Magnetic Resonance Imaging – Measured Blood Flow Change after Antiangiogenic Therapy with PTK787/ZK 222584 Correlates with Clinical Outcome in Metastatic Renal Cell Carcinoma

Cedric de Bazelaire,1 David C. Alsop,1,4 Daniel George,3 Ivan Pedrosa,1,4 Yongyu Wang,5 M. Dror Michaelson,2 and Neil M. Rofsky1,4

Abstract

Purpose: To measure changes in tumor blood flow following treatment with PTK787/ZK 222584, a pan – vascular endothelial growth factor receptor tyrosine kinase inhibitor, and their association with clinical response in patients with metastatic renal cell carcinoma.

Experimental Design: In 10 patients with metastatic renal cell carcinoma treated with PTK787/ZK 222584, tumor blood flow was evaluated by arterial spin labeling (ASL) magnetic resonance imaging before and 1 month on treatment. Changes in blood flow after 1 month of treatment were compared with bidimensional tumor response at 4 months of treatment using the Mann-Whitney test.

Results: Changes in blood flow at 1 month and changes in tumor size measured at 4 months or at time of disease progression were significantly correlated ($P = 0.01$). Patients with progressive disease within 4 months on treatment ($n = 4$) had a nonsignificant increase in tumor blood flow at 1 month ($+25 \pm 33\%$; $P = 0.43$), whereas patients with stable disease or partial response at 4 months ($n = 6$) had a significant decrease in tumor blood flow at 1 month ($-42 \pm 22\%$; $P = 0.02$).

Conclusion: These results suggest that decreasing tumor blood flow with PTK787/ZK 222584 therapy, as shown as soon as 1 month on therapy by ASL, may predict for a favorable clinical outcome. These data are consistent with a hypothetical functional role for tumor ischemia in the mechanism of response to anti-vascular endothelial growth factor therapy. ASL blood flow magnetic resonance imaging shows promise as an early predictor of clinical response to antiangiogenic therapies.

The formation and maintenance of tumor vasculature, so-called angiogenesis, is a hallmark of cancer and has become a very promising target for experimental therapies in oncology (1, 2). Antiangiogenic therapy has shown great promise in preclinical animal models (3, 4), and some agents have shown clinical efficacy in humans. Clinical response to antiangiogenic therapy is highly variable, however, and this variability reduces clinical effectiveness and complicates clinical trials of new compounds. Better control of this variability in clinical trials

and possibly better targeting of compounds to individual patients would be possible if noninvasive markers of angiogenesis and related molecular and functional processes were available. Such markers would also aid identification of better compounds by providing specific information on the mechanisms of success and failure in clinical application.

Unfortunately, noninvasive biomarkers for angiogenesis are still in relatively early stages of refinement. Preliminary results with serum measures, including plasma vascular endothelial growth factor (VEGF) and circulating endothelial cells, show only modest success (5). The most accepted imaging measure of tumor response, tumor size changes according to Response Evaluation Criteria in Solid Tumors or WHO criteria, may be particularly inappropriate for antiangiogenic therapy characterization. To the extent that angiogenic inhibition blocks new vascular formation, tumor regression may be difficult to achieve; most clinical studies to date suggest that the primary effects of antiangiogenic agents are cytostatic (6, 7). Hence, imaging measures that can reflect the antiangiogenic activity of inhibitors in patients more accurately than tumor volume are required.

Several imaging methods for assessment of tumor vascularity and angiogenic activity have been proposed. Molecular imaging methods targeted at highly specific markers of angiogenesis are under development (8, 9) but will require years of testing before use and validation in humans. Measurement of blood

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Requests for reprints: David C. Alsop, Department of Radiology, Ansin 226, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215. Phone: 617-667-0275; Fax: 617-667-7917; E-mail: dalsop@bidmc.harvard.edu.

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volume or microvascular blood volume with magnetic resonance imaging (MRI; refs. 10, 11) has been suggested as an indicator of tumor aggressiveness and response to therapy but methodologic challenges and limited sensitivity have restricted its use in clinical trials. Measurement of the rate of uptake of MRI contrast agents has been evaluated in animals as an indicator of response to antiangiogenic therapy and decreases in uptake have been reported in clinical trials (7, 12, 13). Contrast uptake is determined both by vascular permeability and by blood flow, so the independent effect of antiangiogenic therapy on the two cannot be assessed. The recent appreciation of potential harmful effects from MRI contrast agents in patients with renal insufficiency (14) also discourages the use of contrast in some patients with renal cancer. Although nuclear medicine methods for the measurement of blood flow can be used, low spatial resolution and sensitivity and abnormal uptake of flow tracers in tumors (15) limit their use.

Blood flow can also be measured with a newer MRI technique known as arterial spin labeling (ASL; ref. 16). This technique changes the sign of the water signal in blood outside of the imaged slice. When arterial blood with negative signal enters the tissue, a decrease in signal of the tissue is measurable. A considerable literature on quantification of flow with this technique (16, 17) and validation studies in the brain (18) exists. Here, we report the measurement of blood flow to metastases of renal cell carcinoma in 10 patients undergoing antiangiogenic treatment using PTK787/ZK 222584 (vatalanib, Novartis Pharmaceuticals and Schering AG), an oral angiogenesis and lymphangiogenesis inhibitor that blocks tyrosine kinase signaling from all known VEGF receptors. The feasibility to monitor antiangiogenic therapy using blood flow changes at 1 month of therapy was evaluated.

### Materials and Methods

**Clinical trial.** A phase I/II dose-escalation study of open-label, orally administered PTK787/ZK 222584 in patients with metastatic renal cell carcinoma was approved by the Institutional Review Board of the Dana-Farber Harvard Cancer Center and pursued between May 1999 and May 2002. PTK787/ZK 222584 is an oral angiogenesis inhibitor that blocks vascular endothelial cell growth factor (VEGF) receptor tyrosine kinases, which are important mediators of growth, migration, and survival of endothelial cells. Preliminary data from the open-label, phase I trial, including 49 patients, showed that PTK787/ZK 222584 was well tolerated at once-daily doses of 300 to 1,500 mg (19). Maximum tolerated dose was not defined. At 3 mo, 19% of patients achieved measurable tumor response and an additional 60% had stable disease. Median time to progression was 5.3 mo and median overall survival was estimated at 21 mo.

An expansion cohort of 27 patients was initiated at 1,200 mg/d to determine the biological activity of this dose level using blood flow monitoring with MRI techniques (described below). Inclusion of the

![Fig. 1. Axial T2-weighted (left column) and blood flow (right column) MR images of a metastasis in the right pubic bone before therapy (A), at 1 mo of treatment (B), and at 4 mo of treatment (C). The shrinkage of the tumor became significant (>50%) after 4 mo of therapy. The color scale image shows the blood flow signal in the tumor obtained with ASL. Box, before therapy, ASL detected a high blood flow in the metastasis. After 1 mo of treatment, the blood flow of the tumor had dramatically decreased and was not detectable after 4 mo of treatment. Small arrowhead, the version of ASL used labels all the blood in the arteries, so high signal intensity was present in the large arteries within the slab as seen in the right femoral vessels.](http://www.aacrjournals.org/clinicanal.jpg)
subjects required diagnosis of renal cell carcinoma and evidence of progressive disease. Approximately two thirds of our study population had prior exposure to systemic therapies, including agents with putative antiangiogenic properties such as IFN and thalidomide. All systemic therapies were discontinued at least 4 wk before enrollment. No attempt was made to reduce variability in blood flow by limiting consumption of caffeine or nicotine or excluding subjects taking prescribed drugs that might affect blood flow. Blood flow measurement with ASL MRI and anatomic imaging assessments with MRI and computed tomography (CT) were done at screening as well as at 1-, 2-, and 4-mo time points on study and every 2 mo thereafter.

Computed tomography. Although high-resolution MRI was feasible in abdomen, pelvis, and thorax, anatomic imaging for lung tumors was done using CT with better spatial resolution. Hence, chest and limited upper abdominal CT was done with a helical scanner (Somatom Plus S, Siemens). Slice thickness was 5 mm with a pitch of 1.6. Images were reconstructed at 5-mm intervals. During CT, 100 mL of contrast medium (Ultravist 300, Berlex Laboratories) were administered i.v. at a rate of 2.0 mL/s with a power injector.

Magnetic resonance imaging. All imaging was done in a GE Signa Vh/i 3 Tesla scanner using the body coil for transmit and a surface coil array for reception (Gore Electronics). The imaging protocol included blood flow imaging with ASL and a comprehensive morphologic analysis. The ASL imaging protocol and analysis has been previously described (20). Briefly, single-slice pulsed ASL was done with a single-shot fast spin echo sequence, an in-plane spatial resolution of 6.3 mm, and a slice thickness of 8 mm. Four spin-labeled and control image pairs were acquired within a single breath hold. Background suppression inversion pulses (21) were added to reduce the variance introduced by motion. A quantitative mapping of T1 relaxation was also done (22). For T1 relaxation and blood flow measurements, a single slice through the largest part of the lesion was selected. If more than one lesion was present, the largest lesion was selected.

Morphologic analysis included breath hold axial T2-weighted single-shot fast spin echo (echo time, 64.5 ms; field of view, 40 × 28 cm; Matrix, 256 × 160; section thickness, 4–5 mm) and breath hold three-dimensional ultrafast gradient echo (echo time, 2 ms; repetition time, 4.5 ms; field of view, 38 × 22 cm; Matrix, 288 × 160; section thickness, 4–5 mm), the latter acquired prior to, during, and after injection of 0.1 mmol/kg Gd-DTPA (Magnevist, Berlex Laboratories).

Data analysis. Imaging data were obtained and analyzed blinded to clinical information other than the knowledge that subjects had metastatic renal cancer. All results except the ASL images were reconstructed on the scanner and transferred to workstations for analysis. ASL data were saved as raw signals and separately transferred to the analysis workstation. Custom software written within the Interactive Data Language (Research Systems) was used for subsequent analysis.

For each patient, a single metastatic lesion was selected for longitudinal assessment with ASL. T1 estimates in tumor were obtained from the nonlinear least square fit of the signal intensity measured at each T1 value. The detailed methods for fitting the data and accounting for noisy magnitude data are described elsewhere (22, 23).

ASL blood flow measurement calculation was done within a region of interest drawn to include the entire area occupied by the selected metastatic tumor within the imaged slice (20). Perfusion in the tumor region was often nonuniform. For techniques such as dynamic contrast enhancement where image signal is nonlinearly related to blood flow, analyzing the average of the signal within a nonuniform region to determine blood flow is not equivalent to averaging blood flow within the region. However, quantification of flow with ASL is linear in the difference between label and control images, so averaging of ASL signal across a nonuniform tumor does not corrupt flow calculation.
Conventional, anatomic MRI assessments were done by independent, experienced radiologists during routine examination time. The radiologists were blinded to the results of the ASL MRI findings. Tumor sizes were measured with electronic calipers (Advantage Workstation 4, GE Medical System) on high-resolution anatomic images obtained with MRI and CT. Following WHO methodology (24), each tumor was measured in two dimensions: its maximum diameter in the transverse plane and its largest perpendicular diameter on the same image. These diameters were multiplied to yield a cross-product (25). The sum of the products of all metastases was used for comparison between blood flow and size changes. The drug response diagnostic was based on the Response Evaluation Criteria in Solid Tumors criteria (26) using the sum of the longest axes of all tumors.

**Statistical analysis.** Patients were separated into two groups: the responder group, free of any sign of progressive disease at 4 mo of therapy (by anatomic tumor assessments), and the nonresponder group with significant increase of the tumor size or new lesions diagnosed before 4 mo of therapy. Mean blood flows at 1 mo were compared with values obtained at baseline with a paired Student’s t test in both responder and nonresponder groups. The tumor blood flow changes measured at 1 mo in both groups were compared using the Mann-Whitney test. Similarly, the difference in early tumor size changes between groups with and without progression was evaluated. The consistency between blood flow changes at 1 and 4 mo assessed with ASL MRI was measured with the Wilcoxon signed rank test in those subjects having undergone ASL assessment at 4 mo (n = 6).

Early changes at 1 mo in blood flow and tumor size were compared across subjects to tumor size changes at 4 mo with a Spearman rank correlation. Blood flow changes and size changes were ranked from the highest decrease (lowest rank) to highest increase (highest rank). Because four subjects with early progression left the study before imaging at 4 mo, a 4-mo tumor growth measurement was not available. Their progression was identified by size changes, so their tumor growth at 4 mo would presumably have been the largest of the cohort if imaged. To enable testing for a correlation between 4-mo size change and imaging results at 1 mo, the four progressing subjects were all assigned larger 4-mo size change ranks than the other subjects with the earliest progressor given the highest rank.

The correlation between time to progression and 1-mo blood flow changes was compared with the correlation between time to progression and 1-mo size changes with a Spearman rank correlation.

### Results

Acquisition and analysis of early blood flow changes and later disease progression was successfully done in 10 of 27 patients enrolled. Twelve patients were excluded for nontechnical imaging failures, including subjects not participating in either the baseline or 1-month exam (n = 6), early toxicity before any clinical evidence of response (n = 4), failure to scan same tumor due to respiratory misregistration (n = 1), and tumor size smaller than 10 mm (n = 1). On the other hand, technical imaging failures required excluding five patients. Those were early failures due to readily addressable problems with the ASL sequence (20).

In the 10 patients successfully scanned with ASL, target tumors were located in the lung (n = 2), kidney (n = 2), liver (n = 1), bone (n = 2), adrenal glands (n = 1), and lymph nodes (n = 2) of the abdomen. At baseline, the mean tumor size was 58 ± 48 cm² and the mean relaxation time T1 was 1.14 ± 0.24 s.

Before therapy, the mean tumor blood flow was 260 ± 153 mL/min/100 g. Patients with progressive disease within 4 months after treatment with PTK787/ZK222584 (n = 4) had a nonsignificant increase in tumor blood flow at 1 month (+25 ± 33%; P = 0.43, paired Student’s t test), whereas patients

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**Fig. 3.** Axial T2-weighted (left column) and blood flow (right column) MR images of a metastasis of the 10th right rib obtained with MRI before therapy (A) and after 1 mo of therapy (B), when a clear growth (85%) of the tumor was observed. ASL showed a significant increase of the blood flow in the tumor. However, no substantial blood flow change was observed in the liver (large arrowhead) or in the kidney (small arrowhead).
with stable disease or partial response at 4 months \((n = 6)\) had a significant decrease in tumor blood flow at 1 month \((-42 \pm 22\% ; P = 0.02, \text{paired Student’s } t \text{ test})\). The blood flow decrease in the stable disease or partial response groups was significantly greater than in the progressive disease group \((P = 0.03, \text{Mann-Whitney rank test})\). No significant difference between the two groups in tumor size change at 1 month was found \((P > 0.99, \text{Mann-Whitney rank test})\). The difference in blood flow when baseline and 1-month values were compared was similar to that seen when baseline and 4 months were compared \((P > 0.86, \text{Wilcoxon signed rank test}; n = 6)\).

At 1 month into treatment, ASL MRI was able to show significant reductions in blood flow in the stable disease or partial response group, suggesting biological response, at a time when size changes were not apparent; subsequent size changes at 4 months confirmed response according to Response Evaluation Criteria in Solid Tumors criteria (Figs. 1 and 2). Moreover, early progressive disease apparent after 1 month of therapy showed a blood flow increase (Figs. 3 and 4). Among the 10 patients fully evaluable by ASL MRI, a significant correlation (Spearman \(r = 0.85; P = 0.01\)) between the change in blood flow at month 1 and change in tumor size measured (by WHO criteria) at month 4 or the time of disease progression was found. In contrast, no correlation was found (Spearman \(r = 0.19; P > 0.57\)) between size changes at months 1 and 4 (Fig. 5).

Time to progression was correlated with 1-month blood flow changes across all the subjects (Spearman \(r = -0.87; P = 0.008\)). On the other hand, no correlation was found between 1-month size changes and time to progression (Spearman \(r = -0.25; P = 0.45\)) as shown in Fig. 6.

**Discussion**

These preliminary results highlight the value of blood flow measurement in the characterization of tumor response to antiangiogenic therapy. In these cases, early blood flow increase was associated with tumor growth and early blood flow decrease was associated with tumor shrinkage. A greater than 30% decrease in blood flow at 1 month was found in all patients without progression at 4 months. Although this separation of groups is encouraging, our modest sample size limits the ability to estimate the diagnostic accuracy of the ASL method. Longer scans than the 40 s used here could improve reproducibility if respiratory motion–related inaccuracies were adequately minimized. Although our results suggest that the blood flow monitoring of one tumor reflects the response of all metastasis in patients, the value of assessing several, if not all,
tumors needs to be explored. Indeed, ASL measurements can readily be reproduced on several tumors within the same MRI exam, unlike dynamic contrast studies, where tracer injection cannot be repeated the same day (27).

The fact that ASL evaluations do not involve exogenous contrast medium bestows several benefits and opportunities for tumor assessments. With ASL, assumptions regarding the distribution of contrast medium and the relationship between signal intensity and tracer concentration are bypassed. The short half-life of the magnetic label in ASL allows for a countless number of repeat assessments. This, in turn, paves the way for the assessment of physiologic challenges to interrogate the effects of vasomodulation on the tumor vascular by one or multiple means. We chose to measure the tumor blood flow at 1 month after therapy, when tumor size changes are usually not measurable (26). An earlier blood flow measurement might help identify nonresponding patients in whom additional targeted therapies might be warranted and could further probe the blood flow correlates of the decreased contrast uptake that have been observed at 2 days (12). On the other hand, longitudinal blood flow monitoring could be attractive in the detection of potential acquired drug resistance to antiangiogenic drugs as a tumor transitions its reliance to other growth factors for endothelial cell growth (28). This possibility remains controversial (5, 29, 30) and requires further investigations with biomarkers independent of the antiangiogenic compound used.

Future biomarkers of the antiangiogenic therapy could include plasma VEGF, basic fibroblast growth factor, and platelet-derived

Fig. 5. Early changes at 1 mo in blood flow and tumor size compared with tumor size changes at 4 mo with a Spearman rank order correlation. Higher decrease in blood flow tends to be associated with higher decrease in tumor size. Size changes at 1 mo are not predictive of size changes at 4 mo. White points, responder patients at 4 mo; black points, nonresponder patients at 4 mo; gray points, patients who left the study before 4 mo due to progressive disease. Because tumor size at 4 mo was not available for the early progressive patients, their tumor size change rank was replaced with their time to progression rank in this figure.

Fig. 6. Early changes at 1 mo in blood flow and tumor size compared with delay of progression of the disease after initiation of the treatment. A significant relationship exists between blood flow changes at 1 mo and time to progression, whereas no relationship exists between size at 1 mo and time to progression. White and black points, responder and nonresponder patients, respectively. All responder patients had a decrease in blood flow higher than 30%.
growth factor (6). However, preliminary results of studies using these serum marker levels have mixed results showing no consistent correlation with prognosis (5, 31). Molecular monitoring of antiangiogenic response may require specific markers related to targets of an increasing number of antiangiogenic drugs (5, 7). By measuring blood flow changes, ASL MRI assesses the effect of antiangiogenic therapy on tumor blood supply, the ultimate target of vascular-based therapies, and thus it is unlikely to be affected by the exact target of a therapy.

The results of this study, combined with earlier contrast agent results (7, 12), emphasize that PTK787/ZK222584, and similar agents that also inhibit the VEGF-R2 (KDR) signaling pathway, may affect existing tumor vasculature as well as the development of future blood vessels. The dramatic effects on blood flow shown by MRI were observed just 1 month after therapy. Dynamic contrast MRI has already shown utility for a rapid dose-response evaluation of agents (32). Blood flow measurement with ASL MRI has already shown utility for a rapid dose-response evaluation of agents (32). Blood flow measurement with ASL MRI may be an interesting alternative for such an assessment.

Our results suggest that inhibition of tumor vasculature can result in disease regression that follows the decrease in blood flow. This last point supports the hypothesis that reduction of tumor blood flow with PTK787/ZK222584 causes tumor regression. The strong association between blood flow change and tumor response suggests that variability in success at reducing blood flow with PTK787/ZK222584 is responsible for the variability of tumor response. No patient with progressive disease in the study had a significant decrease of blood flow. This may indicate that renal cell tumors do not readily adapt to the more hypoxic environment created by successful angiogenesis inhibition. However, more patients are required to confirm this hypothesis. Improved response to therapy might be realized by adjusting dose or agent to assure reduction of tumor flow.

The wide availability of MRI, its excellent delineation of tumor anatomy, and the potential ease with which an ASL study could be done and analyzed after further development make ASL MRI an attractive candidate for noninvasive measurement of tumor blood flow and monitoring blood flow changes accompanying antiangiogenic therapy and other oncologic procedures. Further technical development and prospective studies of ASL to realize this potential are merited. The results obtained in this population of patients with renal cell carcinoma provide practical support for this potential.

Disclosure of Potential Conflicts of Interest

Y. Wang is employed by Novartis Pharmaceuticals, and Novartis Pharmaceuticals also provided support for this study. D.C. Alsop is an inventor of patents related to ASL MRI and receives research support from GE Healthcare, an MRI scanner vendor, and Merck & Co.

References

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