Antiangiogenic Agent Sunitinib Transiently Increases Tumor Oxygenation and Suppresses Cycling Hypoxia

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Abstract

Structural and functional abnormalities in tumor blood vessels impact the delivery of oxygen and nutrients to solid tumors, resulting in chronic and cycling hypoxia. Although chronically hypoxic regions exhibit treatment resistance, more recently it has been shown that cycling hypoxic regions acquire prosurvival pathways. Angiogenesis inhibitors have been shown to transiently normalize the tumor vasculatures and enhance tumor response to treatments. However, the effect of antiangiogenic therapy on cycling tumor hypoxia remains unknown. Using electron paramagnetic resonance imaging and MRI in tumor-bearing mice, we have examined the vascular renormalization process by longitudinally mapping tumor partial pressure of oxygen (pO2) and microvessel density during treatments with a multi-tyrosine kinase inhibitor sunitinib. Transient improvement in tumor oxygenation was visualized by electron paramagnetic resonance imaging 2 to 4 days following antiangiogenic treatments, accompanied by a 45% decrease in microvessel density. Radiation treatment during this time period of improved oxygenation by antiangiogenic therapy resulted in a synergistic delay in tumor growth. In addition, dynamic oxygen imaging obtained every 3 minutes was conducted to distinguish tumor regions with chronic and cycling hypoxia. Sunitinib treatment suppressed the extent of temporal fluctuations in tumor pO2 during the vascular normalization window, resulting in the decrease of cycling tumor hypoxia. Overall, the findings suggest that longitudinal and noninvasive monitoring of tumor pO2 makes it possible to identify a window of vascular renormalization to maximize the effects of combination therapy with antiangiogenic drugs. Cancer Res; 71(20): 6350–9. ©2011 AACR.

Introduction

Tumors can grow up to 2 to 3 mm³ in size by relying on passive supplies of nutrients and oxygen. For further growth, tumors activate angiogenesis pathways to develop new vascular networks (1). In normal processes such as wound healing, angiogenesis is tightly regulated and creates a balance between pro- and antiangiogenic factors. However in tumors, the balance is tilted toward promoting angiogenesis, causing the development of architecturally and functionally abnormal vasculature (2, 3). The aberrant tumor blood vessel is neither as efficient nor well organized in delivering oxygen and nutrients (4–6). The consequent hypoxic and acidic microenvironment diminishes tumor responsiveness to treatments (7).

Tumor hypoxia can be categorized into 2 types: chronic or cycling hypoxia. Chronic hypoxia exists in tumor regions beyond the diffusion distance of oxygen. Longitudinal oxygen gradient, where the vascular oxygen concentration remains low, in tumor blood vessels makes the radial oxygen diffusion distance shortened, leading to chronic hypoxia (8). Cycling hypoxia, also known as acute or intermittent hypoxia, has been attributed to fluctuations in tumor perfusion and erythrocyte flux (9, 10). Chaplin and colleagues reported in preclinical studies that at least 20% of solid tumor cells experience cycling hypoxia (11). One consequence of cycling hypoxia is increased resistance to treatments by conferring tumors with cells and endothelial cells of tumor blood vessels with enhanced prosurvival pathways (12, 13). These observations make cycling hypoxia a common hallmark existing in a tumor microenvironment. Baudelet and colleagues have noninvasively observed the characteristic fluctuations of transversal relaxation time (T₂*)-weighted MRI signal in solid tumors attributed to physiologic noise that in turn...
may correlate with instability of tumor oxygenation (14). These fluctuations were suppressed by treatments including carbogen combined with nicotinamide and flunarizine. Although the signal interpretation of $T_2^*$-weighted MRI is complex with regard to the absolute value of partial pressure of oxygen ($pO_2$), such noninvasive and longitudinal imaging approaches to study the temporal dynamics of $pO_2$ would be useful to identify effective treatments targeting cycling tumor hypoxia.

Antiangiogenic drugs have been shown to exhibit efficacy in selectively destroying tumor blood vessels in experimental animals and in humans (1). Although as a monotherapy, antiangiogenic agents yielded modest success in human trials, they are being explored in combination therapies with cytotoxic cancer therapies (15, 16). Although decrease in delivery of oxygen and therapeutics to the tumor should be expected on antiangiogenic treatments, numerous reports point to the significant benefit in patient survival when used with chemotherapy or radiotherapy, suggesting an improvement of tumor oxygenation and perfusion (17–19). To explain this paradox, Jain and colleagues put forward a hypothesis that tumor vasculature transiently normalizes during the course of antiangiogenic treatments, where the immature and ineffective vessels get pruned, making the residual vessels structurally competent with improved function (20). Further research was carried out to serially monitor changes in the vascular normalization process that identify a window, in which chemotherapy or radiotherapy was delivered with maximal therapeutic gain (17, 21, 22). A major challenge identified in this effort is the development of noninvasive imaging biomarkers of the vascular normalization process in tumors (20). Optimization of antiangiogenic therapies is complicated by the fact that these agents are not directly cytotoxic to malignant cells, making tumor growth kinetics an unreliable approach to identify the vascular normalization window (23). Useful criteria for an imaging modality would be noninvasive and capable of longitudinally monitoring tumor physiology and would provide a surrogate quantitative biomarker for tumor blood flow/perfusion (18, 20, 22).

Electron paramagnetic resonance (EPR) is a spectroscopic technique similar to nuclear MRI but detects paramagnetic species. Recent availability of triarylmethyl (TAM) radical derivatives as in vivo compatible paramagnetic tracers made EPR imaging (EPRI) capable of mapping tissue $pO_2$ (24, 25). The sequential imaging with EPRI for tissue oxygen and conventional MRI for anatomy in a system operating at a common resonance frequency provides anatomically overlaid $pO_2$ maps with tumor microvessel density maps (25). In the present study, we report the results from the imaging experiments monitoring tumor $pO_2$ to identify the tumor vascular renormalization window and optimize sequence of combination therapy of antiangiogenic drugs and radiation. The normalization window in tumor oxygenation in response to antiangiogenic treatment was noninvasively visualized, and radiation treatment during this narrow time period resulted in synergistic tumor growth delay. Furthermore, the temporal resolution of EPRI made it feasible to obtain 3-dimensional (3D) $pO_2$ maps every 2 to 3 minutes and enabled to distinguish the phenomenon of cycling hypoxia (12, 26). The results show that the consequence of sunitinib treatment is a decrease in the extent of cycling hypoxia in tumors.

### Materials and Methods

#### Tumor implantation

We established SCCVII tumors (obtained from Dr. T. Phillips, UCSF, San Francisco, CA, and was tested in 2011 by RADIL using a panel of microsatellite markers) in mouse hind leg as described previously (25). Tumor-bearing female C3H mice were treated daily with oral administration of 50-mg sunitinib (LC Laboratories) per kg body weight 6 or 10 days after tumor implantation. X-ray irradiation (10 Gy) was delivered 6 or 10 days after tumor implantation using an X-RAD 320 (Precision X-ray Inc.) with or without pre-treatment of sunitinib. We carried out all our procedures in compliance with the Guide for the Care and Use of Laboratory Animal Resources (27), and experimental protocols were approved by the National Cancer Institute Animal Care and Use Committee.

#### EPRI for $pO_2$

Technical details of the EPR scanner and oxygen image reconstruction were described in Supplementary Data S1. Parallel coil resonators tuned to 300 MHz were used for EPRI and MRI. After the animal was placed in the resonator, TAM (1.125 mmol/kg bolus) was injected intravenously under isoflurane anesthesia. The repetition time was 6.0 microseconds. The free induction decay (FID) signals were collected following the radiofrequency excitation pulses under a nested looping of the $x$, $y$, and $z$ gradients and each time point in the FID underwent phase modulation enabling 3D spatial encoding. Because FIDs last for 1 to 5 microseconds, it is possible to generate a sequence of $T_2^*$ maps, that is, EPR line width maps, which linearly correlate with local concentration of oxygen and allows pixel-wise estimation of $pO_2$.

#### MRI for anatomy and blood volume

MRI scans were conducted using a 7T scanner (Bruker BioSpin MRI GmbH). $T_2^*$-weighted anatomic images were obtained using a fast spin echo sequence (RARE) with an echo time of 13 milliseconds, repetition time of 2,500 milliseconds, RARE factor 8, and resolution of $0.125 \times 0.125$ mm. For convenience of coregistration with EPRI, all MRI images had the same slice thickness of 2 mm and field of view of 3.2 cm with 16 slices or 2.8 cm field of view with 14 slices. For blood volume calculation, spoiled gradient echo sequence images were collected before and 5 minutes after ultrasmall superparamagnetic iron oxide (USPIO; BioPAL Inc., colloidal size of 30 nanoseconds) injection (1.2 μL/g body weight) with the following parameters: matrix $= 256 \times 256$; echo time $= 5.4$ milliseconds; and repetition time $= 250$ milliseconds. The percentage of tumor blood volume was estimated as described previously (28). Coregistration of EPRI and MRI images was accomplished using code written in MATLAB (MathWorks) as described previously (25).
**Immunohistochemical analysis**

Tumor tissues were excised on hour after intravenous injection of a pimonidazole (60 mg/kg). Tumor tissues were fixed with 4% paraformaldehyde and frozen, and 10 μm thick sections were obtained. After blocking nonspecific binding sites, the slides were covered by CD31 antibody (BD Biosciences; 1:250) combined with αSMA antibody (Abcam Inc.; 1:250) or rabbit anti-pimonidazole antisera (Natural Pharmacia International, Inc.; 1:250) overnight at 4°C. The sections were incubated with Alexa Fluor 488 anti-rat and Alexa Fluor 555 anti-rabbit secondary antibody (Invitrogen; 1:500).

**Statistical analysis**

All results were expressed as the mean ± SEM. The differences in means of groups were determined by the Student t test. The minimum level of significance was set at P < 0.05.

**Results**

**Tumor pO2 can be noninvasively and longitudinally monitored by EPRI**

The collisional interaction between molecular oxygen and the paramagnetic TAM leads to broadening of the EPR...
spectral line widths of TAM. By extracting the pO2-dependent EPR line width distribution of TAM, an in vivo pO2 map can be generated where TAM is present at detectable levels (25). Figure 1A (top) shows anatomic images taken on days 8, 10, and 12 and an image representing the microvessel density (MRI) taken on day 12 of a squamous cell carcinoma (SCC) tumor–bearing control (untreated) mouse. The corresponding pO2 maps from the same time points are shown in Fig. 1A (bottom), along with an independent immunohistochemical analysis of microvessel density (CD31, green) and pericytes (αSMA, red). Longitudinal pO2 imaging showed that the extent of hypoxia increased with tumor size. The hypoxic fraction (pO2 <10 mmHg) was 24.2% ± 6.8% on day 8 and increased to 40.9% ± 6.1% and 47.4% ± 8.3% on days 10 and 12, respectively. Blood volume images obtained by MRI using the blood pool contrast agent USPIO as an in vivo marker of microvessel density (28) showed substantial vascularization in this tumor. However, immunohistochemical analyses indicated that the tumor blood vessels had inadequate pericyte coverage, suggesting inefficient oxygen delivery by these vessels (4, 6), consistent with the hypoxic nature of this tumor.

**Early treatment with an antiangiogenic drug delays progression of tumor hypoxia**

In earlier preclinical studies with antiangiogenic drugs, a renormalization of tumor vasculature along with a transient improvement in oxygen status was observed (17, 22). We hypothesized that if the vascular normalization is occurred by sunitinib treatment, tumor oxygenation improves simultaneously with reduced vascular microvessel density under the assumption of unchanged cellular oxygen consumption. To noninvasively study this phenomenon, daily sunitinib...
treatment was initiated at an early stage before tumors became hypoxic (6 days after SCC tumor implantation, hypoxic fraction <3%). In mice receiving early antiangiogenic treatment initiation, sunitinib significantly delayed the SCC tumor growth (Fig. 1B). When the pO2 status and microvessel density were evaluated by consecutive EPRI and MRI scans, a different pattern emerged. The pO2 levels in treated tumors were transiently higher than in untreated mice (P < 0.01) followed by a monotonous decrease (Fig. 1C), whereas approximately 40% reduction in the microvessel density as assessed by MRI (Fig. 1D) and immunohistochemistry (Fig. 1B, bottom) was observed after 2 to 6 days of sunitinib treatment. The increase in tumor pO2, which was accompanied by a decrease in microvascular density, after sunitinib treatment compared with untreated controls may be attributed to a phenomenon known as transient vascular renormalization, where the delivery of nutrients including oxygen improves as a result of pruning immature blood vessels and the subsequent recruitment of pericytes (19, 22). Further continuation of sunitinib treatment resulted in hypovascularity for up to 2 weeks after initiating treatment, at which time the tumors became severely hypoxic compared with the size-matched control tumors.

**Antiangiogenic treatment at a later stage improves tumor oxygenation by vascular normalization**

In addition to vascular normalization, sunitinib-induced suppression of tumor growth might contribute to the improved tumor oxygenation. To investigate this, sunitinib treatment was initiated at a later stage (SCC day 10), when the tumors became significantly hypoxic (hypoxic fraction ~35%). Figure 2 shows images of tumor pO2 and microvessel density from SCC tumors treated with sunitinib 10 days after tumor implantation. Day 0 corresponds to images before treatment. The other images were taken on days 2 and 4 after initiating sunitinib treatment. Even in this tumor, which had significant hypoxic regions, tumor oxygen levels increased 2 and 4 days after sunitinib treatment compared with oxygen level before treatment (Fig. 2A, top). It should be noted that no significant change in tumor size occurred during these time points (835 ± 44 mm3 before treatment and 822 ± 89.5 mm3 4 days after treatment), despite a 45% reduction in tumor blood volume [Fig. 2A (bottom) and E]. In frequency histograms of this tumor, a right shift of tumor pO2 was observed (Fig. 2B) with a concomitant left shift of the blood vessel density (Fig. 2C). The transient increase in tumor pO2 was quantified (Fig. 2D), as was the loss in microvessel density (Fig. 2E).

In 2 instances (Figs. 2A and 3A), the tumor size and shape difference between before and 4 days after sunitinib treatment were less than 3%. This permitted monitoring individual voxel-based changes in pO2 (Fig. 3A) and blood volume (Fig. 3B) before and after sunitinib treatment. In the scatter plot of data from these 2 experiments (Fig. 3C), a left shift of the data in each row from the untreated (left column) to the treated (right column) indicates a decrease in tumor...
microvessel density and an upward shift of the data from the left column to the right column in each row would indicate an improvement in tumor pO₂. The analyzed data were classified into 4 groups on the basis of pO₂ levels before and after sunitinib treatment as follows: (i) pO₂ levels more than 10 mm Hg before and after treatment (black circles), which may represent tumor regions that are normoxic and covered with functional vessels; (ii) pO₂ levels less than 10 mm Hg before and after sunitinib treatment, which may represent hypoxic regions whose vasculature was not responsive to sunitinib treatment (red circles); (iii) pO₂ levels less than 10 mm Hg before and more than 10 mm Hg after sunitinib treatment (blue circles), which may represent regions with normalized vasculature after sunitinib treatment; and (iv) pO₂ levels more than 10 mm Hg before and less than 10 mm Hg after sunitinib treatment (green circles), regions where sunitinib may have destroyed the vasculature substantially such that the effect of the hypovasculature may have overwhelmed the beneficial effect of vascular normalization. The results from mouse 1 (the same mouse shown in Fig. 2) showed that a significant fraction of pixels in the tumor that was hypoxic before sunitinib treatment (blue circles) displayed pO₂ increase to a level more than 10 mm Hg with a concomitant decrease in blood vessel density. In addition, the average pO₂ values of all groups showed an increase, suggesting a global vascular normalization in the tumor (Supplementary Data S2). Similar results were observed in mouse 2 (Fig. 3C), but the improvement in tumor pO₂ was relatively limited and also some regions of significant hypoxia were established after treatment (green circles).

Vascular normalization with sunitinib increases oxygen diffusion distance and reduces hypoxic fraction in tumors

The noninvasive observation of transiently improved tumor oxygenation by EPRI after antiangiogenic treatment was further validated by histologic analysis. Immunohistochemical evaluation of pimonidazole (Fig. 4A), a hypoxia marker, shows that the hypoxic fraction of SCC tumors decreased by 9% and 7% after 4 days of sunitinib treatment initiated at days 6 and 10, respectively. Microvascular density (CD31 staining, Fig. 4B) decreased 40% 4 days after sunitinib treatment, whereas no

Figure 4. Vascular normalization with sunitinib increases oxygen diffusion distance and reduces hypoxic fraction in tumors. A, immunostaining of hypoxia marker pimonidazole of representative tumors. Left, untreated control (SCC day 10); middle, 4 days after sunitinib treatment initiated on SCC day 6; and right, 4 days after sunitinib treatment initiated on SCC day 10. B, double staining of endothelial cell (CD31, green) and hypoxia (pimonidazole, red) show elongated oxygen gradients after 4 days sunitinib treatment. C, summary of immunohistochemical analysis. *, P < 0.05; **, P < 0.01. Sunit, sunitinib; pimonidazole, pimo.
significant change was observed in the number of pericytes (αSMA, Fig. 4C). The average distance between microvessels (CD31) and the edge of hypoxic regions (pimonidazole) was measured to represent oxygen gradient distance and was determined to be 75 μm in untreated control tumors, which increased to 110 μm 4 days after sunitinib treatment (Fig. 4B and C). These histologic results are consistent with previous observations and support noninvasive assessment of transient vascular normalization and resultant improvement of tumor oxygenation after antiangiogenic treatment.

**Transient increase in tumor oxygenation by antiangiogenic treatment enhances outcome of radiotherapy**

As hypoxic cells show resistance to radiation, a transient increase in tumor oxygenation has a potential to improve treatment effect of radiation. A combination of 10 Gy radiation at the end of 4 days sunitinib treatment synergistically delayed the tumor growth (8 days) compared with monotherapy of radiation (2-day delay) or 4 days of sunitinib treatment (2-day delay, Fig. 5). Collectively, microenvironmental changes resulting from normalization of tumor blood vessels by antiangiogenic treatment contributed to the augmented efficacy of radiotherapy during the window of improved tumor oxygenation that can be directly monitored with EPRI.

**Vascular normalization with sunitinib suppresses cycling hypoxia**

In recent studies (12, 26), the temporal fluctuations of tumor pO2 in the various tumor subregions were examined to distinguish chronically hypoxic tumor regions from transiently hypoxic regions, a phenomenon known as cycling hypoxia. It was found that there was a significant spatiotemporal heterogeneity in the dynamics of tumor pO2. To examine the effect of antiangiogenic agents in modifying these spatiotemporal fluctuations in tumor pO2, dynamic oxygen imaging experiments were carried out in treated and untreated mice. Figure 6A shows a series of snapshot EPRI images taken every 3 minutes over a period of 30 minutes in untreated animals. For the 4 regions of interest (ROI), marked ROI-1 to ROI-4, the temporal pO2 changes are displayed in Fig. 6B. The results show that there were no significant temporal fluctuations in ROI-2, typical of chronically hypoxic regions, whereas ROIs 1, 3, and 4 displayed features characteristic of cycling hypoxia, with pO2 fluctuations of approximately 20 mm Hg were noticed. When similar experiments were carried out on sunitinib-treated animals and the various ROIs examined (Fig. 6C), it can be seen that in addition to the chronically hypoxic region, ROI-2, which displays steady levels of pO2 less than 10 mmHg, ROIs 1, 3, and 4 show an improved and more stable pO2 levels (Fig. 6D). Similar results were obtained in 4 independent mice for both untreated and sunitinib-treated groups. Figure 6E shows SDs of tumor pO2 (pO2 SD) maps of the treated and untreated mice calculated from the 10 images taken in the 30-minute time window. This parametric image can visualize the locations and extent of temporal pO2 fluctuations, and regions with high pO2 SD (>6 mmHg) were observed in the large majority of untreated tumors whereas limited area of high pO2 SD in sunitinib-treated tumors. Averaged pO2 SD values in tumor regions decreased by 25% 4 days after sunitinib treatment compared with size-matched untreated control tumors (Fig. 6E, right). This observation from EPRI experiments provides new information that the vascular normalization by antiangiogenic treatment minimizes cycling hypoxia resulting from temporal pO2 instability.

**Discussion**

The transient vascular normalization resulting from antiangiogenic cancer treatments presents a window of opportunity to augment treatment with radiation and/or chemotherapy to realize additive or synergistic responses in treatment (20–22). Noninvasive imaging biomarkers that can quantitatively and longitudinally monitor physiologic changes in tumor microenvironment in response to antiangiogenic therapies will be of significant value where the classic endpoints in cancer treatment such as tumor shrinkage may not apply (23, 29). Such capabilities will be especially useful when planning combination therapies (1, 22). By using the image contrast provided by molecular oxygen to a paramagnetic tracer such as TAM, EPRI can provide quantitative maps of tissue pO2, a key determinant of radiotherapy. Anatomic guidance with MRI allows the spatial coregistration of tissue/tumor oxygenation in a straight forward manner (25).

Figure 5. Transient increase in tumor oxygenation by antiangiogenic treatment enhances outcome of radiotherapy. Tumor growth kinetic study was carried out on groups of tumor-bearing mice that include untreated control (black circles), single 10 Gy radiation at SCC day 10 (blue circles), 4 days sunitinib treatment during 6 to 10 days after SCC implantation (red circles), and 4 days sunitinib followed by single 10 Gy radiation (green circles). Sunit, sunitinib. p. o., per os.
The results from the present imaging and histologic studies show that, after starting sunitinib treatment, there is a time window when tumor oxygenation in treated mice is significantly higher than in the untreated controls. The improved oxygenation, which is accompanied by a decrease in blood vessel density, suggests that the residual blood vessels had improved function in terms of delivering oxygen and nutrients, in agreement with earlier reports (17, 22, 30). Interestingly, Ansiaux and colleagues reported that antiangiogenic drugs SU5416 and vandetanib increased tumor oxygenation by a decrease in oxygen consumption (31, 32). Such other mechanism of transient increase in tumor oxygenation may be also involved in the case of sunitinib and further investigations remain required. Even when sunitinib treatment was started at a later stage in the tumor growth where there is already significant hypoxia, a similar profile of improved oxygenation was observed. The large proportion of hypoxic tumor regions that became oxygenated after sunitinib treatment (blue circles in Fig. 3C) represent tumor regions that can be expected to be responsive to radiotherapy. In addition, a synergistic delay in tumor growth was observed when radiation was delivered during the improved tumor oxygenation after 4 days of sunitinib administration (Fig. 5). These results support the capability of EPRI to longitudinally and noninvasively visualize tumor pO2, allowing us to monitor and adjust the impact of antiangiogenic drugs on individual tumors and optimize benefit of combined therapy of antiangiogenesis and other treatments.

Cycling hypoxia is now a well-recognized hallmark of solid tumors (9, 12). The cycle of hypoxia/normoxia induces accumulation of hypoxia-inducible transcription factor-1 in both tumor cells as well as supporting endothelial cells, promotes cancer cell phenotypes with enhanced prosurvival pathways, and acquires resistance to therapy with increased malignant potential (13, 33, 34). However, until now, treatments currently did not consider the existence of cycling hypoxia, nor examine the consequence of antiangiogenic agents on cycling tumor hypoxia. The phenomenon of cycling hypoxia has been originally observed as a consequence of radiobiological experiments and investigated in detail using histologic approaches and subsequent window-chamber experiments (9, 33). Because a priori knowledge of location and frequency of cycling hypoxia may help plan the treatment regimens, noninvasive imaging techniques are being actively explored to monitor this phenomenon with required spatial and temporal resolutions.

Figure 6. Vascular normalization with sunitinib improves cycling hypoxia in SCC tumors. A, dynamic 3D EPRI images obtained every 3 minutes noninvasively visualized fluctuating pO2 in an untreated control SCC tumor. B, four ROIs, 1 to 4, were selected and the pO2 fluctuations were plotted. C, in independent experiment, 4 days of sunitinib treatment reduced extent of temporal fluctuations in tumor pO2. D, temporal pO2 fluctuations in ROIs 1 to 4 were plotted. E, SD of pO2 fluctuation in tumor calculated from sequential 10 scans for 30-minute measurement time. Sunit, sunitinib.
(12). EPRI provided a noninvasive capability to obtain 3D \( pO_2 \) maps within 3 minutes. Serial oxygen mapping with EPRI in a time window of 30 minutes enabled to spatially distinguish cycling hypoxic regions from chronically hypoxia regions in the tumors (26). To quantitatively visualize the extent of cycling hypoxia, the parametric image (SD of \( pO_2 \) map) was calculated from 10 individual images in 30-minute time window. The results in this study show, for the first time, that cycling hypoxia in tumors can be suppressed by sunitinib. Thus antiangiogenic treatment might, in addition to its well-known modes of action alone or in combination therapy, prevent prosurvival pathways that can be acquired by cycling hypoxia.

Jain and colleagues reported that the combination of MRI-based parameters with circulating collagen IV can predict survival of glioblastoma patients after anti-VEGF treatment (30). Combination of such blood markers with the \( pO_2 \) and blood vessel density values may improve the accuracy of these types of predictions. The present study showed that the methodology developed here has the capability to non-invasively and longitudinally monitor spatial and temporal changes in tumor \( pO_2 \) before and after antiangiogenesis treatment and to successfully visualize the improvement in cycling hypoxia during the vascular normalization window, which results in enhanced efficacy of combined radiation therapy. EPRI can use the experience from MRI to scale up for human use, making it a promising modality for integration into clinical settings.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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