Introduction

Bevacizumab, a VEGF-specific antibody, was conditionally approved in 2009 by the U.S. Food and Drug Administration for treatment of patients with recurrent glioblastoma, based on 2 phase II trials showing notable antitumor activity alone or in combination with adjuvant chemotherapy (1, 2). However, whether bevacizumab treatment leads to longer survival in recurrent glioblastoma patients in a phase III trial is not known, nor are the precise mechanisms of survival benefit. A recent preclinical study has shown that anti-VEGF therapy reduces tumor blood perfusion and increases invasiveness in glioblastoma, casting doubt on any survival benefit (3). In contrast, a number of preclinical 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, reduces the blood vessels of recurrent glioblastoma (8), and more crucially, the extent of vascular normalization by day 1 correlated with both progression-free survival (PFS) and overall survival (OS; ref. 9). Furthermore, serial magnetic resonance spectroscopy showed that cediranib also had a direct metabolic effect on recurrent glioblastoma 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 recurrent glioblastomas 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 a 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 Fig. 1, antiangiogenic therapy might affect tumor vessels in 3 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 recurrent glioblastoma 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.
Materials and Methods

Patient population

We included 30 subjects with confirmed recurrent glioblastoma in this prospective study of cediranib sponsored by the National Cancer Institute (NCT00035656; ref. 12). The study was approved by the Institutional Review Board, and informed consent was obtained from all patients. After study termination, 9 of the 30 patients received 1 subsequent cycle of salvage chemotherapy, 8 patients received 2 cycles, 1 patient received 3 cycles, 2 patients had undisclosed information, and 1 patient received stereotactic radiosurgery.

MRI

Our MRI protocol including (1H-MRS; ref. 10) spectroscopy has previously been described (8–10). In addition, arterial spin-labeling (ASL) perfusion images (QUIPSS II; ref. 13) were acquired with repetition time $= 2.0$ s, echo time $= 12$ ms, resolution $= 3.44 \text{ mm}/3.44 \text{ mm}/5 \text{ mm}$, matrix-size $= 64/64, 6/180$ slices/volumes, and inversion-times $= 700 \text{ ms}/1,800 \text{ ms}$. Metabolic 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 interest were drawn by an experienced neuroradiologist on fluid-attenuated inversion recovery (FLAIR)- and contrast-enhanced $T_1$-weighted images (8, 9). The dynamic contrast-enhanced (DCE) data were processed to create $K^*_{\text{trans}}$ maps (8), a measure of the permeability-surface area product. Blood perfusion [cerebral blood flow (CBF)] and blood volume were calculated using established models on the dynamic susceptibility contrast (DSC) data in nordicICE and corrected for contrast-agent leakage (14). Also, to minimize $T_1$-shortening effects, the contrast-agent predose from DCE was used to saturate leaky tissue from the blood–brain barrier breakdown or resection. Patient-specific variations were reduced by automatic arterial input function selection and partial volume correction, and tumor DSC values were normalized to normal-appearing gray- and white-matter tissue (14). Blood perfusion by ASL was 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 (CI) of the variations across patients (98%–107%; baseline set to 100%), derived from the within-patient percentage perfusion changes between the 2 baseline time points. Also, a perfusion increase or decrease had to be consistent for 2 or more consecutive time points equal to 1 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. Values of $P < 0.05$ were considered statistically significant.

Results

The median PFS and OS durations from time of enrollment for the 30 patients were 111 days (95% CI; 71–140 days) and 220 days (168–285 days), respectively, with 23.3% alive and progression free at 6 months (12). Figure 2A shows examples...
of serial anatomic MRIs of a patient with increased perfusion (Fig. 2B) compared with perfusion in reference tissue (Fig. 2C).

Correspondingly, Supplementary Fig. S1A shows serial anatomic MRIs of a patient with decreased perfusion (Supplementary Fig. S1B) compared with perfusion in reference tissue (Supplementary Fig. 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 Fig. S2A), decreasing peritumoral vasogenic edema (Supplementary Fig. S2B), and decreasing permeability (Supplementary Fig. S2C).

Durable increase in tumor perfusion of at least 1 month duration was seen in 7 patients (Supplementary Fig. S3A), stable perfusion in 12 patients (Supplementary Fig. S3B), and durable decrease in tumor perfusion in 11 patients (Supplementary Fig. 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 pretreatment perfusion values, after 1 or 2 months of imaging (8). Compared with 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 Fig. S3. These perfusion metrics focus on capillary-level (microvessel) blood perfusion, but similar, supporting results are present for total (macrovessel) blood perfusion (Supplementary Fig. S4A and using microvessel perfusion groups in Supplementary Fig. S4B) and ASL (macrovessel perfusion groups, Supplementary Fig. S5A; and microvessel perfusion groups, Supplementary Fig. S5B). Test-retest (Supplementary Fig. S6A) and between-baseline (Supplementary Fig. S6B) reproducibility analysis showed minimal variability of the microvessel blood perfusion technique.

An increase in tumor perfusion was associated with prolonged PFS (Fig. 3B) and OS (Fig. 3C). Patients with increased tumor blood perfusion had a median OS of 348 days, as compared with 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 T1-weighted and FLAIR tumor volume changes during treatment and salvage chemotherapy and stereotactic radiosurgery after study termination (P < 0.05).

Potential prognostic factors for outcome (9), including age, pretreatment T1-weighted tumor volume, extent of resection, neurologic performance, and mental status were not
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 Fig. S7).

Discussion

The advent of antiangiogenesis 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 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. In addition, 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 with 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 preclinical studies have shown that antiangiogenic 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 3 key insights. First, vascular changes in recurrent glioblastoma after antiangiogenic therapy, including increased perfusion, clearly occur and occur 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 (VNI)—an early predictor of PFS and OS after 1 day of antiangiogenic treatment (9), patients with an increase in microvessel perfusion showed significantly higher VNI values (0.48 ± 0.24 mean ± SEM) compared with patients with stable (−0.44 ± 0.13) or decreased perfusion (−0.51 ± 0.26; Mann–Whitney; P < 0.05; Fig. 4). Furthermore, for patients with increased perfusion and compared with 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 Fig. S7).
durably. Importantly, perfusion does not increase in all patients, only in about 25% of them. Second, vascular changes occur not only in regions most traditionally associated with recurrent glioblastoma—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, that 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 preclinical 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 recurrent glioblastomas in some of the longer-surviving patients (10). There are 2 potential explanations for this metabolic response.

First, because cediranib is a multireceptor tyrosine kinase inhibitor and some of these receptors are present on glioblastoma cells (8), it is conceivable that the normalized vessels permit a better delivery of cediranib to the glioblastoma cells, leading to a better antitumor 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 anticaner 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 anticancer 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 anticancer 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 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.

Disclosure of Potential Conflicts of Interest

A.G. Sorensen is a consultant and is on the advisory boards of ACR-Image Metrics, BayerScheringPharma, Bristol-Myers Squibb, BiogenIdec, Merrimack Pharmaceuticals, Olea Medical, Mitsubishi Pharma, GE Healthcare, Regeneron, Novartis, Roche, Siemens Medical, Takeda, AstraZeneca, and Kit, Inc. T.T. Batchelor is a consultant and is on the advisory boards of Merck, Roche/Gerntech, Amgen, Spectrum, and Exelixis. R.K. Jain is a consultant on the advisory boards of Dyax, Noxxon, SymDevRx, and Xtuit.

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References

Correction: Increased Survival of Glioblastoma Patients Who Respond to Antiangiogenic Therapy with Elevated Blood Perfusion

In this article (Cancer Res 2012;72:402–7), which was published in the January 15, 2012, issue of Cancer Research (1), all instances of the word "flow" in the Supplementary Figures should have been "perfusion." The corrected Supplementary Figures for this article are available online at http://cancerres.aacrjournals.org. The authors regret this error.

Reference


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Increased Survival of Glioblastoma Patients Who Respond to Antiangiogenic Therapy with Elevated Blood Perfusion

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