Abstract

Late stage or aggressive cancers exhibit metastatic growth at multiple sites, and the characterization of treatment response in various organs to drugs with potentially wide-ranging efficacy is needed. Tumor cells that induce angiogenesis are a common characteristic of metastatic disease, and clinically, antiangiogenic therapies have shown value in the setting of advanced cancer. However, recent preclinical studies have suggested that exposure to antiangiogenic drugs can increase tumor invasiveness and metastasis, making it important to determine which contexts antiangiogenic therapy is most appropriate. We describe here the effects of cediranib, a receptor tyrosine kinase inhibitor, in a model of advanced prostate cancer metastatic to skeleton and brain. Treatment with cediranib decreased metastatic tumor burden in the brain and bone, decreased cerebral vasogenic edema, and improved survival, despite increasing the invasive histology of brain metastases. Short-duration cediranib treatment given at the time of tumor cell dissemination was sufficient to inhibit the establishment and subsequent growth of bone metastases, although brain metastases were subject to rebound growth after the discontinuation of cediranib. Distinct growth patterns at different organ sites in the same animal showed that certain tumor microenvironments such as bone may be most amenable to interventions by anti–vascular endothelial growth factor (VEGF) therapies. In addition, anti-VEGF treatment may be of utility in decreasing the rapid growth of solid brain metastases and vasogenic edema in patients with advanced cancer, leading to reduced morbidity and associated clinical benefit. Cancer Res; 70(21): 8662–73. ©2010 AACR.

Introduction

Metastasis is the major cause of morbidity and mortality for cancer patients. Advanced cancers frequently metastasize to distant organs, and multiorgan metastasis is not unusual (1). A common feature of metastases is vascular endothelial growth factor (VEGF)–initiated angiogenesis in the tumor microenvironment (2). Antiangiogenic therapies targeting VEGF and VEGF receptors (VEGFR) have provided survival benefits when combined with chemotherapy for treating patients with late-stage disease, although the benefits are mostly short-lived (3). Recent preclinical studies have suggested that antiangiogenic therapies increase tumor invasiveness and decrease overall survival (4, 5). Although the models that have been analyzed to date are limited, the implications of such preclinical investigations for future clinical trial designs are significant. Thus, it is important to expand the range of metastasis models and to analyze various parameters of response to anti-VEGF therapies, including the tumor microenvironment context of metastatic growth in different organs. This is a consideration for assessing organ-specific risks and benefits and especially relevant in designing therapies for treating multiorgan metastasis.

Bone and brain are common metastatic sites for breast, lung, and prostate cancer, as well as melanoma among others, and patients with such cancers are at risk for suffering metastases in both sites simultaneously (6, 7). Brain is a well-vascularized tissue, and many of the properties expressed by tumors growing in the brain such as endothelial cell proliferation and increased vascular permeability are dependent on VEGF (8). Thus far, most of the studies concerning the interactions of tumor cells and brain vasculature as well as the response of tumors to anti-VEGF therapy are evaluations of malignant gliomas (9, 10). Much less data are available concerning the development of clonal brain metastases and their response to small molecule VEGFR2 inhibitors that penetrate the blood-brain barrier. Similarly, relatively little is known about the vascular remodeling that occurs in developing bone metastases and the response of such metastases to anti-VEGF treatment.

The DU145 line was originally isolated from a brain metastasis of a prostate cancer patient, but the parental DU145 does not form metastases in xenograft models. Previous work has established that the introduction of an activated Ras effector mutant (RasV12G37) resulted in a gain of bone metastatic capability following intracardiac inoculation (11). To
improve metastatic efficiency, the DU145(RasV12G37) cells were passaged in vivo, and the DU145/RasB1 cell line was isolated from a bone metastasis (12). This cell line produces high levels of VEGFA and platelet-derived growth factor B (PDGFB) and metastasizes to bone and brain at a high frequency. In this study, we used cediranib (AZD2171), a tyrosine kinase inhibitor directed against VEGFR1/2 and PDGF receptor, to determine the efficacy of various treatments and to characterize the organ-specific responses to antiangiogenesis regimens in the setting of multiorgan metastasis.

Materials and Methods

Cell culture

DU145/RasB1 cells were infected with the retrovirus pSFGnesTGL, and positive cells were isolated by fluorescence-activated cell sorting (12).

Animal studies

Animal work was performed in accordance with a protocol approved by NIH Animal Care and Use Committee. Intracardiac inoculation and bioluminescent imaging (BLI) were as described (12). See Supplementary Fig. S1 for the correlation analyses between BLI and tumor burden determined histologically. For survival studies, mice were euthanized when one of the following situations applied: 10% loss of body weight, paralysis, or head tilting. All animal studies were repeated three times.

Experimental design

Cediranib was provided by AstraZeneca. Cediranib was dissolved with 1% (w/v) aqueous polysorbate 80 in deionized water and given at 6 mg/kg body weight, a therapeutic dose, by gavaging mice daily. To study the effect of cediranib on mortality, morbidity, and tumor progression, an experimental design as shown in Fig. 1A was used. Each of the four final

![Figure 1](cancerres.aacrjournals.org)
experimental groups contained 9 or 10 animals at the initiation. For combined cediranib and Zometa (Novartis) therapies, the treatments were started 3 weeks after tumor cell inoculation. To quantify osteolytic destruction, long bones were imaged with a Faxtron X-ray machine. The numbers of hyperintensive regions were counted for each long bone and confirmed with histology.

**Magnetic resonance imaging**

T2-weighted axial images, encompassing the whole brain (16 slices), were acquired using a fast spin echo sequence to delineate anatomic details (field of view, 19.2 mm; in-plane resolution, 75 μm; TE/TR, 50 ms/3,000 ms; echo train length, 8; number of averages, 8; slice thickness, 1 mm; 16 slices). Quantitative T2-weighted images of nine 1-mm axial slices (TE/TR, 15/3,000 ms; number of echo images, 16; in-plane resolution, 150 μm; interslice gap, 1.5 mm), with the first slice positioned 1.5 mm anterior to the Bregma, were acquired. Magnetic resonance imaging (MRI) data were processed and analyzed using software routines written in MATLAB (Mathworks, Inc.).

**Histology and immunohistochemistry**

Brain tissue was collected and fixed in 10% buffered formalin for H&E staining or 4% paraformaldehyde for immunohistochemical staining. For morphometric analysis, each mouse brain was coronally cut into four equal quarters; one section from each quarter was analyzed. Mice were injected with bromodeoxyuridine (BrdUrd; Sigma, 70 mg/kg i.p.) 30 minutes before euthanasia. The following antibodies were used: rat anti-BrdUrd (OBT), ApopTag in situ apoptosis detection kit (Millipore), CD34, and αSMA (Abcam). For histomorphometric analysis, bright field microscopic images were collected using an Axioplan microscopy system (Zeiss). Tumor cell proliferation (BrdUrd labeling) and apoptosis were quantified using AxioVision software (Zeiss). Histomorphometric analysis of CD34-stained vessels was performed based on previously described protocols with modifications (13). Blood vessel area was measured at 200× magnification. Coregistration of endothelial cells and pericytes was analyzed through double staining with CD34 and αSMA followed by rhodamine- and FITC-conjugated secondary antibodies, respectively. Fluorescent cells were counted at 400× magnification. To measure tumor invasiveness, the number of invasive edges, defined as clusters of cells outside the contour of the tumor mass, was counted for each tumor and corrected for total tumor area. Tumors were divided into four groups based on the total area of each tumor: extra large (>10 × 10^5 μm^2), large (5–10 × 10^5 μm^2), medium (1.5–4.9 × 10^5 μm^2), and small (<1.5 × 10^5 μm^2). Bones were decalcified in 10% EDTA for 2 weeks before processing.

**Data analysis**

Data were analyzed using Prism software (GraphPad Software, Inc.) by repeated measures ANOVA. Survival was analyzed by log-rank test. Data are expressed as the mean ± SEM, with P < 0.05 considered statistically significant.

**Results**

**Cediranib improved mortality and morbidity of tumor-bearing mice**

It has been previously shown that DU145/RasB1 cells form bone metastasis and highly vascularized brain metastasis after intracardiac inoculation into immunocompromised mice. Moreover, DU145/RasB1 cells secrete VEGFA, PDGFB, and angiogenin, but not angiopoietin-2, epidermal growth factor (EGF), basic fibroblast growth factor, heparin-binding EGF, leptin, or placental growth factor, as determined by ELISA-based quantibody array (Raybiotec, Inc.; ref. 12). To determine the physiologic response of brain and bone metastasis to antiangiogenic therapy, mice were treated with cediranib, a small molecule VEGFR antagonist that is permeable to the blood-brain barrier (9, 14). A graphic depicting the experimental design is shown in Fig. 1A. To determine if cediranib inhibits the growth of established brain and bone metastasis, treatment group mice were given cediranib from week 3 onward when metastases can first be detected using BLI. To determine if cediranib prevents metastatic colonization, prevention group mice were treated at the time of systemic tumor cell inoculation and thereafter for 3 weeks. Mice also were continuously treated in the prevention/treatment group from the time of tumor cell inoculation.

The effects of cediranib treatment on morbidity, one measure of which is body weight, and mortality were evaluated in tumor-bearing mice. As shown in Fig. 1B, the mice in the treatment groups maintained their body weight for the study period, whereas the mice in the control and the prevention groups began losing weight at ∼4 weeks. Consistent with this, cediranib treatment, either continuously or starting after 3 weeks, significantly improved the survival rate of tumor-bearing mice (Fig. 1C). Seventy percent of mice in the vehicle control group did not survive beyond 5 weeks, whereas 50% or more of mice in the treatment groups were still alive after 7 weeks. There was no statistically significant difference between the survival of the control and prevention groups. Most mice in the control group were euthanized as a result of neurologic disorders associated with edema, focal brain dysfunction secondary to multiple nodular tumors in the cerebrum and cerebellum. By contrast, cediranib-treated mice rarely showed neurologic symptoms. The percentage of animals that developed metastasis is detailed in Supplementary Table S1.

**Cediranib decreased brain metastasis burden by inhibiting solid tumor growth**

Continuous treatment with cediranib starting at the time of tumor cell dissemination resulted in significantly less brain metastatic burden after 3 weeks as measured by BLI compared with nontreated animals (Fig. 2A, left). In the prevention arm, which encompasses treatment for the first 3 weeks after inoculation, tumor growth inhibition was maintained for the first week after discontinuation of treatment (week 4), but 2 weeks after the withdrawal of cediranib (week 5), the burden of brain metastasis was similar to the untreated group, suggesting that the inhibitory effects did not last (Fig. 2A, right). In the treatment arm, cediranib inhibited the
growth of established brain metastasis (Fig. 2A, right). The inhibitory effect was obvious even after 1 week of treatment (week 4). At the end point, the brains from each experimental group were analyzed histologically in four coronal sections, distributed equally throughout the brain area. The number of mice displaying tumors and the total number of mice evaluated, as well as the absolute number of specific histologic types of brain metastases. The ratio below the graph is the incidence of brain metastasis in each group.

To evaluate the effect of cediranib treatment on various parameters of tumor morphology, tumors were allowed to develop for 3 weeks after tumor cell inoculation, and subsequently, mice were treated with cediranib for 1 week. Vessel morphology was analyzed using CD34 staining (Fig. 3A). Vessels in both types of tumors seemed dilated, and endothelial cells seemed hypertrophic relative to normal brain. The density of blood vessels in solid tumors in control and cediranib-treated mice is quantified in Fig. 3B. Following cediranib treatment, solid tumors lose blood vessel density mainly in their center while retaining a rim of vessels at the tumor border (Fig. 3A). By contrast, the vessels in infiltrative tumors were relatively insensitive to cediranib (data not shown).

To determine the effects of drug treatment on tumor proliferation and apoptosis, BrdUrd (Fig. 3A) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining (data not shown) were separately performed on serial histologic sections from both control and cediranib-treated mice. BrdUrd staining showed that the presence of...
Figure 3. The effects of cediranib on tumor blood vessel density and tumor cell proliferation. A, CD34-positive endothelial cells and BrdUrd-positive proliferating cells in consecutive sections of brain metastases from control (left) and cediranib-treated (right) mice. Top, large solid tumors; bottom, infiltrative tumors. Scale bar, 100 μm. B, blood vessel areas (left; n = 12) and BrdUrd-positive cells (right) were quantified in solid brain tumors from control and cediranib-treated groups. C, representative double-stained images for CD34-positive endothelial cells in red (left) and αSMA-positive pericytes in green (middle) in a solid tumor. Right, merged image. Scale bar, 20 μm. D, quantification of the ratio of αSMA- and CD34-positive blood vessels in solid and infiltrative tumors. *, P < 0.05.
dividing cells correlated spatially with the pattern of blood vessel density observed on CD34 staining. Solid tumors in the cediranib-treated group showed BrdUrd-labeled cells at the tumor border with significantly fewer BrdUrd+ cells in the body of the tumor compared with tumors in the control group (Fig. 3A). By contrast, there were no significant differences in BrdUrd-labeled cells for the smaller infiltrative tumors when comparing control and cediranib-treated mice (Fig. 3A). The quantification of BrdUrd+ cells in multiple tumors with or without 1 week of cediranib treatment is shown in Fig. 3B. The number of apoptotic cells was not significantly increased in the cediranib-treated mice, although some tumors from cediranib-treated animals displayed necrotic centers (data not shown).

Because VEGF withdrawal can cause angiogenic vessels lacking pericytes to undergo regression whereas those stabilized by pericytes undergo survival, we investigated whether vessel survival was correlated with coregistration of a pericyte marker (15). Endothelial cells and pericytes were costained for CD34 and αSMA, respectively (Fig. 3C). Approximately, 60% and 30% of vessels in solid and infiltrative tumors, respectively, stained for αSMA (Fig. 3D). The reason for the reduced staining in infiltrative vessels is not known. One possibility is that a more elongated morphology of pericytes in infiltrative tumor vessels leads to the lower percentage of sectioned vessels scored as positive. Importantly, the ratio of αSMA/CD34 did not change before and after cediranib treatment (Fig. 3D). Therefore, association with αSMA did not correlate with selective survival of vessels following antiangiogenic treatment.

Finally, an important observation for antiangiogenic therapy is the phenotype of tumors affected by inhibition of VEGFR2 in the tumor vasculature resulting in increased tumor invasiveness. A quantitative analysis of tumors categorized with respect to tumor area and the relative density of invasive edges is shown in Fig. 4B for control and mice treated with cediranib. Although cediranib treatment leads to fewer tumors larger than $10 \times 10^5 \text{ mm}^2$, those large tumors that do develop have an increased number of invasive edges (Fig. 4A). All smaller tumors have an invasive morphology and showed relatively constant numbers of invasive edges between control and cediranib-treated mice. Because the absolute numbers of small tumors did not change with treatment, it seems that the growth of newly seeded tumors was not significant during the 1-week observation period.

**Cediranib treatment decreased vasogenic edema**

Cediranib has been shown to prevent edema in glioblastoma patients, and we hypothesized that the amelioration of edema secondary to brain metastases was a factor in
the improved survival of cediranib-treated mice (9, 16). To address the effects of cediranib on vasogenic brain edema in the DU145Ras/B1 model system, BLI and MRI were used between 3.5 and 4.5 weeks after the initiation of experimental metastasis to evaluate tumor burden, anatomic structures, and edema. Tumor-bearing mice without evidence of significant edema were randomized to control and cediranib-treated groups. After 7 days, five control and three treated mice were reimaged. As shown by a representative example in Fig. 5A and quantified in Fig. 5B, the T2 maps showed accumulation of edema in the control mice along the white matter tracts of the brain. The animals treated with cediranib, however, showed no discernable difference in T2 values after 1 week, although the morphology scans showed an increase in tumor diameter during the study period (Fig. 5A and B). The prevention of edema by cediranib was confirmed by Luxol fast blue staining for myelin in histologic sections from control and treated mouse brains (Fig. 5C). Demyelination, which is indicative of edema, was seen along the corpus callosum of control mice, matching the hyperintensive areas in the T2 maps (Fig. 5A).

To test whether cediranib can ameliorate established edema, three tumor-bearing mice with radiological...
evidence of edema were treated with cediranib for 7 days. Control mice were not included in this arm, as they would not survive for an additional 7 days with established brain edema. Cediranib-treated mice survived without significant morbidity to the second scan date, which revealed stabilization or reduction of edema, despite expansion of the metastatic lesions as determined by morphology scans (Fig. 5D).

Figure 6. Cediranib inhibited the development and progression of bone metastases. A, bone tumor burdens were measured by BLI at weeks 2 and 3 (left) and at weeks 4 and 5 (right; note scale differences). *, P < 0.05; n = 9-10. B, histomorphometric analysis of bone metastases on H&E-stained sections. **, P < 0.05; n = 9-10. C, CD34 stains of endothelial cells in bone metastases from representative control and cediranib-treated mice. D, quantification of blood vessel area on CD34-stained sections. ***, P < 0.001. Numbers indicate the bones analyzed in each group.
Cediranib inhibited bone metastasis even after short duration treatment

There has been relatively little preclinical evaluation of the effects of antiangiogenic agents on the development and progression of bone metastases. Intracardiac inoculation of DU145/RasB1 cells leads to osteolytic bone metastases, and BLI of the long bones and spine was assessed in the four groups shown in Fig. 1A. The inhibition of bone metastatic...
growth was evident after 2 and 3 weeks of continuous treatment (Fig. 6A, left). In the prevention group, bone metastasis did not relapse at week 5, following withdrawal of cediranib at week 3 (Fig. 6A, right). This contrasts with the renewed growth of brain metastasis at this same time point. Also, treatment of established bone metastases either continuously from their initiation or starting at week 4 onward inhibited BLI at least 10-fold (Fig. 6A, right). The BLI data were confirmed with histomorphometric analysis of bone sections (Fig. 6B) and X-rays (not shown).

Bone metastases were stained for CD34 to determine the effect of cediranib on vessel number and morphology (Fig. 6C and D). Nontreated mice displayed a uniform high density of CD34+ vessels within bone metastases, and many vessels appeared dilated. The vessel density as well as the dilated appearance decreased in bone metastases from cediranib-treated animals. The vessel area relative to total tumor area decreased ~6-fold as a result of cediranib treatment (Fig. 6D).

VEGFA binding to VEGFR1 has been shown to stimulate osteoclast differentiation, migration, and activity (17). Because cediranib has some inhibitory activity for VEGFR1 (14), the osteoclast number per millimeter normal bone or per tumor bone interface was quantified in non-tumor-bearing and tumor-bearing mice, respectively, either untreated for 4 weeks or cediranib-treated between weeks 3 and 4. In non-tumor-bearing mice, cediranib did not affect osteoclast number (Supplementary Fig. S2). Although cediranib-treated mice developed smaller and fewer bone metastases, the osteoclast number per millimeter tumor bone interface was not affected.

Bisphosphonates, such as Zometa, are currently used for a variety of patients with metastatic bone lesions, including lesions originating from prostate cancer (18). Because cediranib did not seem to inhibit osteoclast activity, the effects of cediranib and Zometa were directly compared as monotherapy agents and as potentially synergistic drugs in combination. BLI revealed that continuous treatment from the time of tumor cell inoculation with cediranib, Zometa, and a combination of both drugs inhibited tumor burden significantly and approximately equally (Fig. 7A). Histopathologic analyses of bone sections showed that there were fewer bone metastases in cediranib-treated mice compared with controls, and those metastases that did develop were generally smaller. The bone metastases growing in the presence of Zometa showed metaphyseal sclerosis with tumor cells embedded in the trabecular bone (Fig. 7B). Consistent with the histologic analysis, Zometa treatment resulted in almost complete inhibition of radiologically evident osteolytic lesions (Fig. 7C). Parallel BLI of the brain at 5 weeks showed that Zometa alone had no statistically significant effect on the brain metastatic burden (Fig. 7D).

Discussion

We investigated the efficacy of various cediranib prevention and treatment regimens using an experimental model of hematogenously disseminated prostate cancer that metastasizes predominantly to brain and bone. This system models critical steps in metastatic spread including dissemination, extravasation, and colonization of selected prostate cancer cells. The DU145/RasB1 model is specifically useful as brain and bone are major sites of metastasis for many commonly occurring cancers, and co-occurrence at the two sites is not unusual (7, 8). Cediranib treatment starting either from the time of DU145/RasB1 intracardiac inoculation or starting after the establishment of micrometastases led to a significant survival benefit that correlated with decreased tumor burden and decreased cerebral vasogenic edema.

The response of brain metastases to cediranib was multifaceted and displayed distinct histologic features. Untreated mice were more likely to develop rapidly growing and expansive solid brain metastases, which contributed to most of the brain tumor burden, whereas the occurrence of small invasive tumors was more rare. Cediranib treatment led to regression of the vessels in the center of large tumors. By contrast, neither the tumor vasculature of the invasive tumors nor the tumor cells at the rim of the large expansive tumors regressed after cediranib treatment. The perivascular pattern of growth following antiangiogenic treatment was characteristic of vessel co-option, which has been observed also for glioblastoma and melanoma (19, 20).

Preclinical models have implications for understanding both the mechanisms of treatment response and resistance, as well as potential morbidity and mortality benefits. Outside of glioblastoma, there are limited examples analyzing the response of tumor cells in the brain to antiangiogenic treatment. Kim and colleagues described the response of MDA-231 breast cancer brain metastases to PTK 787, resulting in decreased tumor burden but no obvious survival benefit (21). Vessel co-option was not specifically addressed. Leenders and colleagues assessed the response of cerebral melanoma metastases to ZD6474, wherein a decreased tumor burden, no survival benefit, and evidence of vessel co-option were observed (22). The preclinical model presented here adds to the small body of data showing that vessel co-option is a general response of different cell types to antiangiogenic treatment in the highly vascularized brain microenvironment.

Vessel co-option following treatment may represent strong selective pressure on the propensity of tumor cells in the brain to colonize preexisting vessels as a result of their adhesion to the vascular basement membrane (23). In the DU145/RasB1 model, a morphologic difference was not apparent to explain the sensitivity of endothelial cell survival in the co-opting versus solid tumors. The vasculature in both expansive and invasive tumors appeared dilated, and there did not seem to be a selective survival of vessels with demonstrably different pericyte coverage following antiangiogenic treatment. The lack of response of the perivascular brain metastases seen here is consistent with a limited amount of imaging data from clinical trials suggesting increased infiltrative growth in advanced glioblastoma treated with bevacizumab (10). Increasing long-term survival in patients with brain metastasis may require combination therapies to inhibit several functions including adhesion between tumor cells and cerebral vessels, infiltrative growth, and angiogenesis. However, for palliative therapy, it seems likely that
cediranib treatment will be useful for mitigating the morbidity associated with progressive brain metastasis, similar to the situation with advanced glioblastoma (9).

Recent attention has focused on observations from genetically engineered mouse tumor and experimental metastasis models showing that inhibition of the VEGF/VEGFR2 axis leads to increased tumor invasiveness and micrometastasis formation (4, 5). Consistent with these studies, histologic analyses of established DU145/RasB1 brain metastases revealed increased invasive projections after 1 week of cediranib treatment, which were most apparent in the largest tumors. On the other hand, increased survival and decreased metastatic tumor burden in DU145/RasB1 inoculated mice treated continuously with cediranib contrasts with the results of Ebos and colleagues (4). In the Ebos experimental design, sunitinib treatment accelerated metastatic burden and decreased overall survival in mice following introduction of MB-MDA-231 breast cancer cells via tail vein injection or following removal of primary orthotopically grown tumors. It is likely that these contrasting end points are in part a result of tumor-specific contributions. In addition, we suggest that the tumor microenvironment in various organs will be differently affected by antiangiogenic treatments. Specifically, it seems that the major site of metastatic burden was the lungs in the Ebos studies with MB-MDA-231. The lungs may be most susceptible to "metastatic conditioning," which is thought to reflect circumstances that increase tumor cell extravasation and initial colonization, such as in response to stress/injury, including antiangiogenic treatment (4, 24).

An interesting observation to emerge from our studies is the different sensitivity of brain or bone resident tumor cells to cediranib treatment and subsequent withdrawal. After 3 weeks of cediranib treatment from the time of tumor cell dissemination, cediranib withdrawal led to rebound growth of brain metastases, whereas bone metastases were significantly inhibited. Thus, individual or micrometastatic tumor cells in the brain compared with the brain seemed to either not survive a VEGF signaling deficient microenvironment or, alternatively, were not able to emerge from dormancy after cediranib withdrawal.

One interpretation of these results is that tumor-initiating cell niches in the brain and bone microenvironment are affected differently by inhibition of the VEGF axis. The source of such differential effects could be differences in the endothelial cells themselves as well as the differentiated cells making up the parenchyma of the organ (25). Vascular niches that contribute to the survival and establishment of glioma-initiating cells in the brain and leukemia-initiating cells in the bone marrow have been described (26, 27). An alternative interpretation is that cediranib directly disrupts osteogenic cells involved in bone remodeling through effects on VEGFR1-expressing hematopoietic cells, such as osteoclasts. However, we found no evidence for functional effects of cediranib on osteoclasts in control or tumor-bearing mice. Thus, we favor the interpretation that, at the dosage used in this study, cediranib influences the growth of bone metastasis via an effect on VEGFR2 and bone endothelium.

In summary, our work provides evidence that antiangiogenic treatment not only inhibited the growth of aggressive prostate cancer metastases in bone and brain but also reduced the morbidity and mortality of tumor-bearing mice. These results support a value for clinical trials investigating appropriate combination therapies that include antiangiogenic drugs for treatment of patients with advanced cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Cediranib/AZD2171 Inhibits Bone and Brain Metastasis in a Preclinical Model of Advanced Prostate Cancer

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