

MR spectroscopic imaging predicts early response to anti-angiogenic therapy in recurrent glioblastoma

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Abstract

Background. Determining failure to anti-angiogenic therapy in recurrent glioblastoma (GBM) (rGBM) remains a challenge. The purpose of the study was to assess treatment response to bevacizumab-based therapy in patients with rGBM using MR spectroscopy (MRS).

Methods. We performed longitudinal MRI/MRS in 33 patients with rGBM to investigate whether changes in N-acetylaspartate (NAA)/Choline (Cho) and Lactate (Lac)/NAA from baseline to subsequent time points after treatment can predict early failures to bevacizumab-based therapies.

Results. After stratifying based on 9-month survival, longer-term survivors had increased NAA/Cho and decreased Lac/NAA levels compared to shorter-term survivors. ROC analyses for intratumoral NAA/Cho correlated with survival at 1 day, 2 weeks, 8 weeks, and 16 weeks. Intratumoral Lac/NAA ROC analyses were predictive of survival at all time points tested. At the 8-week time point, 88% of patients with decreased NAA/Cho did not survive 9 months; furthermore, 90% of individuals with an increased Lac/NAA from baseline did not survive at 9 months. No other metabolic ratios tested significantly predicted survival.

Conclusions. Changes in metabolic levels of tumoral NAA/Cho and Lac/NAA can serve as early biomarkers for predicting treatment failure to anti-angiogenic therapy as soon as 1 day after bevacizumab-based therapy. The addition of MRS to conventional MR methods can provide better insight into how anti-angiogenic therapy affects tumor microenvironment and predict patient outcomes.

Key Points

- Anti-VEGF therapy limits conventional MR methods to assess treatment response.
- Using MR spectroscopy (MRS), shorter-term survivors had higher tumor Lac/NAA and lower NAA/Cho.
- MRS can identify early biomarkers of treatment failure to anti-VEGF therapy.

Importance of the Study

Patients with recurrent glioblastoma are treated with anti-angiogenic therapy to improve survival and quality of life. Conventional MR techniques have had limited efficacy in predicting treatment response. Here, we perform a longitudinal MR spectroscopy study in patients with recurrent glioblastoma on anti-angiogenic therapy to show that shorter-term survivors

have higher levels of Lac/NAA and lower levels of NAA/Cho compared to longer-term survivors at multiple time points. These results suggest that Lac/NAA and NAA/Cho may serve as early biomarkers of treatment response. Future studies may need to consider the impact of differential dosing of anti-angiogenic therapy to alter tumor physiology and improve survival.

Glioblastoma (GBM) is an aggressive primary brain tumor with a grim prognosis.¹ With radiographic recurrence characterized by new areas of contrast enhancement or T2-weighted fluid-attenuated inversion recovery (FLAIR) changes on MRI, patients are started on bevacizumab, an anti-vascular endothelial growth factor (VEGF) agent that decreases contrast enhancement via normalizing or pruning tumor vasculature.^{2,3} This medication has been shown to improve progression-free survival and quality of life compared to historical controls,^{2,4-6} but its impact on overall survivorship has been less clear. For example, one study in 310 GBM patients suggested that bevacizumab treatment did not improve survival⁷ while another study in 61 patients suggested that bevacizumab improved survival, with the median overall survival being 10.3 months for the bevacizumab-treated group and 4.2 months for the non-bevacizumab-treated group.⁸ Due to the absence of a well-established consensus with regards to bevacizumab's effects on overall survival, there is an unmet clinical need to determine responders to bevacizumab-based therapy.

Much research has focused on advanced MR methods to explore better biomarkers that differentiate pseudo-response from true treatment response after anti-angiogenic therapy.⁹ Perfusion-weighted imaging assesses tumor hemodynamics such as blood volume (CBV) and flow (CBF); these parameters can become abnormal in aggressive tumors due to malformed vasculature and are affected by anti-VEGF therapy. For example, a few studies that investigated perfusion changes in recurrent GBM (rGBM) after anti-angiogenic therapy found that early changes in CBV were predictive of survival,¹⁰⁻¹² although the findings are not consistent.¹³ The variability among findings could be because perfusion-weighted imaging does not adequately capture the underlying tumor biology after anti-VEGF therapy.

MR spectroscopy (MRS) provides clinically relevant information about the tumor microenvironment after anti-VEGF therapy independent of contrast-enhancement, FLAIR changes, and tumor hemodynamics. Data from a prospective, randomized, phase II multicenter trial evaluating bevacizumab-based therapy suggests that changes in N-acetylaspartate (NAA), a marker for neuronal integrity, and choline (Cho), a marker for tumor proliferation, are useful imaging biomarkers to assess response to anti-angiogenic therapy. In 13 patients, increases in NAA/Cho ratios at 8 weeks post-treatment compared to baseline (BL) levels were predictive for progression-free survival at 6 months (PFS-6) and overall survival at 12 months.¹⁴ Lactate (Lac) was not a focus of the study but merits

investigation in aggressive tumors that survive in a relatively hypoxic environment.¹⁵ Anti-VEGF agents can affect vascular growth, leading to alterations in lactate: higher levels may suggest more aggressive tumor growth while lower levels could suggest treatment response.

To build on our previous work by validating our data findings in a larger patient population, investigating new potential metabolic biomarkers of tumor hypoxia or necrosis (ie, Lac/NAA), and interrogating earlier time points, we performed a prospective, longitudinal study in patients with rGBM receiving bevacizumab-based therapy. We also investigated whether other metabolic biomarkers, especially Lac/NAA, were predictive of treatment outcomes and explored whether earlier time points can predict overall survival in patients with rGBM.

Material and Methods

Recruitment

This study was approved by our center's institutional review board and was conducted in accordance with the principles of the "World Medical Association Declaration of Helsinki: Research involving human subjects." Patients with rGBM were recruited in collaboration with their treating neuro-oncologist and provided written informed consent. This study was registered at clinicaltrials.gov (NCT02843230). Eligible participants were at least 18 years old with a BL KPS \geq 50. Patients had confirmed GBM pathology and recurrence with a treatment plan including bevacizumab monotherapy or combination therapy. The treating neuro-oncologist, in collaboration with a multidisciplinary tumor board, determined patient progression based on increasing tumor size and/or clinical decline. Patients underwent serial MRI scans at the following time points: BL (before bevacizumab treatment), 1–2 days, 2 weeks, 4 weeks, 6–8 weeks, and 12–16 weeks after bevacizumab initiation. The 1–2 days, 6–8 weeks, and 12–16 weeks time points are referenced as 1 day, 8 weeks, and 16 weeks in the manuscript for simplicity.

Imaging Acquisition

MRS was performed on either 3T Siemens (Siemens Healthineers) or 1.5T GE (GE Healthcare) MRI scanners and included the following sequences: FLAIR resolution = 0.85 ×

0.85 × 5 mm with 1 mm slice gap (Siemens/GE: repetition time (TR) 9000/9000 ms, echo time (TE) 80/135 ms, inversion time (TI) 2600/2200 ms), T1-weighted imaging res 0.85 × 0.85 × 5 mm with 1 mm slice gap (Siemens/GE: TR 525/475 ms, TE 9/13 ms), and high-resolution 3-dimensional magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and brain volume imaging (BRAVO) post-contrast imaging (Siemens/GE: resolution 1 × 1 × 1 mm/0.5 × 0.5 × 1 mm, TR 2300/8.4 ms, TE 2.3/3.2 ms). Patients were scanned in either GE or Siemens scanners throughout the study to minimize scanner variability.

On the 3T Siemens scanners, 3D MRS data were acquired using a Localization by Adiabatic SElective Refocusing (LASER) pulse sequence with fast SPIRAL k-space acquisition.^{16,17} Acquisition parameters include: TE = 135 ms, TR = 1700 ms, NA = 4, Matrix = 16 × 16 × 8, FOV = 160 mm, isotropic resolution 1.0 cm³, duration: 7 min. Water suppression was achieved with a modified chemical selective saturation method known as water suppression enhanced through T1 effects (WET), with automatic and subsequent manual shimming of the region of interest (ROI) prior to acquisition to minimize linewidths artifacts. Manual shimming was performed for ~30 s to 1 min.

On the 1.5T GE scanners, 2D MRS data were acquired using a product point-resolved spectroscopy (PRESS) excitation pulse sequence for signal localization with phase encoding. Acquisition parameters included TE 135 ms, TR = 1500 ms, NA = 1, phase encoding = 18 × 18, FOV = 220, resulting in nominal voxel sizes of 1.2 × 1.2 × 1 cm, duration: 8 min. Water suppression was achieved with Chemical Shift Selective (CHESS) methods, with the ROI undergoing automatic and manual shimming to minimize field inhomogeneity. On all MRS acquisitions, scalp lipid contribution was minimized, and the same ROI was selected across time points by using anatomical landmarks (eg, anterior commissure-posterior commissure line) in conjunction with staff annotations for previously selected voxels and automated software such as Auto-Align.¹⁸

MRS Data Analysis

The raw data were processed using LCModel 6.3 Software¹⁹ to quantify various brain metabolites (NAA, Creatine [Cr], Cho, and Lac). GE data were preprocessed with Spectroscopy Analysis by General Electric (SAGE) software (GE Healthcare). A clinical spectroscopist with over 18 years of experience visually inspected all spectra. Spectra with full width at half maximum (FWHM) < 15 Hz and signal to noise ratio (SNR) ≥ 2 defined as the highest peak divided by the standard deviation of the noise of the residual between the spectrum and the fit (generated by LCModel), were included in the analyses. LCModel has been shown to systematically underestimate the true SNR calculated from the recent MRS consensus paper.^{20,21} For example, the spectrum from [Figure 1F](#) has an LCModel-generated SNR of 8 but a true SNR of 21, suggesting that the true SNR for the data is much higher.

ROI Selection

The areas of tumor enhancement on the T1-weighted post-contrast image were semi-automatically segmented using

3D Slicer^{32,23} as previously described.²⁴ Areas of necrosis and resection cavity were excluded.

The MRS data were overlaid on the post-contrast T1-weighted images. Voxels were classified as contrast-enhancing tumor, nonenhancing tumor periphery, and contralateral normal appearing white matter ([Figure 1](#)). Mean metabolite ratios in those regions were calculated as previously described.¹⁴ Of note, tumor voxels identified in the BL scan were followed throughout the visits regardless of enhancement. Occasionally, voxel shifting was applied to enable better overlay of baseline scans with follow-up scans.

Statistical Analyses

Based on our previous published work,¹⁴ we focused on NAA/Cho at the 8-week time point because it had discriminated between progression-free survival at 6 months and overall survival at 1 year. We also focused on Lac/NAA since other studies have highlighted the importance of hypoxia in GBM.^{25,26} We also performed exploratory analyses on other metabolites including NAA/contralateral Cr, Cho/contralateral Cr, and Lac/contralateral Cr.

Our previous work chose a 1-year overall survival for classification. However, only 6 patients were alive at this time point, so we chose the 9-month overall survival time point (OS-9). Receiver operating characteristic (ROC) curves for NAA/Cho and Lac/NAA were constructed at OS-9; areas under the ROC curves (AUC), Kaplan–Meier curves, and all analyses, were performed using JMP Pro 15 (SAS, Singapore). A marker was considered effective when the AUC lower 95% CI was greater than 0.50. For demographic data, categorical variables underwent Chi-square testing while continuous variables underwent Mood's MedianTest for between group comparisons.

Results

Subject Demographics

Of the 45 patients enrolled in this study, 3 patients were excluded (1 withdrew, 2 missed the baseline scan). Of the 42 patients who underwent a baseline scan, 4 patients did not receive bevacizumab-based therapy as part of their treatment plan, and 2 patients did not complete serial follow-up scans. Of the remaining patients, 3 subjects were excluded from analysis because their data did not meet quality control criterion due to issues involving voxel placement (too close to skull with lipid contamination, susceptibility artifact resulting in poor SNR with inadequate shimming and water suppression). Thirty-three were included in the final data set ([Figure 2](#)). The following patients were scanned at each time point: BL (33), 1 day (13), 2 weeks (10), 4 weeks (25), 8 weeks (21), and 16 weeks (16). There were no group differences when comparing longer-term with shorter-term survivors across age, gender, race, BL KPS, and tumor genetics ([Table 1](#)). Patients received bevacizumab monotherapy (10 mg/kg every 2 weeks) or combination therapy with lomustine (90 mg/m² every 6 weeks), temozolomide (dosing regimen varied; patients either received 50 mg/m² daily, 150–200 mg/m² for 5/28 days, or 50 mg-70 mg/m²

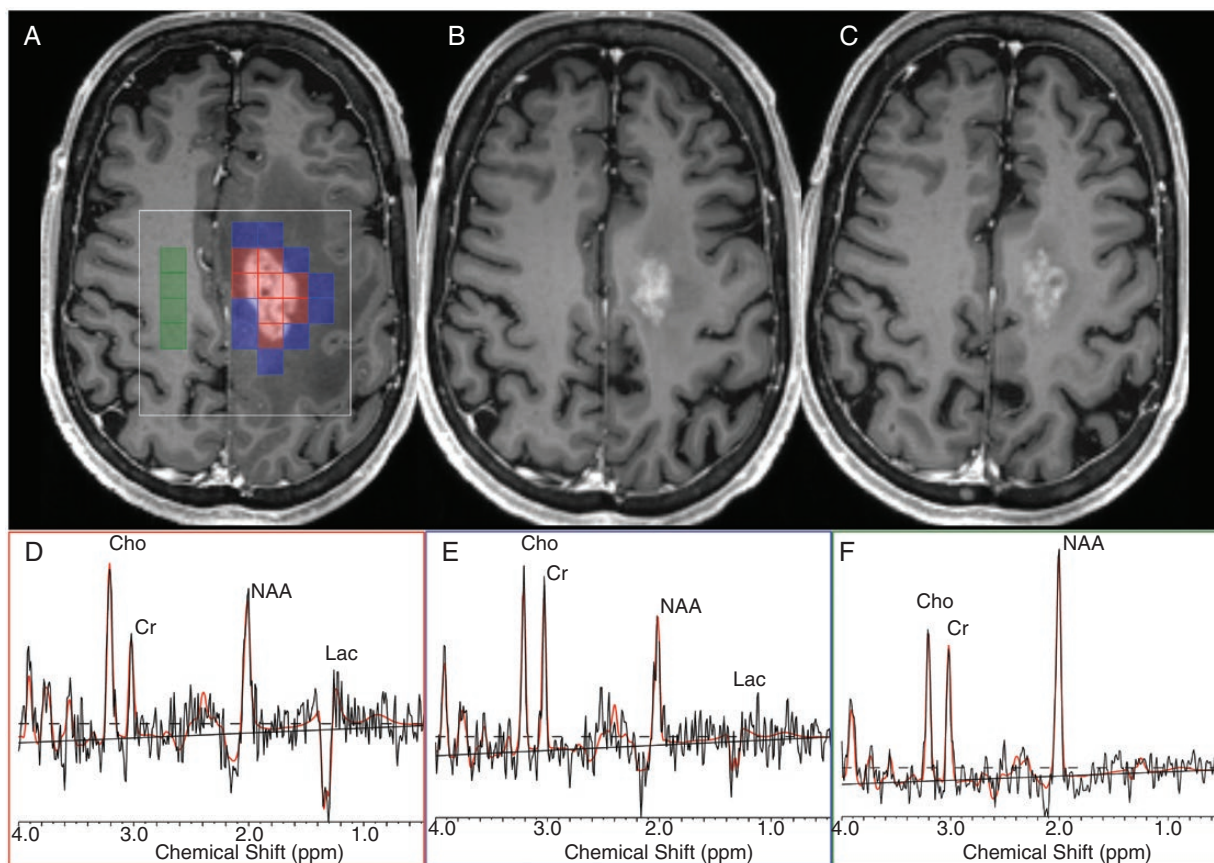


Figure 1. Representative MRS voxel selection. On an axial T1-weighted post-contrast image (A), intratumor voxels (red), peritumoral (blue), and contralateral normal voxels (green) are selected for analyses. The white square represents the MRS volume of interest. Serial imaging at 4 weeks (B) and 8 weeks (C) after the administration of antiangiogenic therapy is shown for the same patient. Representative spectra for the tumor (D), periphery (E), and contralateral (F) voxels are shown with labeling of NAA, lactate (Lac), creatine (Cr), and choline (Cho) peaks where appropriate with the LCMoel fit in red.

day one week on and one week off), and pembrolizumab (2 mg/kg every 3 weeks). Notably, there were no survival differences across the 4 treatment groups.

Longitudinal Metabolic Changes Across Different Treatment Groups

Since patients underwent different bevacizumab-based therapies as prescribed by their neuro-oncologists, we first investigated if there were any group differences in NAA/Cho and Lac/NAA across treatment groups. As there was only one patient treated with bevacizumab and pembrolizumab, this subject was excluded from this analysis. We did not find any effects of treatment on intratumoral or peritumoral metabolites across different time points ($P > .05$; [Supplementary Figure 1](#)), so treatment groups were combined for subsequent analyses.

Tumor Volume

We compared T1-weighted enhancing tumor volume across various time points. Longer-term survivors (OS-9)

had an average BL tumor burden volume of 54.81 cm^3 while shorter-term survivors (non-OS-9) had an average volume of 61.79 cm^3 ($P = .65$). Regardless of survival, enhancing brain tumor volume significantly decreased from BL at all time points ($P < .05$) except 1 day. The change in tumor volume discriminated survivorship only at 2 weeks and 16 weeks ([Figure 3](#)). ROC analysis for changes in tumor volume showed the following AUCs (confidence interval [CI]): 0.57 (0.43–0.70) for 1 day, 0.93 (0.84–1.0) for 2 weeks, 0.50 (0.39–0.60) at 4 weeks, 0.52 (0.40–0.63) at 8 weeks, and 0.7 (0.58–0.82) at 16 weeks).

NAA/Cho as a Predictor of OS-9

Intratumoral changes in NAA/Cho relative to BL are shown in [Figure 4A](#). Longer-term survivors had a higher level of NAA/Cho compared to shorter-term survivors at 8- and 16-week time points in the tumor and periphery ([Figure 4B](#)). ROC analysis for intratumoral NAA/Cho changes compared to BL to assess for stratification based on survival revealed an AUC of 0.71 at 8 weeks and 0.85 at 16 weeks ([Table 2](#)). In the tumor periphery, the 8- and 16-week time points were also predictive of survival (AUC 0.75 and 0.88, respectively,

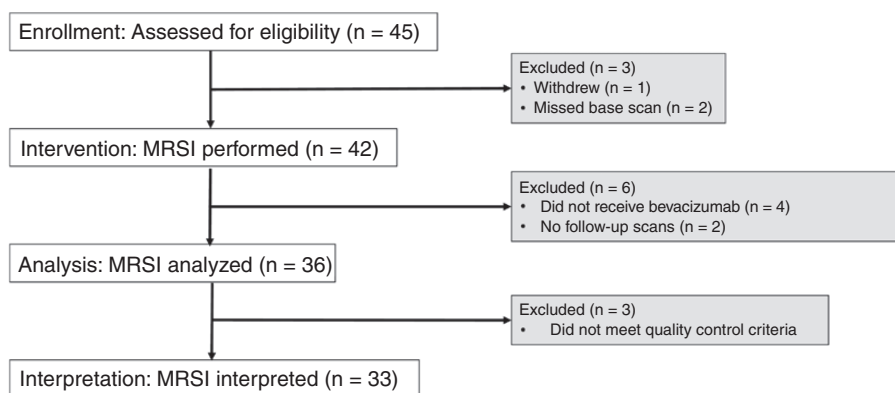


Figure 2. Consort flowchart. Forty-five patients are enrolled in the study with 42 patients undergoing MRS imaging. Thirty-six patients have analyzable data, and 33 patients have appropriate quality scans for analyses.

Table 1. Patient Demographics

Demographics	Longer-term Survivor	Shorter-term Survivor	Total	P-value
Age in years, median \pm SD (range)	62.5 \pm 7.0 (54–78)	63 \pm 12.3 (28–81)	63 \pm 10.9 (28–81)	0.69
Male gender (%)	5 (15%)	18 (55%)	23 (70%)	0.11
Race (white/Asian/black/other)	9/0/1/0	19/1/2/1	28/1/3/1	0.68
Baseline KPS, median \pm SD (range)	81 \pm 3 (50–90)	80 \pm 2 (50–90)	81 \pm 11 (50–90)	0.12
IDH1 mutant/wildtype/unk	1/9/0	2/20/1	3/29/1	0.49
MGMT methylated/unmethylated/unk	3/6/1	3/17/3	6/23/4	0.53
Treatment type:				0.43
Bev monotherapy	2	5	7	
Bev + lomustine	3	11	14	
Bev + temozolomide	5	6	11	
Bev + pembrolizumab	0	1	1	

There are no group differences between overall longer-term and shorter-term survivors across a variety of demographics including age, gender, tumor genetics, race, BL metabolite level, and treatment type. Unk denotes unknown and Bev denotes bevacizumab.

Table 2). Contingency analysis at the 8-week time point showed that 88% of the participants with a decrease in NAA/Cho by 0.074 relative to BL did not survive at 9 months.

We then performed exploratory analyses to determine if NAA/Cho can distinguish longer-term from shorter-term survivors at earlier time points. Longer-term survivors had increased NAA/Cho in the tumor while shorter-term survivors had decreased NAA/Cho compared to BL (**Figure 4**). NAA/Cho was predictive of survival at 1 day and 2 weeks (AUC 0.92 and 0.75, respectively) but not at 4 weeks (**Table 2**). In the tumor periphery, changes in NAA/Cho levels compared to BL were associated with longer-term survival at all earlier time points (**Table 2**, **Figure 4B**). In sum, changes in intratumoral NAA/Cho were significantly different between longer-term and shorter-term survivors except at the 4-week time point,

while changes in peritumoral NAA/Cho were significantly different between shorter-term and longer-term survivors at all time points evaluated.

Survival Analyses

We split our cohort into 2 subgroups, one with a change in NAA/Cho greater than 0.074 and the other with a change in NAA/Cho less than 0.074 compared to BL. After performing a Kaplan-Meier survival analysis, we found that subjects with increased NAA/Cho had a median overall survivorship of 274 days compared to 175 days for subjects with decreased NAA/Cho (**Supplementary Figure 2**). Generalized Wilcoxon test demonstrated a statistically significant difference between the 2 groups ($P = .04$).

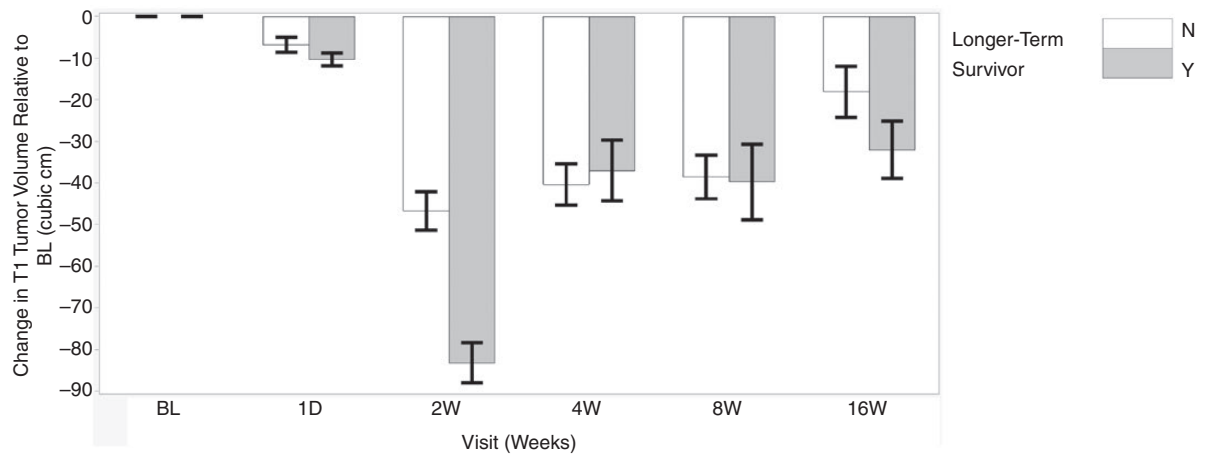


Figure 3. Longitudinal changes in tumor volume compared to BL. Regardless of survival, brain tumor volume significantly decreases from BL to the 2-week, 4-week, 8-week, and 16-week time points. T1-weighted enhancing tumor volume does not discriminate between overall survivorship except at the 2-week and 16-week time points.

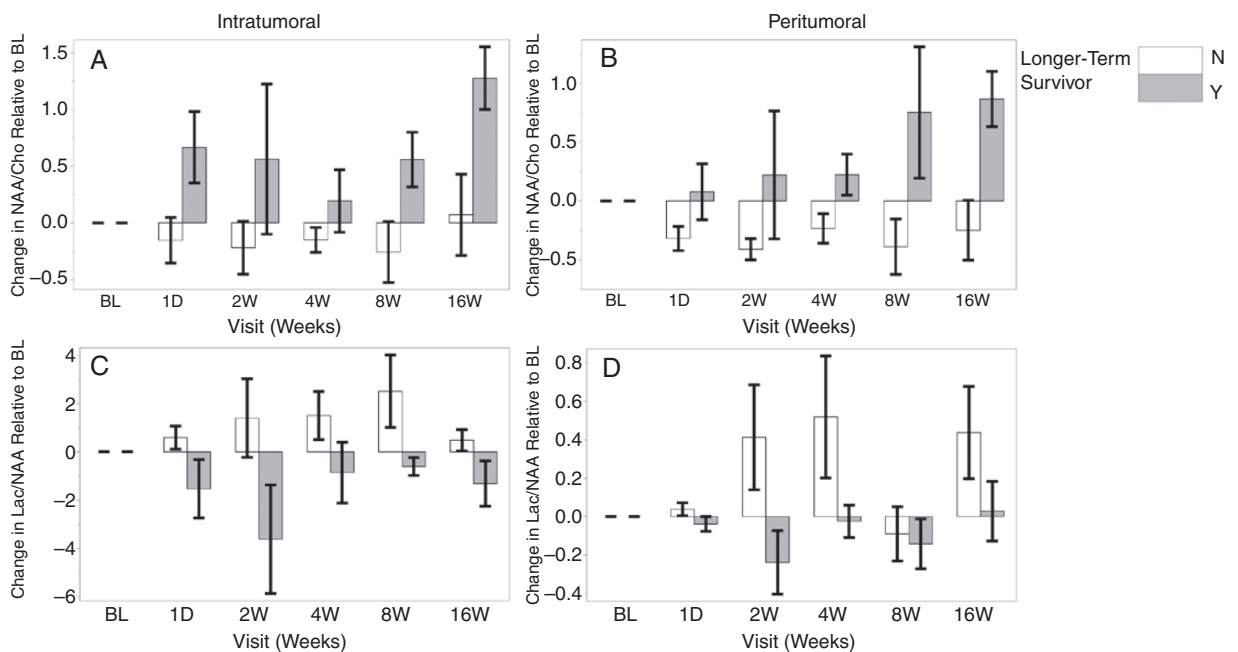


Figure 4. Intratumoral and peritumoral changes in NAA/Cho and Lac/NAA relative to BL scan. Longer-term survivors have higher NAA/Cho levels relative to shorter-term survivors in the tumor (A) and peritumoral area (B) across different time points. Lac/NAA is higher in shorter-term survivors compared to longer-term survivors in tumor voxels (C) and in most time points in the peritumoral area except the 8-week time point (D). Error bars represent standard error.

Lac/NAA as a Predictor of OS-9

Intratumoral changes in Lac/NAA relative to BL are shown in [Figure 4C](#); shorter-term survivors had increased changes in Lac/NAA compared to longer-term survivors at 8 and 16 weeks. ROC analyses revealed an AUC of 0.76 at both time points ([Table 2](#)). In the peritumoral voxels, shorter-term

survivors were associated with higher Lac/NAA values compared to BL at 16 weeks (AUC 0.72) but not the 8 weeks ([Figure 4D](#), [Table 2](#)). Contingency analysis at the 8-week time point revealed that 90% of individuals with an increased Lac/NAA from BL did not survive at 9 months.

Similar to NAA/Cho, we investigated changes in Lac/NAA at earlier time points to see if this could be predictive

Table 2. AUCs for Various MRS Metabolites Stratified by 9-Month Survival

Region	Metabolite	1 day AUC (95% CI)	2 week AUC (95% CI)	4 week AUC (95% CI)	8 week AUC (95% CI)	16 week AUC (95% CI)
Tumor	NAA/Cho	0.92 (0.78,1)	0.75 (0.54,1)	0.60 (0.41, 0.78)	0.71 (0.52, 0.90)	0.85 (0.68, 1)
	Lac/NAA	0.83 (0.64,1)	1 (1,1)	0.76 (0.69, 0.92)	0.76 (0.58, 0.93)	0.76 (0.56, 0.96)
Periphery	NAA/Cho	0.75 (0.53, 0.97)	0.88 (0.69,1)	0.75 (0.57, 0.93)	0.75 (0.57, 0.93)	0.88 (0.73, 1)
	Lac/NAA	0.55 (0.30, 0.79)	1 (1,1)	0.68 (0.51, 0.85)	0.61 (0.41,0.80)	0.72 (0.51, 0.93)

The table illustrates AUCs of NAA/Cho and Lac/NAA in the tumor and tumor periphery with 95% CI at different time points. Effective classifications defined by a lower bound of the 95% CI greater than 0.5 are bolded.

of survival. ROC analyses within tumor voxels showed that Lac/NAA predicted survival to bevacizumab-based therapy at all earlier time points (Table 2). Within peritumoral voxels, ROC analyses showed an AUC of 1 at the 2-week time point and 0.68 at 4 weeks but did not predict survival at 1 day (Table 2).

Survival Analyses

Similar to before, we generated 2 subgroups, one with increased Lac/NAA and the other with decreased Lac/NAA relative to BL. Kaplan-Meier analysis showed that subjects with increased Lac/NAA had a median overall survivorship of 197 days compared to 292 days for subjects with decreased Lac/NAA (Supplementary Figure 3), although this finding was not significant ($P = .11$).

Other Metabolic Predictors of OS-9

Finally, we explored whether other metabolites also changed during anti-VEGF treatment. Within the tumor, we analyzed NAA/contralateral Cr, Lac/contralateral Cr, and Cho/contralateral Cr and did not find any group differences across the different time points (Supplementary Figure 4).

Discussion

Our longitudinal MRS study in patients with rGBM illustrates that intratumoral changes in NAA/Cho and Lac/NAA can differentiate longer-term survivors from shorter-term survivors as early as 1 day after initiation of bevacizumab-based therapy and persist at the 2- and 8-week time points. In the tumor periphery, the NAA/Cho was higher in longer-term compared to shorter-term survivors at the early time points, but this was not true for Lac/NAA. Taken together, these results suggest that NAA/Cho and Lac/NAA may serve as timely biomarkers for predicting early treatment failure (or response) to bevacizumab-based therapy.

Our findings that metabolite levels derived from MRS may be robust early biomarkers in assessing patient

response to anti-angiogenic therapy are consistent with emerging literature. We previously showed that increased levels of NAA/Cho compared to BL at 8 weeks post-treatment were predictive of overall survival at 12 months,¹⁴ and we show that these findings are also present even sooner. In a study by Kim et al., 20 patients with rGBM were treated with cediranib, a tyrosine kinase inhibitor of the VEGF receptor; the authors showed that intratumoral increases in NAA/Cho were higher at 56 days (8 weeks) post-treatment and predictive of 6-month overall survival.²⁷ A longitudinal prospective study in 40 newly diagnosed GBM patients receiving cediranib and comitant chemoradiation showed that Cho/hCr (healthy creatine) at 4 weeks correlated with overall survival and had AUC larger than tumor volumetrics and cerebral blood flow.²⁸ In another study involving 31 patients with GBM receiving anti-angiogenic therapy and radiation, the authors showed that reductions in Cho/NAA and lower Lac/NAA at 16 weeks correlated with progression-free and overall survival.²⁵ Lower Cho/NAA suggests reduced tumor cellularity while decreased Lac/NAA reflects vascular normalization and increased tumor oxygenation. Our data are able to stratify treatment failure sooner than 16 weeks. At 8 weeks, we find that 88% of patients with decreased NAA/Cho from BL and 90% of individuals with an increased Lac/NAA from BL did not survive at 9 months.

Our MRS results illustrate that changes in tumor microenvironment after treatment with anti-angiogenic therapy impact patient survival. GBM has a necrotic core with cells migrating away from the hypoxic environment toward a vascular supply.²⁹ The hypoxic environment helps tumor cells evade the immune system while selecting for more malignant tumor cells.³⁰ This survival advantage is balanced with upregulation of angiogenic factors to maintain adequate perfusion for survival.^{31,32} Targeting angiogenic factors, such as VEGF, can lead to either pruning of fragile tumor vessels or vascular normalization. Pruning results in increased tumor hypoxia and lactate while normalization has the opposite effect and can improve survival.³⁰ Our results suggest the underlying mechanism of shorter-term survivors could be due to vascular pruning, leading to increased hypoxia or necrosis (and Lac/NAA), and increased tumor cellularity (decreased NAA/Cho levels). Consistent with

this,³¹ phosphorous MRS has shown that shorter-term survivors had higher markers of tumor cell proliferation when measuring phospholipid membrane metabolism.³³ Therefore, MRS may be a dynamic biomarker of vascular structure and function and could help tailor bevacizumab dosing. Consistent with this framework, mouse studies have shown dose-dependent effects of bevacizumab on tumor cells and vasculature.^{34,35} Future studies will need to explore whether shorter-term survivors have fewer VEGFA receptors and would benefit from reduced bevacizumab dosing to shift tumor physiology from vascular pruning to normalization.

Our finding that changes in intratumoral NAA/Cho and Lac/NAA as well as peritumoral NAA/Cho were predictive of survivorship as early as 1 day suggests that anti-angiogenic therapy may have an immediate effect on tumor microenvironment, a finding reported by other groups. A previous study by Gerstner et al. showed that 1 day after BEV treatment, readily detectable changes in contrast-enhancing tumor volume, tumor Ktrans values, and tumor SUV were seen.³⁶ Furthermore, another phase II trial by Sorensen et al. showed that cediranib, a VEGF receptor tyrosine kinase inhibitor, results in extensive normalization of tumor vasculature.³⁷ The extent of this normalization was predictive of PFS and OS.³⁷ Taken together, these results may suggest that anti-angiogenic therapy can result in dramatic changes in tumor microenvironment as early as 1 day after treatment initiation. This is also supported by the rapid decrease in contrast enhancement seen one day after bevacizumab treatment.

We also investigated correlations between tumor volumes and survival and found that enhancing tumor volume decreases at all tested time points regardless of survival. The magnitude of this change was able to distinguish between longer-term and shorter-term survivors at 2 weeks and 16 weeks after bevacizumab-based therapy, which is consistent with reports by other groups who have been able to distinguish between longer-term and shorter-term survivors based on enhancing tumor volume.³⁸ The finding's clinical relevance is limited by the fact that tumor volume decreased in all subjects regardless of survivorship status and was not significant at other time points. However, our findings regarding Lac/NAA and NAA/Cho are of greater clinical significance because longer-term and shorter-term survivors displayed opposite trajectories. Namely, longer-term survivors display increases in NAA/Cho and decreases in Lac/NAA, the opposite of shorter-term survivors. Furthermore, metabolic changes as detected by MRS were predictive at all time points, further increasing the clinical robustness of these findings by making it easier for clinician evaluation.

Despite its potential to provide meaningful biomarkers to improve diagnosis, better define the natural history of a disease process, or monitor metabolic responses to therapy, there is a wide notion that MRS is difficult to implement within the clinical workflow and lacks reproducibility. Here, we use a non-product 3D MRSI sequence that has been used in several clinical trials,^{26,39} yields robust findings, and is available to other hospitals and research sites by way of a C2P agreement. Since there is considerable technical expertise that limits methodological

implementation, our group has been working with other national and international sites to provide a turn-key package of advanced MRS technology for end users for multi-site/vendor trials and routine clinical use (Siemens, GE, and Philips 3T platforms). To address the variable data quality that may be inherent to standard clinical MRS packages, our group is developing a protocol using a semi-LASER sequence,⁴⁰ utilizing automated atlas-based voxel positioning selection for the ROI,⁴¹ and implementing Automated FASTMAP to improve shimming and line width reduction.⁴² The goal would be to generate metabolic color maps which would depict increases and decreases in metabolites such as NAA/Cho or Lac/NAA from baseline.

We also acknowledge the limitations common to longitudinal studies. First, there was some patient attrition across scan time points due to changes in goals of care (despite lack of tumor progression) or inability to make some scan time points. This led to a smaller than anticipated sample size during some study visits (ie, 1 day and 2 weeks), making a type 1 error more likely. However, our results still remain significant at later time points with larger samples, highlighting the robustness of the findings. Patients had MRS sequences added onto clinical MRI scans at locations that were convenient for them. We made sure to scan patients using the same type of scanner (GE or Siemens), and we focused on changes in metabolites compared to BL scan to minimize scanner effect and any large systematic confounds. Although only voxels that were clearly defined in the tumor or tumor periphery were included, there could be partial volume effects due to the large voxel size of the data. Given our experience with the method, we feel confident that the voxels selected best represent each of the areas indicated with minimal overlap or shift across time. Furthermore, a diagnosis of recurrence was not determined by histological biopsy but rather a multidisciplinary tumor board in conjunction with the patient's treating neurooncologist. Although unlikely, pseudoprogression from radiation necrosis may have been a confound when enrolling patients for this study.

Conclusion

Although conventional MR methods can assess for rGBM, they are susceptible to pseudo-response after anti-angiogenic therapy, making them unreliable. Addition of advanced MR methods such as MRS more accurately reflects tumor burden by assessing tumor microenvironment and can work in synergy with conventional MR methods to understand how anti-angiogenic therapy affects the tumor environment, which may lead to better treatment combinations. Our study shows that changes in tumoral and peripheral NAA/Cho and tumoral Lac/NAA can be robust biomarkers for predicting response to anti-angiogenic therapy and are predictive of patient outcomes as early as 1 day and 2 weeks. Incorporating MRS into clinical scans can distinguish between pseudo-response and treatment response early in the course of anti-angiogenic therapy in patients with rGBM.

Supplementary Material

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

Keywords

bevacizumab | biomarker | brain tumor | lactate | MR spectroscopy

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