Vessel architectural imaging identifies cancer patient responders to anti-angiogenic therapy

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Measurement of vessel caliber by magnetic resonance imaging (MRI) is a valuable technique for in vivo monitoring of hemodynamic status and vascular development, especially in the brain. Here, we introduce a new paradigm in MRI termed vessel architectural imaging (VAI) that exploits an overlooked temporal shift in the magnetic resonance signal, forming the basis for vessel caliber estimation, and show how this phenomenon can reveal new information on vessel type and function not assessed by any other noninvasive imaging technique. We also show how this biomarker can provide new biological insights into the treatment of patients with cancer. As an example, we demonstrate using VAI that anti-angiogenic therapy can improve microcirculation and oxygen saturation and reduce vessel calibers in patients with recurrent glioblastomas and, more crucially, that patients with these responses have prolonged survival. Thus, VAI has the potential to identify patients who would benefit from therapies.

Anti-angiogenic therapeutic agents target solid tumors by vessel pruning and normalization of vascular structure and function, thereby contributing to the improved outcome of simultaneously administered chemotherapy, radiation therapy and immunotherapy1–3. In a trial using cediranib, an oral pan–vascular endothelial growth factor (pan–VEGF) receptor kinase inhibitor4, patients with recurrent glioblastomas whose tumor perfusion increased during treatment survived approximately 6 months longer compared to those whose perfusion did not increase5. Although promising, the exact microvascular mechanism by which these drugs increase perfusion and subsequently improve survival in patients is not fully understood. In the brain, MRI is the modality of choice for soft tissue imaging and has not been described to date. Here, we first illustrate the power of the VAI technique in a range of vessel types under different conditions by using Monte Carlo simulations and then show data from subjects with recurrent glioblastomas. Using VAI, we found that patients who responded to anti-angiogenic therapy with reduced tumor vessel calibers and improved hemodynamic efficiency and relative oxygen saturation survived longer than patients without these responses.

Vessel caliber is estimated by comparing the changes in observed proton relaxation from simultaneously acquired contrast agent–enhanced gradient-echo and spin-echo MR16,7,9. The gradient-echo and spin-echo readouts have different sensitivity to the so-called susceptibility effect (the magnetization induced in a medium when exposed to a magnetic field), and the highly susceptibility-sensitive gradient-echo images are sensitive to both microscopic and macroscopic vessels, whereas spin-echo images are predominantly sensitive to microscopic vessels (radius < 10 µm)7,10,11.

Here we build on this concept and exploit an overlooked temporal shift in the magnetic resonance signal that forms the basis for vessel caliber estimation and have coined the technique VAI. In practice, cerebral vessel caliber by MRI is assessed using the quotient of gradient-echo to spin-echo blood volume or direct assessment of the point-by-point difference in the contrast agent–enhanced relaxation rate curves4,9,12,13. However, depending on the hemodynamic properties of the tissue, the different sensitivities of the gradient-echo and spin-echo images to the susceptibility effect will result in an apparent variation in the respective MRI signal readouts (Fig. 1a–d). The outcome of this is a relative shift in the shapes and peak positions of the two relaxation rate curves. When visualized in a parametric plot, depending on tissue type, the pairwise gradient-echo and spin-echo data points may form a vortex curve of a certain shape and transverse in a clockwise or counterclockwise direction9.

The origin of this phenomenon, its exact relationship to the underlying tissue and its implication for imaging in patients with cancer have not been described to date. Here, we first illustrate the power of the VAI technique in a range of vessel types under different conditions by using Monte Carlo simulations and then show data from subjects with recurrent glioblastomas. Using VAI, we found that patients who responded to anti-angiogenic therapy with reduced tumor vessel calibers and improved hemodynamic efficiency and relative oxygen saturation survived longer than patients without these responses.

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Figure 1 Vessel architectural imaging in a healthy volunteer. (a–d) Simultaneously acquired gradient-echo (GE) (a) and spin-echo (SE) (b) contrast enhanced relaxation rate images showing the gradient-echo and spin-echo magnetic resonance signals peaking at different image readouts during the contrast agent bolus passage. Typically, in areas with fast inflow of the contrast agent, such as in the feeding branches of the middle cerebral artery (red arrows), the gradient-echo signal peaks earlier than the spin-echo signal, resulting in a clockwise vortex when plotting the relaxation rate curves in a point-by-point parametric plot (c). Correspondingly, in slow-inflow areas, such as in the venules leading to the internal cerebral veins (blue arrows), the spin-echo signal peaks earlier than the gradient-echo signal, resulting in a counterclockwise vortex (d). The contrast agent–induced relaxation rates in c and d are scaled relative to their baseline rates (before contrast agent arrival) and will increase and decrease with a full-width half-maximum proportional to the mean transit time. $V_f$ is defined as the area under the relaxation rate curves (percentage of blood in the image voxel – blood volume), whereas perfusion ($\sim$ flow) can be estimated using the central volume principle stating that $V_f$ is the product of flow and mean transit time.

RESULTS
Intravascular magnetic susceptibility perturbations in VAI
To gain insight into the VAI technique for different vessel types and calibers, we conducted Monte Carlo simulations to derive parametric vessel vortex curves for physiologically meaningful capillary vessels of 3.5-µm radius and for noncapillary vessels with radii ranging from 7.5 µm to 40 µm (Fig. 2). The vessel vortices transverse in a counterclockwise direction if, and only if, the vascular system contains both slow-inflow, larger-caliber venule-like vessel components and faster-inflow, smaller-caliber arteriole-like or capillary-like vessel components. In contradistinction, if the vascular system consists of arterioles and capillaries only—or, for some geometrical, pathological or physiological reason, fast-inflow arterioles with larger calibers than venules8,14—the vessel vortices transverse in a clockwise direction. For vessels of identical caliber, because of differences in...
tissue-specific oxygen saturation (SO₂)\textsuperscript{15,16}, the vessel vortices traverse in a counterclockwise direction if both arterioles and venules are included. However, if all vessels have identical calibers and SO₂ concentrations, there is no vortex (shown for capillaries in Fig. 2). Similarly, if a vascular system with a fixed SO₂ concentration contains arterioles, capillaries or venular structures only, there is no vortex, even if the vessels have different radii (Supplementary Fig. 1a).

The shape of the vessel vortex curve depends not only on the vessel types included but also on their relative difference in vessel radius, as discussed below (Supplementary Fig. 1b,c). The slope of the vortex curve is assumed proportional to the vessel caliber and tilted toward the gradient-echo axis for vascular systems with larger average vessel calibers\textsuperscript{9}. We provide a schematic illustration of the VAI analysis procedure, including an explanation of how the various parameters are derived from the vessel vortex curves (Supplementary Fig. 2).

**VAI response to changes in volume fraction and SO₂ concentration**

We used Monte Carlo simulations to gain insight into the VAI technique for different blood volume fractions (\(V_f\) ~ blood volume; Fig. 1c,d) and for varying concentrations of SO₂. Increased \(V_f\) by vessel recruitment (Supplementary Fig. 3a) results in a proportional increase in the length of the long axis of the vessel vortex curve\textsuperscript{16}.

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**Figure 3** Responses in parametric vessel vortex curves to changes in oxygen saturation. Monte Carlo simulations showing resulting parametric vessel vortex curves for a uniform system of arterioles (\(R = 10\) \(\mu\)m), capillaries (\(R = 3.5\) \(\mu\)m) and venules (\(R = 10\) \(\mu\)m) from changes in SO₂ (\(V_f\) fixed at 3.5%). (a,b) In a, the SO₂ concentration in the arterioles is kept at 93%, with capillary and venule SO₂ concentrations ranging from 93% (no consumption, that is, from local shunting) to 0% (full consumption). In b, the SO₂ concentrations in the arterioles range from 93% to 0%, with capillary and venule SO₂ concentrations fixed at 0%. The slope, as would be identified by a linear fit of the vessel vortex curve and historically used as a measure proportional of vessel caliber\textsuperscript{9}, is higher in b compared to a at pathologic concentrations of SO₂, even though the vessel caliber is unchanged (shown for 0–0% and 93–93%, respectively, with trend lines indicating no vortex curves). GE, gradient-echo; SE, spin-echo.

**Figure 4** Parametric vessel vortex curves of a responding subject with recurrent glioblastoma. (a) Contrast agent–enhanced MRI (T1-weighted) at baseline (days −5 and −1) and during anti-angiogenic therapy (days 1, 28, 56 and 112). (b) Contrast-enhanced tumor regions outlined on MRI showing tumor center (blue) and tumor edge (red). (c) Vessel caliber MRI. (d) Corresponding average vessel vortex curves from all pairwise gradient-echo (GE) and spin-echo (SE) relaxation rate curves in the tumor center (blue vortex curves) and tumor edge (red vortex curves). Following anti-angiogenic drug administration, the contrast agent–enhanced tumor area recedes, and the average vessel vortex direction changes from being predominantly counterclockwise at baseline to clockwise during treatment (days 1 and 28) before reversing at day 56. This effect is most prominent in the tumor center, and the subject was identified as a responder to the anti-angiogenic therapy by a relative increase in image voxels with a clockwise vortex direction compared to the arithmetic mean of all of the subjects. GE\textsubscript{ref} and SE\textsubscript{ref} represent scaling to GE and SE reference curves, respectively.
Vessel architectural imaging during anti-angiogenic therapy in subjects with recurrent glioblastomas. (a) Example of anatomical MRI and VAI of a subject with recurrent glioblastoma at baseline (day −1) and at day 28 after therapy onset. The images show (from top to bottom) anatomical contrast-enhanced T1-weighted images, volume fraction maps, vessel vortex area maps and vessel vortex direction maps. At baseline, larger-vessel calibers are observed in the tumor center compared to the tumor edge, with low oxygen extraction (low vessel vortex area values) and few voxels with a clockwise vessel vortex direction. (b) Corresponding vessel architecture in tumor edge, tumor center and reference tissue at baseline and day 28, respectively. The resulting vessel structures are based on average values from all 30 subjects, including vessel caliber, \( V \), vessel vortex direction and vessel vortex area (Supplementary Table 1). Responding subjects \( (n = 10) \) show a move toward a more competent microcirculation during therapy identified by a relative increase in image voxels with a clockwise vessel vortex direction in the tumor center, with reduced vessel calibers and improved \( \text{SO}_2 \) concentrations. Similarly to normal tissue, red, violet and blue indicate normal-appearing arteriole, capillary and venule hemodynamic status, respectively. (c) Kaplan-Meier survival curves show prolonged survival for responding subjects (median progression-free survival = 153 d, overall survival = 341 d) compared to nonresponding subjects \( (n = 12) \); median progression-free survival = 64 d, overall survival = 146 d), the latter identified by a relative decrease in voxels with a clockwise vessel vortex direction.

(Supplementary Fig. 4a), an exponential decrease in slope value (Supplementary Fig. 4d) and a proportional increase in the corrected vessel vortex area (Supplementary Fig. 4g). For vessel distention (Supplementary Fig. 3b), similar although less pronounced effects are observed (Supplementary Fig. 4b,e,h). The only exception is an exponential increase in slope value with increased \( V \) for tissue without functioning or missing capillary vessels or for vessel shunting. The vessel vortex direction was not affected by the induced changes in vessel recruitment nor distention.

We assessed the resulting vessel vortex curves for varying concentrations of \( \text{SO}_2 \) using different combinations of arterioles, capillaries and venules (Fig. 3a,b). Here, the length of the long axis and the slope of the vessel vortex curve increase with increasing amounts of deoxygenated blood (Supplementary Fig. 4c,f). In a vascular system with relatively unchanged or fixed vessel calibers and inflow rates, the corrected vessel vortex area reflects the different baseline susceptibility states in oxygenated and deoxygenated blood and thus the concentration of \( \text{SO}_2 \). Here, under normal conditions (venule calibers > arteriole calibers), the corrected vessel vortex area shows a Gaussian, bell-shaped response to changes in \( \text{SO}_2 \) amount (Supplementary Fig. 4i), expressed by an increase in the corrected vessel vortex area for increased absolute differences in \( \text{SO}_2 \) between well-saturated, oxygenated arterioles (\( \text{SO}_2 > 90\% \)) and deoxygenated capillaries and venules (\( \text{SO}_2 < 90\% \)). Correspondingly, for anoxic \( \text{SO}_2 \) concentrations and toward a theoretical and fully deoxygenated hemodynamic environment (arterioles, \( \text{SO}_2 < 75\% \); capillaries and venules, \( \text{SO}_2 = 0\% \)), the corrected vessel vortex area decreases.

Improved tumor microcirculation prolongs survival

We show a clinical application of VAI by retrospective analysis of 30 human subjects with recurrent glioblastomas enrolled in a phase 2 clinical trial of cediranib\(^4,17\). Collectively, we observed a significant increase (pairwise Wilcoxon signed-ranks test; \( P < 0.05 \)) in the relative number of image voxels with a clockwise vortex direction in the tumor after therapy onset, which thereby mimics normal-appearing tissue values (Supplementary Fig. 5a and Supplementary Table 1). We saw a more dominant effect in the tumor center (pairwise Wilcoxon signed-rank test; \( P < 0.01 \)) compared to the tumor edge (Supplementary Fig. 5b). We were able to identify ten subjects as responders to the anti-angiogenic therapy by a relative increase in image voxels with a clockwise vortex direction compared to the arithmetic mean of all of the subjects and at a minimum of two consecutive imaging time points (Fig. 4a−d and Supplementary Fig. 5c). Twelve subjects were identified as nonresponders by a relative decrease in image voxels with a clockwise vortex direction (Supplementary Figs. 5d and 6).
DISCUSSION
Assessment of the topological and structural heterogeneity of tumor microcirculation is important for monitoring of disease progression and treatment response. Tumor vessels are characterized by increased leakiness and regional, inefficient, closed or blind vascular pathways with or without hypoxia\(^2,18–22\). The VAI technique described in our study is capable of measuring these effects \textit{in vivo}, ranging from well-functioning, well-oxygenated normal-appearing tissue to the vascular collapse observed in anoxic tumor tissue. Our results provide several new insights. Overall, the temporal shift in the magnetic resonance signal can be readily observed with a standard combined gradient-echo and spin-echo contrast-enhanced MRI acquisition technique. In normal tissue, the resulting vessel vortex curve propagates in a counterclockwise direction if large, slow-inflow vessels and faster-inflow vessels with smaller calibers are present. The slope of the vessel vortex curve is indeed influenced by the average vessel caliber of the tissue\(^9\), but the traditional view of increasing slope values for bigger-vessel calibers is dependent on changes in \(V_f\) and \(SO_2\) concentration. The highest slope values were observed for theoretical vessel systems with local shunting, where big, disorganized, fast-flow arterioles aberrantly connect to disorganized venous structures\(^19,23\). Unlike in vascular systems with functioning capillaries, local shunting will also result in a relatively constant corrected vessel vortex area, indicative of little or no difference in oxygen saturation between the tissue types.

The subnormal vascular function and nonuniform branching hierarchy of recurrent glioblastomas\(^2,20\) were identified by a higher relative ratio of larger-caliber, deoxygenated venule-like vessels compared to other vessel types. This higher ratio was more pronounced toward the tumor center and in line with a vascular gradient moving from a neoangiogenic tumor border of normal or dilated vessels toward a hypoxic or anoxic core with scarce, inefficient and very large vessels\(^1,22,24\). During anti-angiogenic therapy, a higher ratio of image voxels with a clockwise vortex direction was observed in responding subjects, mimicking the ratio seen in normal-appearing tissue. This change in vessel vortex direction requires a higher quantity of vessels with fast inflow rates combined with a reduction of large vessels with slow inflow. This is consistent with data from studies in animal and human solid tumors where proper doses of anti-angiogenic drugs lead to improved tumor microenvironment and more effective delivery of exogenously administered therapeutics by reduced tumor hyperpermeability, vessel caliber, hypoxia and interstitial fluid pressure and increased vascular pericycle coverage\(^2,5,25–27\). Correspondingly, the improved microcirculation identified by the VAI technique was predictive of progression-free survival and overall survival. Notably, although perfusion has a key role in the response to therapy\(^5\), average perfusion values alone could not explain the observed difference between responders and nonresponders. This is in line with previous work showing that changes in perfusion are not likely to have a substantial influence on the relaxation rate curves\(^7\) (that is, vessel vortex curves) and indicates that VAI is a different and potentially more sensitive biomarker than traditional MRI. This may, in part, be explained by the VAI’s apparent sensitivity to changes in \(SO_2\). For responding subjects on day 1 of treatment, the average vessel vortex curve slope did not increase even though the average \(V_f\) decreased, which, at a hypothetical fixed \(SO_2\) concentration, should have resulted in an increased slope value (Supplementary Fig. 4d–f). This suggests that anti-angiogenic therapy improves and normalizes oxygen concentrations, thereby providing benefit to these subjects\(^2,19,23,27\).

In summary, whereas traditional MRI of cancer \textit{in vivo} is confounded by haphazard and heterogeneous vessel architecture with limited or redundant perfusion, the VAI technique exploits these properties and provides further insights into the complex nature of tumor vascularity.

METHODS
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
K.E.E. wrote the manuscript. K.E.E., K.M., A.B., P.Y.W., P.I., T.T.B., B.R.R., R.K.J. and A.G.S. designed the study. D.J., B.R.R. and A.G.S. acquired all of the MRI data. P.Y.W. and T.T.B. acquired all of the clinical data. K.E.E. and A.B. performed the Matlab simulations. K.E.E., K.M., A.B., C.T.F., D.J., R.J.H.B., B.R.R., R.K.J. and A.G.S. analyzed and interpreted the simulations and human data. K.E.E. performed the statistical analysis. K.E.E., K.M., A.B., C.T.F., D.J., R.J.H.B., P.Y.W., P.I., T.T.B., B.R.R., R.K.J. and A.G.S. revised the manuscript critically. K.E.E., K.M., A.B., C.T.F., D.J., R.J.H.B., P.Y.W., P.I., T.T.B., B.R.R., R.K.J. and A.G.S. approved the final version of the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.
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ONLINE METHODS

Monte Carlo simulations. We performed Monte Carlo simulations in MatLab (MathWorks) to assess variations in transverse relaxation rates for the gradient-echo and spin-echo signals as a function of contrast agent concentration and microvascular structure. The general theory behind the relaxation rate simulations has been previously described. In short, the intravascular fraction of tissue is approximated by randomly oriented water-impermeable cylinders with a defined blood volume fraction \( V_B \), radius \( R \), water diffusion \( D \) and susceptibility difference \( \Delta \chi \) between the intracylindrical and extracylindrical space. Vessels were modeled as infinite cylinders under the assumption that the average proton diffusion length during the observation time, equal to the echo time (TE) of the respective gradient-echo and spin-echo sequences, is much shorter than the typical vessel segment length. The \( z \)-component of the magnetic field perturbation of the external magnetic field \( B_0 \) (oriented along the horizontal \( z \) axis of the magnetic resonance magnet) due to each segment can then be approximated by:

\[
\frac{\Delta B_z(\phi, \theta)}{B_0} = \begin{cases} 
\frac{2\pi}{2} \Delta \chi \left( \frac{R^2}{7} \cos 2\theta \sin^2 \theta, \quad r \geq R \right) \\
\frac{2\pi}{3} \Delta \chi (3 - \cos^2 \theta - 1), \quad r < R 
\end{cases}
\]

where \( \theta \) is the angle between \( B_0 \) and the cylinder axis, and \( (\phi, \theta) \) are the polar coordinates of the proton location relative to the projection of \( B_0 \) in a plane orthogonal to the cylinder axis.

A single proton was placed at the origin of the closed simulation space and allowed to randomly diffuse in a plane orthogonal to \( B_0 \) through the intra- or extracylindrical space with total diffusion duration equal to the echo time. The random walk was simulated by arbitrarily changing the orientation of the spin every 0.1 ms using a Gaussian displacement distribution (with mean \( \mu = 0 \) and variance \( \sigma = \sqrt{2D\Delta t} \)) along the orthogonal directions at each time step. The magnetic field perturbation at the proton position from a predefined set of cylinders and the corresponding phase shift were recorded every 0.5 ms. This procedure was repeated for \( n = 5,000 \) protons, and the complex signal, due to the accumulated phase of all of the protons, was defined as follows:

\[
S(t) = \frac{1}{N} \sum_{n=1}^{N} \phi_n(t) = \Im \{ \Delta R \}
\]

where \( \phi_n(t) \) is the phase of the \( n \)th proton at time \( t \). The proton phase accumulated during a time step from the presence of each cylinder was given by \( \Delta \phi_n(t) = \gamma \Delta R \Delta t \), where \( \gamma \) is the gyromagnetic ratio.

For estimations of \( \Delta \chi \) as a function of contrast agent concentration, the baseline magnetic susceptibility of fully oxygenated blood and tissue was assumed to be equal, and \( \Delta \chi \) was assumed to be directly proportional to \([C] \Delta \chi_{\text{Gd}}\), where \([C] \) is the intravascular gadolinium concentration and \( \Delta \chi_{\text{Gd}} = 0.32 \times 10^{-6} \text{ mm}^{-1} \text{ s}^{-1} \), as previously shown. The transverse relaxation effect due to deoxygenated blood was modeled by inclusion of an additional intra- and extravascular susceptibility difference in the resulting arterial, capillary and venous relaxation rate curves, which could be varied between \( \Delta \chi_{\text{Hb, B}} = 2.5 \times 10^{-6} \text{ for fully deoxygenated blood and } \Delta \chi_{\text{Hb, D}} = 0 \text{ for fully oxygenated blood} \). The relationship between arteriole and venule blood hematocrit were considered linear for our study, and the two-dimensional model has previously been shown to provide relaxation rate estimates in very good agreement with in vivo data obtained with an intravascular contrast agent in a rat model.

Representative relaxation rate curves following a simulated contrast agent injection (similar to the curves in Fig. 1c,d) were estimated by coupling the resulting gradient-echo and spin-echo relaxation rates at physiologically meaningful values of \( V_B \) radius and water diffusion to synthetic and typical arteri- capillary- and venous-shaped curves using jSim (National Simulation Resource Physiome initiative). Parametric vessel vortex curves were derived by point-by-point parametric plots of the gradient-echo and spin-echo relaxation rate curves (Fig. 1c,d and Supplementary Fig. 2). The effect of contrast agent extravasation due to the disrupted blood-brain barrier was assumed negligible or corrected for.

Human subjects. The study was approved by the institutional review board and informed consent was obtained from all of the subjects. Subject data included 13 females and 17 males diagnosed with a recurrent glioblastoma, average age 52 years, range 20 to 78 years. After study termination, nine subjects received one subsequent course of salvage chemotherapy, eight subjects received two cycles, one subject received three cycles, two subjects had undisclosed information on salvage chemotherapy, and one subject received stereotactic radiosurgery.

MRI acquisition. Baseline MRI examinations were acquired before therapy onset (days and 1 and 112 after cediranib (AstraZeneca Pharmaceuticals) anti-angiogenic therapy onset or until disease progression, according to the Macdonald criteria. All of the imaging was performed on a 3 Tesla Magnetom Trio MRI system (Siemens Medical Solutions) as follows: T1-weighted images. Axial images were acquired before and after contrast agent injection (gadopentetate dimeglumine (Gd-DTPA), Magnevist, Bayer Schering Pharma AG). Repetition time, 600 ms; echo time, 12 ms; slice thickness, 5 mm; inter-slice distance, 1 mm; in-plane resolution, 0.45:0.45 mm; matrix size 384:512; 23 slices.

T2-weighted (FLAIR) images. Axial images with repetition time 10 s, echo time 70 ms, slice thickness 5 mm, inter-slice distance 1 mm, in-plane resolution 0.60:0.45 mm, matrix size 384:512 and 23 slices.

Dynamic contrast-enhanced (DCE) images. Axial, fast gradient-echo images with repetition time 5.7 ms, echo time 2.73 ms, slice thickness 2.1 mm, inter-slice distance 0.4 mm, in-plane resolution 2.90:2.00 mm, matrix size 128:87 and 20 slices. After approximately 52 s of imaging, a 0.1 mmol kg\(^{-1}\) dose of Gd-DTPA was injected at 5 cc s\(^{-1}\). Also, spoiled gradient-recalled-echo images with five different flip angles (2°, 5°, 10°, 15° and 30°) were initially acquired for T1 mapping.

Dynamic susceptibility contrast (DSC) perfusion images. Axial gradient-echo, spin-echo echo-planar images with repetition time 1.33 s, echo times 34 ms and 103 ms (respectively), slice thickness 5 mm, inter-slice distance 2.5 mm, in-plane resolution 1.70:1.70 mm, matrix size 128:128, 10 slices and 120 volumes. After approximately 85 s of imaging, a 0.2 mmol kg\(^{-1}\) dose of Gd-DTPA was injected at 5 cc s\(^{-1}\).

MRI post-processing. An experienced neuroradiologist identified tumor by outlining enhancing regions on the contrast-enhanced T1-weighted images and peritumoral vasogenic edema on the FLAIR images. The anatomical magnetic resonance images were realigned to the DSC and DCE images using normalized mutual information coregistration. On the T1-weighted tumor outlines, areas corresponding to the tumor center and edge were derived using three-dimensional connectivity morphologic analysis in MatLab, where an
image voxel was assumed to be a center voxel if all neighboring cubical voxels were also outlined as tumor.

The DCE data were processed using custom-made software in MatLab, applying standard approaches to create $K_{trans}$ maps, a measure of the permeability that roughly corresponds to wash-in rates of the contrast agent in tissue.

We obtained relaxation rate curves for VAI analysis, perfusion values, blood volumes and mean transit times using established tracer kinetic models on the DSC images, corrected for contrast agent leakage (from blood-brain barrier breakdown or resection) and fitted to a $\gamma$-variante curve for better visualization of vessel vortex effects. It has been speculated that contrast agent leakage is the reason for the clockwise vortex effect. Not correcting for leakage resulted in an average 5% drop in the clockwise-to-counterclockwise vortex direction ratio with minimal influence on our results. Here, the pre-dose of Gd-DTPA during DCE imaging saturated potential leaky tumor tissue in the DSC images, thereby minimizing the influence of leakage-induced $T_1$-shortening effects.

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Relaxation rate curves not suited for analysis, conveying highly fluctuating time courses from partial volume effects, voxel shifts and physiological pulsations, were excluded from further analysis. Across all 30 subjects, an average of 78.21% ± 12.78% (s.d.) of all of the tumor voxels met the inclusion criteria. To account for potential global systemic effects from hypertension, tumor relaxation rate curves were scaled with the corresponding slice-specific mean normal-tissue reference curves. In all figures showing vessel vortex curves, the tails of the vortex curves have been cut short to better visualize vortex direction. VAI analysis was performed using custom-made software in MatLab, and traditional MRI was analyzed in nordicICE (NordicNeuroLab AS).

Statistical analyses. A subject was assumed to have an increase (decrease) in voxels with a clockwise vortex direction if the clockwise-to-counter-clockwise ratio was higher (lower) than the 95% confidence interval of the population. A subject was assumed to have an increase (decrease) in voxels with a clockwise vortex direction if the clockwise-to-counter-clockwise ratio was higher (lower) than the 95% confidence interval of the population. Differences in VAI parameters during therapy were assessed using pairwise Wilcoxon signed-rank test. Differences in tumor volumes, vessel caliber, permeability, perfusion and mean transit times were assessed using Mann-Whitney tests. Associations between changes in vessel vortex direction ratios and progression-free survival and overall survival were assessed using multinomial logistic regression, Kaplan-Meier survival analysis and Cox regression after adjustments for age, extent of resection, neurological performance, salvage chemotherapy and stereotactic radiosurgery after study termination as well as changes in permeability ($K_{trans}$), $T_2$-weighted contrast-enhanced tumor volume and $T_2$-weighted FLAIR tumor volume before and during antiangiogenic therapy. For all of the tests, $P = 0.05$ was considered significant (with Holm-Bonferroni correction for multiple comparisons), and pixel values below the fifth percentile and above the ninety-fifth percentile were removed before analysis to reduce the influence of outliers. Reproducibility tests were assessed using Spearman Rank correlations and Bland-Altman plots. Statistical analysis was performed using SPSS 17 (SPSS Inc.).

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Erratum: Vessel architectural imaging identifies cancer patient responders to anti-angiogenic therapy

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In the version of this article initially published, the labeling of Figure 2 was incorrect. The three top labels for arterioles should have read as follows (from bottom to top): \( R_{\text{art}} = 7.5 \, \mu m \), \( R_{\text{art}} = 10 \, \mu m \) and \( R_{\text{art}} = 20 \, \mu m \), respectively. The error has been corrected in the HTML and PDF versions of the article.