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Increased Survival of Glioblastoma Patients who Respond to Anti-angiogenic Therapy with Elevated Blood Perfusion

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Abstract

The abnormal vasculature of the tumor microenvironment supports progression and resistance to treatment. Judicious application of anti-angiogenic therapy may normalize the structure and function of the tumor vasculature, promoting improved blood perfusion. However, there has been a lack of direct clinical evidence for improvements in blood perfusion after anti-angiogenic therapy. In this study, we used MRI to assess tumor blood perfusion in 30 recurrent glioblastoma patients who were undergoing treatment with cediranib, a pan-VEGF receptor tyrosine kinase inhibitor. Tumor blood perfusion increased durably for more than one month in 7 of 30 patients where it was associated with longer survival. Together, our findings offer direct clinical evidence

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Conflicts of interest

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in support of the hypothesis that vascular normalization promotes tumor regression and longer patient survival.

Keywords

Glioblastoma; anti-VEGF; vascular normalization; blood perfusion; survival

INTRODUCTION

Bevacizumab, a vascular endothelial growth factor (VEGF)-specific antibody, was conditionally approved in 2009 for treatment of patients with recurrent glioblastoma (rGBM), based on two phase II trials showing notable anti-tumor activity alone or in combination with adjuvant chemotherapy[1,2]. However, whether bevacizumab treatment leads to longer survival in rGBM patients in a phase III trial is not known, nor are the precise mechanisms of survival benefit. A recent pre-clinical study has shown that anti-VEGF therapy reduces tumor blood perfusion and increases invasiveness in GBM, casting doubt on any survival benefit[3]. In contrast, a number of pre-clinical studies have shown that judicious application of anti-VEGF agents can transiently “normalize” the abnormal tumor vessels, and this normalization can reduce vascular permeability, edema and hypoxia as well as improve the delivery and efficacy of various therapies[4–7]. Indeed, a phase II trial showed that cediranib, a pan-VEGF receptor tyrosine kinase inhibitor, can normalize the blood vessels of rGBM[8], and more crucially, the extent of vascular normalization by day 1 correlated with both progression-free survival (PFS) and overall survival (OS)[9]. Furthermore, serial MR spectroscopy showed that cediranib also had a direct metabolic effect on rGBM in patients who survived longer[10].

Although the first human evidence for vascular normalization in rectal carcinomas[11], increased vessel maturation and decreased interstitial fluid pressure, was followed by further evidence for normalization in rGBMs by less permeable and more normal-sized vessels and reduced edema[8], direct evidence for increased blood perfusion in human tumors - a potential consequence of vascular normalization - is not yet available. Glioblastomas have inefficient, irregular vessels that are leaky and dilated with haphazard pattern of interconnection and their baseline blood perfusion rate on average is lower than that of the surrounding normal brain[5,6].

As depicted in Figure 1, anti-angiogenic therapy might affect tumor vessels in three different ways: no effect at all; excessive destruction of blood vessels and reduction in perfusion leading to increased hypoxia, necrosis and/or invasion; or after pruning of some abnormal vessels the structure of remaining tumor vessels might become closer to normal vessels potentially resulting in an increase in absolute blood perfusion[5]. To this end, we measured the changes in tumor blood perfusion during the course of treatment with cediranib using advanced MRI methods in 30 rGBM patients, and show for the first time that tumor blood perfusion indeed increased in a subset of patients undergoing VEGF-treatment and that these patients survived approximately 6-months longer than patients whose tumor blood perfusion did not increase.

METHODS

Patient Population

We included 30 subjects with confirmed rGBM in this prospective study of cediranib (AstraZeneca) sponsored by the NCI (NCT00035656)[12]. The study was approved by the institutional Review Board and informed consent was obtained from all patients. After study

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termination, 9 of the 30 patients received one subsequent cycle of salvage chemotherapy, 8 patients received two cycles, 1 patient received three cycles, 2 patients had undisclosed information and 1 patient received stereotactic radiosurgery.

MR Imaging

Our MRI protocol including (^{1}H-MRS) spectroscopy has previously been described[8–10]. In addition, arterial spin-labeling (ASL) perfusion images (QUIPSS II[13]) were acquired with repetition-time=2.0s, echo-time=12ms, resolution=3.44mm/3.44mm/5mm, matrix-size=64/64, 6/180 slices/volumes and inversion-times=700ms/1800ms. Metabolitic concentrations of N-acetylaspartate (NAA), choline (Cho) and normal-side creatine (norCre) were assessed to derive ratios for NAA/Cho, NAA/norCre and Cho/norCre and normalized to healthy tissue[10].

Volumetrics, Permeability maps and Perfusion analysis

Tumor regions-of-interests were drawn by an experienced neuroradiologist on FLAIR- and contrast-enhanced T_1-weighted images[8,9]. The DCE data were processed to create K^{trans} maps[8], a measure of the permeability-surface area product. Blood perfusion and blood volume were calculated using established models on the DSC data in nordicICE and corrected for contrast- agent leakage[14]. Also, to minimize T_1-shortening effects, the contrast-agent pre-dose from DCE was used to saturate leaky tissue from blood-brain barrier breakdown or resection. Patient-specific variations were reduced by automatic arterial input function selection and partial volume correction before normalized to normal-appearing gray- and white-matter tissue[14]. Blood perfusion by ASL were derived in Matlab as previously described[13] - including quantitative T_1 mapping.

Statistical analysis

Changes in perfusion after therapy onset were assessed by applying a highly conservative threshold in which changes in the tumor-to-reference tissue perfusion ratios had to be higher or lower than the 95% confidence interval of the variations across patients [98%–107%; baseline set to 100%], derived from the within-patient percent perfusion changes between the two baseline time points. Also, a perfusion increase or decrease had to be consistent for two or more consecutive time points equal to one month of imaging or more. We used paired Wilcoxon test, with Holm-Bonferroni corrections for multiple comparisons, to assess changes over time. Groups were compared using Mann-Whitney tests, log-rank test, and Wald test in Cox regression analysis of survival data. *P*-values <0.05 were considered statistically significant.

RESULTS

The median PFS and OS from time of enrollment for the 30 patients were 111 days (95% confidence-interval; 71–140days) and 220 days (168–285days), respectively, with 23.3% alive and progression free at six months[12]. Figure 2A show example serial anatomic MRI of a patient with increased perfusion (Figure 2B) compared to perfusion in reference tissue (Figure 2C). Correspondingly, supplementary Figure S1A show serial anatomic MRI of a patient with decreased perfusion (supplementary Figure S1B) compared to perfusion in reference tissue (supplementary Figure S1C). Here, baseline alterations and especially changes in blood perfusion were neither subtle nor limited to regions of contrast enhancement. Importantly, the changes occurred even when the conventional imaging showed signs of tumor response, with decreasing contrast enhancement and mass effect (supplementary Figure S2A), decreasing peritumoral vasogenic edema (supplementary Figure S2B) and decreasing permeability (supplementary Figure S2C).

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Durable increase in tumor perfusion of at least one month duration was seen in 7 patients (supplementary Figure S3A), stable perfusion in 12 patients (supplementary Figure S3B) and durable decrease in tumor perfusion in 11 patients (supplementary Figure S3C). Figure 3A shows the group means over time, also showing that all groups tended to eventually exhibit increased perfusion, or reverse and return to pre-treatment perfusion values, after one or two months of imaging[8]. Compared to pretreatment values, patients with an increase in perfusion showed an average increase in perfusion of >5% (day +1), >10% (days +28 and +56) and >15% (day +112). Individual time courses for all patients are shown in supplementary Figure S3. These perfusion-metrics focus on capillary-level (microvessel) blood perfusion, but similar, supporting results are present for total (macrovessel) blood perfusion (supplementary Figure S4A and using microvessel perfusion groups in supplementary Figure S4B) and ASL (macrovessel perfusion groups; supplementary Figure S5A and microvessel perfusion groups; supplementary Figure S5B). Test-retest (supplementary Figure S6A) and between baseline (supplementary Figure S6B) reproducibility analysis showed minimal variability of the microvessel blood perfusion technique.

An increase in tumor perfusion was associated with prolonged PFS (Figure 3B) and OS (Figure 3C). Patients with increased tumor blood perfusion had a median OS of 348 days, as compared to those with decreased tumor blood perfusion (213 days) and stable tumor perfusion (169 days) (Mann-Whitney; $P<0.01$, supplementary Table S1). Using Cox regression with time dependent covariates, the effect of increased blood perfusion remained a significant predictor of PFS and OS after adjusting for T_1-weighted and FLAIR tumor volume changes during treatment as well as salvage chemotherapy and stereotactic radiosurgery after study termination ($P<0.05$). Potential prognostic factors for outcome[9], including age, pretreatment T_1-weighted tumor volume, extent of resection, neurological performance and mental status were not statistically significant in Cox regression that included perfusion changes. Tested against a vascular normalization index (VNI) - reflecting decreases in permeability and microvessel blood volume and increase in circulating collagen IV levels – an early predictor of PFS and OS after one day of anti-angiogenic treatment[9], patients with an increase in microvessel perfusion showed significantly higher VNI values (0.48±0.24 mean±SEM) compared to patients with stable (−0.44±0.13) or decreased perfusion (−0.51±0.26) (Mann-Whitney; $P<0.05$) (Figure 4). Furthermore, for patients with increased perfusion and compared to pretreatment ratios, tumor metabolic ratios of NAA/norCre were significantly higher at days +28 (1.89±0.28) and +56 (1.38±0.51) and at day +28 (1.52±0.37) for Cho/norCre (Wilcoxon signed-rank; $P<0.05$) (supplementary Figure S7).

DISCUSSION

The advent of anti-angiogenesis therapy has been a welcome advance in cancer treatment, yet it has been associated with some controversy. The initially proposed mechanism of benefit, namely, starving the cancer by elimination or reduction of tumor vasculature, does not seem to fit with clinical observations, particularly the of lack of a clear dose-response relationship and the lack of benefit in the absence of concomitant cytotoxic therapy[5,6,8,9]. Theoretically, administration of anti-VEGF should reduce the effect of chemotherapy by reducing the supply of drug via elimination of blood vessels. Additionally, the resulting hypoxia should reduce the effectiveness of drugs[5,6]. Yet, no anti-VEGF trial in patients with metastatic disease has shown a decline in OS compared to chemotherapy alone[6]. One possible explanation for these findings is vascular normalization whereby anti-VEGF treatments, when used in judicious doses, can normalize abnormal vessel structures, potentially leading to increased blood perfusion. In fact, a number of pre-clinical studies

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have shown that anti-angiogenic agents can improve oxygenation and/or drug delivery[6]. However, human data on increased blood perfusion, oxygenation or drug levels are lacking.

To this end, our data provide three key insights. First, vascular changes in rGBM after anti-angiogenic therapy, including increased perfusion, clearly occur and occur durably. Importantly, perfusion does not increase in all patients, only in about one quarter of the patients. Second, vascular changes occur not only in regions most traditionally associated with rGBM – that is, in the area of blood-brain barrier breakdown – but also in surrounding areas. Third, and most provocative, this increase in blood perfusion is associated with prolonged survival.

The most straightforward explanation for these observations is that the increased tumor blood perfusion is simply a result of decreased permeability of normalized blood vessels – as the patient group with increased tumor blood perfusion had the highest VNI. This is consistent with a mathematical model showing that high vascular permeability can lead to perfusion stasis, and conversely, a decrease in permeability can increase perfusion[15] and another model showing that the decreased permeability also leads to a reduction in edema[16]. We have previously shown in pre-clinical data that edema reduction alone by cediranib can account for increased survival without affecting tumor growth[4]. However, edema control alone does not fully explain the improved survival – as we also observed direct metabolic effects of cediranib in rGBMs in some of the longer surviving patients[10]. There are two potential explanations for this metabolic response.

First, since cediranib is a multi-receptor tyrosine kinase inhibitor and some of these receptors are present on GBM cells[8], it is conceivable that the normalized vessels permit a better delivery of cediranib to the GBM cells leading to a better anti-tumor effect. Killing of cancer cells surrounding blood vessels can open up compressed blood vessels, and in turn, also increase blood perfusion[17]. Thus cediranib acts as a combined vascular normalizing agent and anti-cancer agent both contributing to increased tumor blood perfusion. Consequently, the patients with increased blood perfusion - and a higher VNI - benefit from both better anti-edema and anti-cancer effects. This could potentially explain why some patients with decreased blood perfusion had no OS gain - despite decreased vascular permeability and edema – suggesting a lack of anti-cancer effect by cediranib in these patients.

A second explanation might be that vascular remodeling and resulting increased perfusion and delivery improve the innate immune response[18,19], an emerging and compelling concept. A recent study offers evidence that targeting abnormal polarization of tumor-associated macrophages (TAMs) can normalize tumor vessels and also enhance antitumor immunity[19]. Also, a more even distribution of blood perfusion with a subsequent reduction in areas of hypoxia and acidosis[6] can further increase immune response as hypoxia and also low pH compromise the cytotoxic functions of tumor-infiltrating immune cells[20]. Thus, patients whose tumor blood perfusion did not increase did not benefit from immunostimulation resulting from reduced hypoxia.

In summary, our data are consistent with the vascular normalization hypothesis, and suggest that improvement in survival in response to anti-VEGF therapy may be mediated by mechanisms other than vascular pruning and tumor “starvation”. Whether bevacizumab has similar effects in glioblastoma remains to be determined and is a high-priority research question for the field.

Supplementary Material

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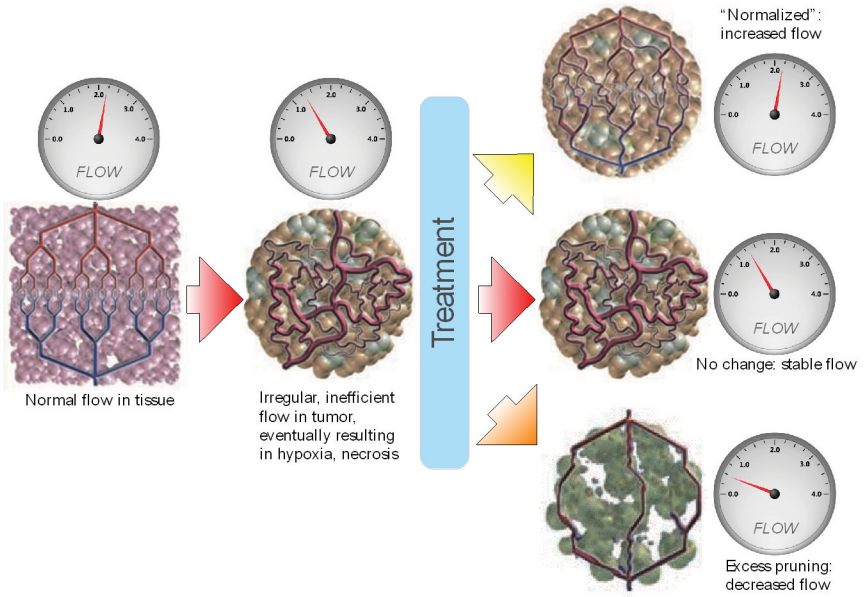

Figure 1. Vascular Normalization Hypothesis

Schematic of the effects of anti-angiogenic therapy on tumor vascular structure and blood perfusion[5]. Compared to normal vessels **(left)**, the tumor vasculature **(center)** is less efficient. After anti-angiogenic therapy, the tumor vasculature might become "normalized", resulting in increased blood perfusion **(top right)**; or not respond to therapy **(center right)**; or be markedly pruned leading to decreased blood perfusion **(bottom right)**.

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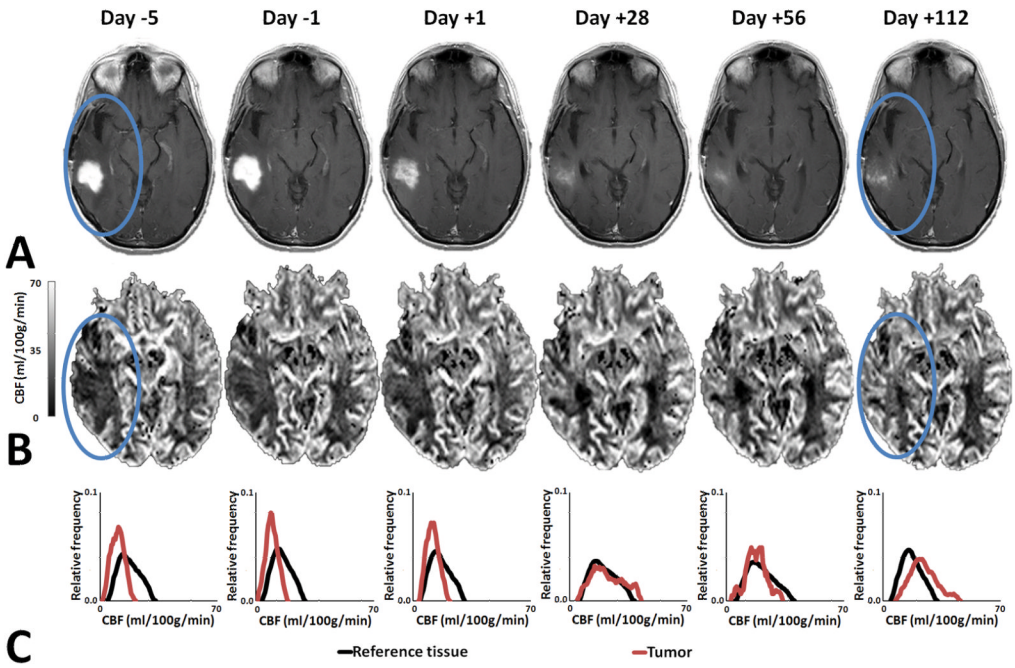

Figure 2. Increased Perfusion

Representative example of a patient with perfusion increase. **(A)** Anatomic MRI showing decrease in the contrast enhanced tumor area with blue ovals indicating tumor region. **(B)** Blood perfusion maps showing increasing perfusion. **(C)** Histogram analysis of enhancing tumor showing increase and normalization of perfusion compared to reference tissue.

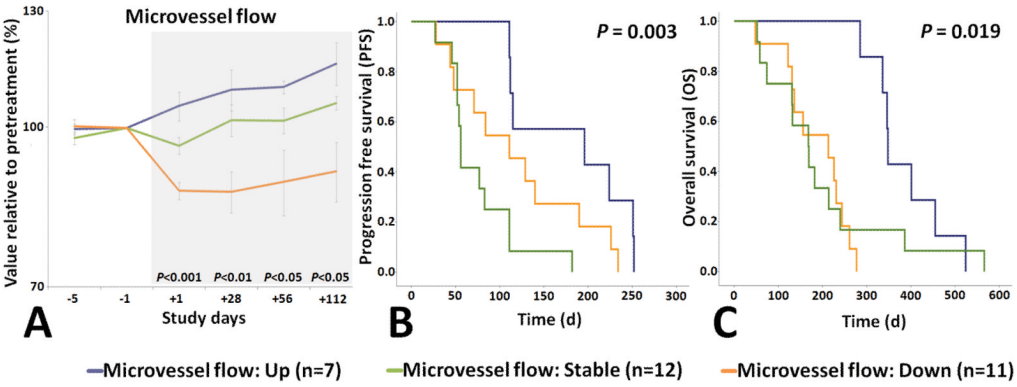

Figure 3. Perfusion Response to Treatment and Survival analysis

(A) Three types of perfusion response to anti-angiogenic treatment are evident: perfusion increase, stable perfusion or perfusion decrease. Figure show log-scaled averaged values (±SEM) and *P*-values from Kruskal-Wallis tests (Holm-Bonferroni corrected). Values at day −1 were set as 100%. **(B)** Kaplan-Meier analysis for progression-free survival. **(C)** Kaplan-Meier analysis for overall survival. Patients with an increase in tumor perfusion had prolonged PFS compared patients with stable perfusion, and prolonged OS compared to patients with stable and decreased perfusion (Mann-Whitney; $P<0.01$, Holm-Bonferroni corrected). Differences between PFS and OS may be attributed to the inherent uncertainty of the PFS estimate by the Macdonald criteria – since anti-VEGF agents decrease vascular permeability resulting in decreased contrast in the absence of an anti-tumor effect[4].

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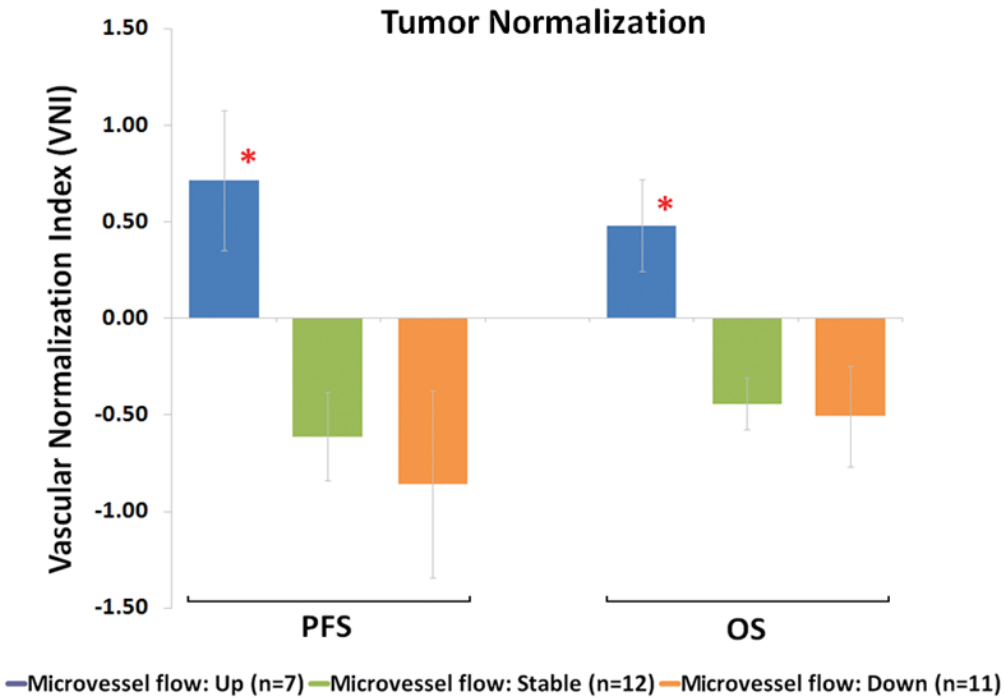

Figure 4. Relationship Between Microvessel Perfusion and a Vascular Normalization Index (VNI)

Patients with an increase in perfusion showed significantly higher VNI values compared to patients with stable or decreased perfusion for PFS and OS (*Mann-Whitney; $P<0.05$, Holm-Bonferroni corrected). A higher VNI value is also associated with increased PFS and OS[9]. Although perfusion and blood volume are inherently associated, but not identical, the relationship between perfusion changes and VNI indicate that increased perfusion could be a result of decreased vascular permeability.