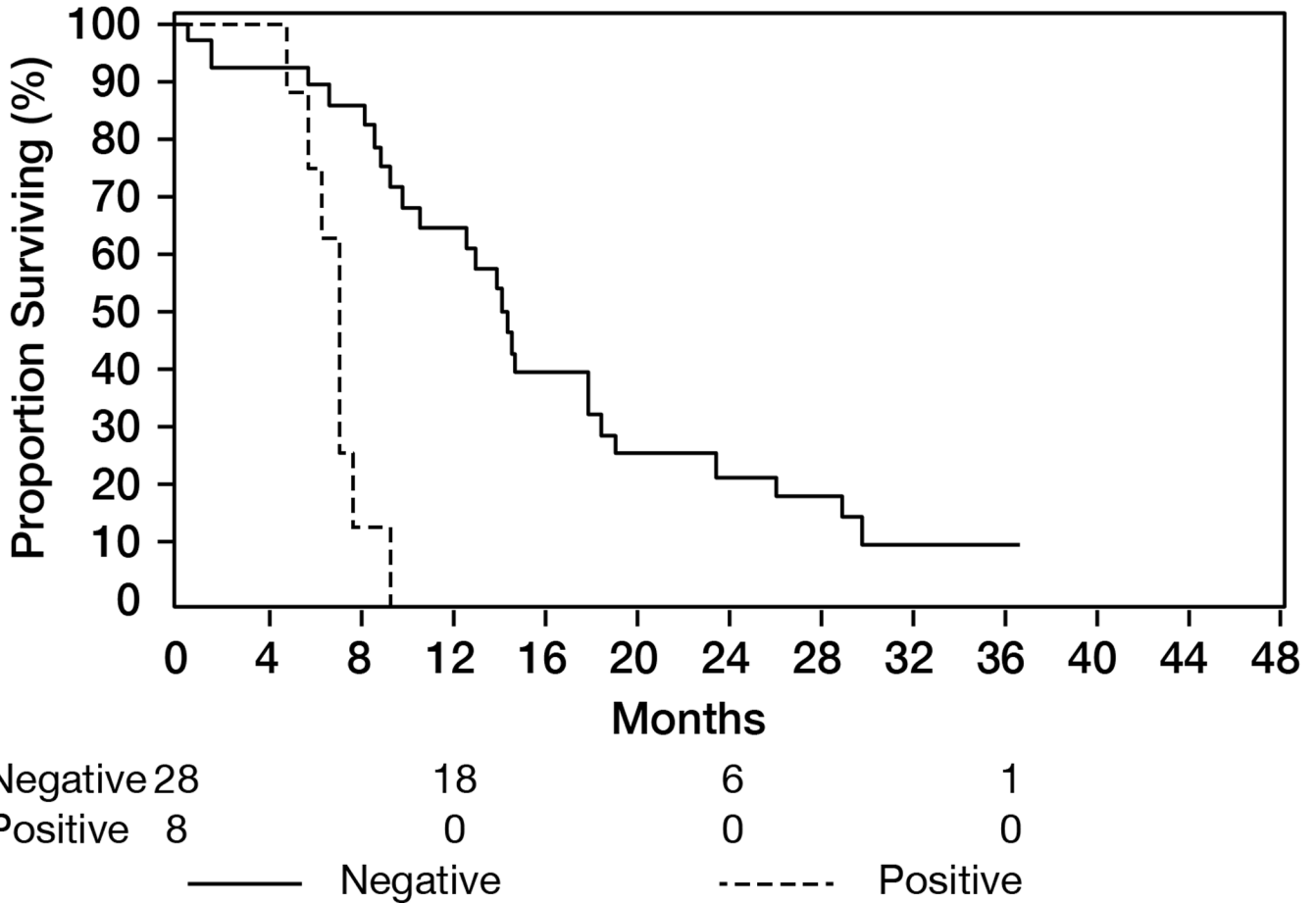


Unmethylated	28	17	11	4	0
Methylated	9	8	1	1	0

— Unmethylated      - - - - - Methylated

E

### Overall Survival by Residual Hypermetabolism on FDG-PET



**Figure 2.**

A: Overall survival (OS) for the entire population (N= 40). 1-y OS = 93% (95%CI 84–100); median OS= 19 months (95%CI 15–23). B: Progression-free survival (RANO criteria) for all patients (N=40). Median PFS= 10 months (95%CI: 8–11); 1-year PFS= 28% (95%CI 14–41). C: Overall survival in patients age>50 (median 17 months) versus age ≤ 50 (median 26 months); p=0.02. D: Overall survival by MGMT promoter methylation status. Median OS in unmethylated tumors= 22 months; median OS in methylated tumors= 18 months; p=0.56. E: Overall survival according to presence or absence of hypermetabolism in the FDG-PET performed at the 6-month time point. Median OS from date of FDG-PET was 7 months in patients with hypermetabolism, versus 14 months in patients without; p<0.0001.

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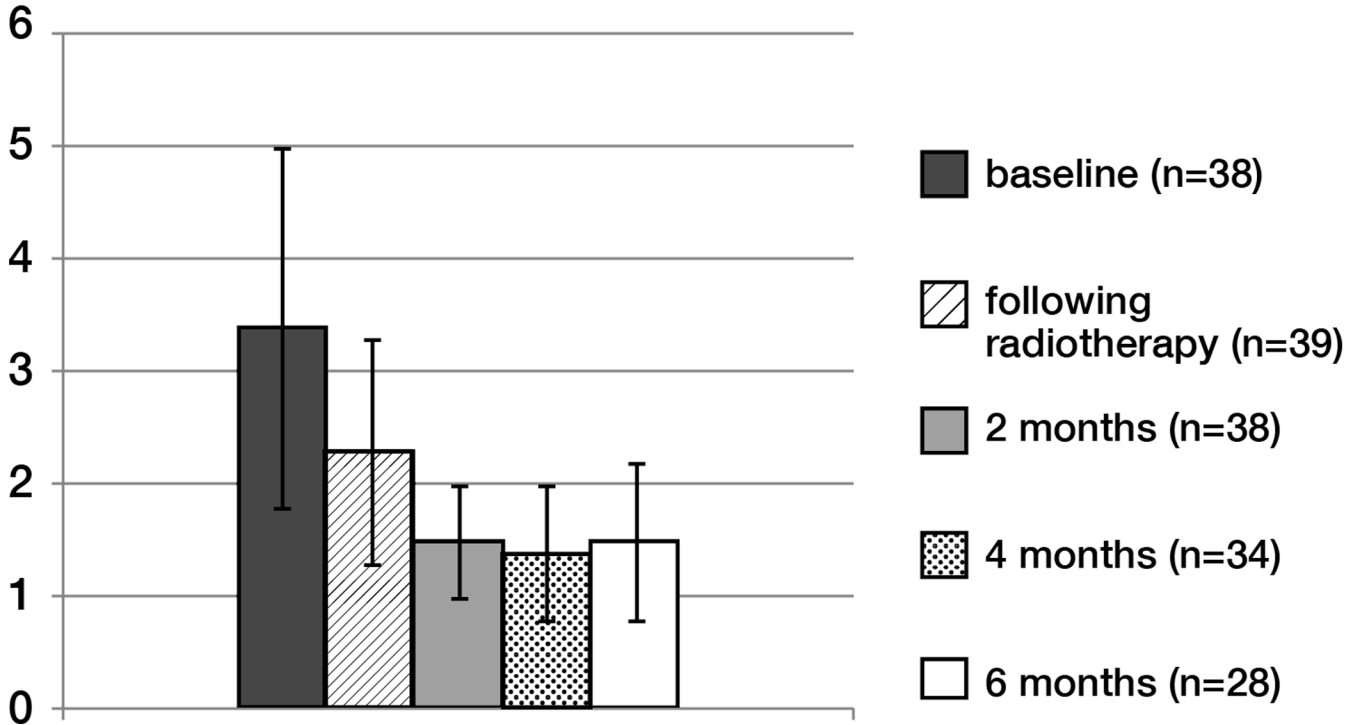
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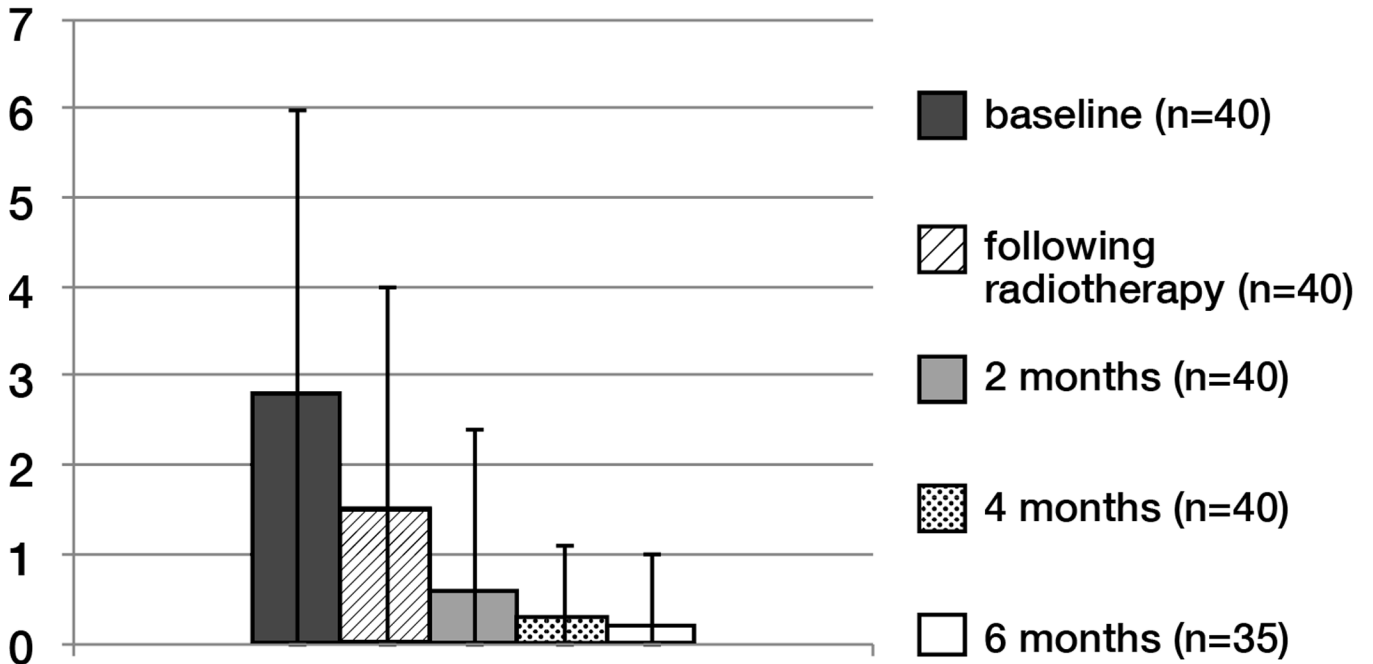
A

### Mean rCBV by MRI Timepoint



B

### Mean daily dexamethasone dose (mg)



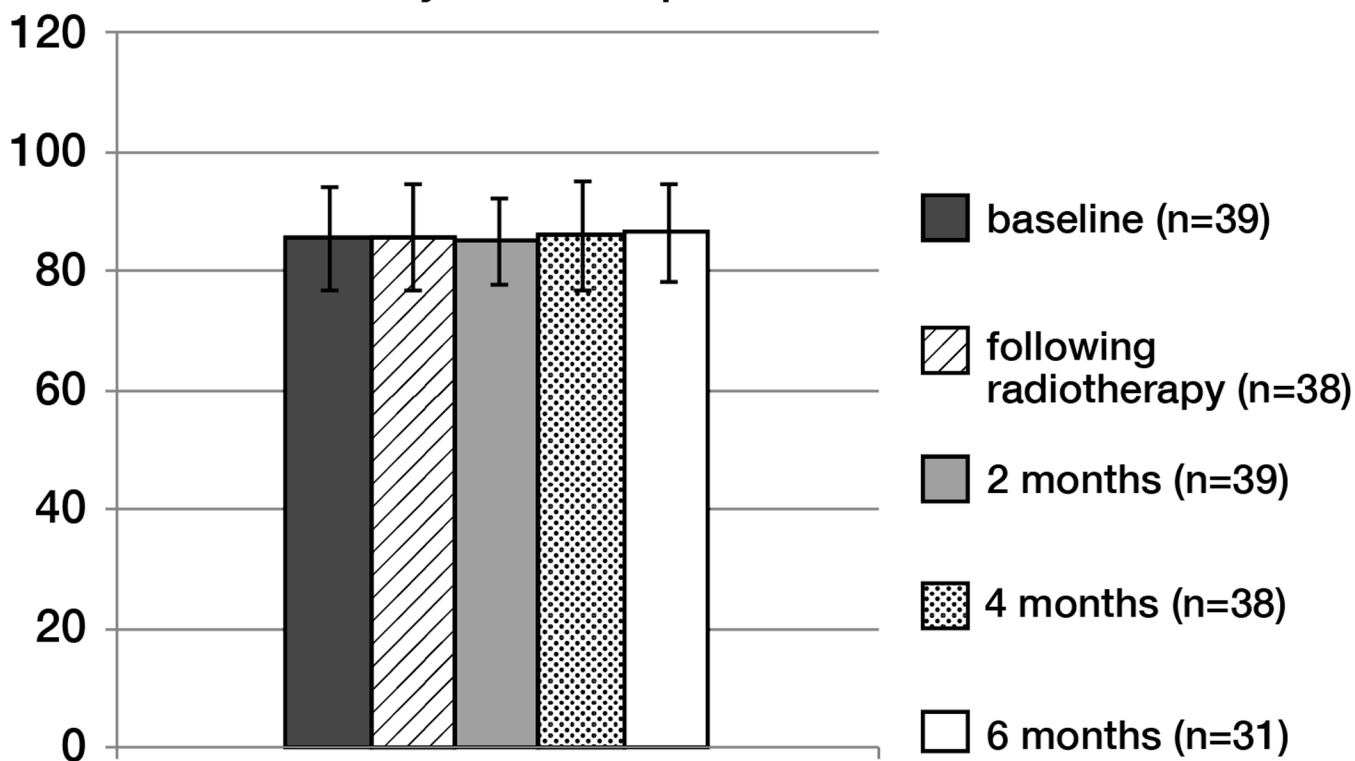
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### Mean KPS by MRI Timepoint



**Figure 3.** Mean rCBV (A), corticosteroids use (B) and KPS (C) over time (error bars: standard deviation).

A- Mean rCBV

B- Mean corticosteroid dose (mg of dexamethasone)

C- Mean KPS

**Table 1**

Patient characteristics (N=40).

Age	Median: 55 (Range: 17–75)
KPS	Median: 90 (Range: 70–100)
Women	14 (35%)
Men	26 (65%)
Complete surgical resection	10 (25%)
Partial resection or biopsy	30 (75%)
MGMT promoter methylation	
Methylated	9 (23%)
Unmethylated	28 (70%)
Unknown	3 (8%)

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**Table 2**

Response rates according to Macdonald and RANO criteria.

N=30 <sup>a</sup>	Macdonald		RANO	
	%	95% CI	%	95% CI
CR	27%	12–46	13%	4–31
PR	60%	41–77	43%	26–63
SD	7%	1–22	37%	20–56
PD	7%	1–22	7%	1–22
ORR	87%	69–96	57%	37–75

<sup>a</sup>N=10 patients had no measurable disease post-operatively and accordingly were not evaluable for radiographic response.

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**Table 3**

Distribution of transcriptional glioblastoma subclasses and corresponding age, response rate (RANO) and overall survival

	<b>N (%)</b>	<b>Median age</b>	<b>Objective response rate by RANO (95% CI)</b>	<b>Median overall survival</b>
Proneural	8 (26%)	62	14% <sup>a</sup> (0–53%)	15m <sup>b</sup>
Mesenchymal	13 (42%)	55	70% (35–93%)	20m
Classical	9 (29%)	58	83% (36–100%)	22m
Neural	1 (3%)	N/A	N/A	N/A
All patients with evaluable tissue	31	57	58% (37–78%)	20m

<sup>a</sup>P= 0.009 (Fisher's exact test, proneural vs others);

<sup>b</sup>P= 0.56 (Logrank test, Proneural versus others);

N/A: Not applicable (only one patient presented with this phenotype)

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