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Cediranib Inhibits both the Intraosseous Growth of PDGF D-Positive Prostate Cancer Cells and the Associated Bone Reaction

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Abstract

BACKGROUND—The major cause of death in prostate cancer (PCa) cases is due to distant metastatic lesions, with the bone being the most prevalent site for secondary colonization. Utilization of small molecule inhibitors to treat bone metastatic PCa have had limited success either as monotherapies or in combination with other chemotherapeutics due to intolerable toxicities. In the current study, we developed a clinically relevant *in vivo* intraosseous tumor model overexpressing the platelet-derived growth factor D (PDGF D) to test the efficacy of a newly characterized VEGFR/PDGFR inhibitor, cediranib (also called AZD2171).

METHODS—An intratibial-injection model was established utilizing DU145 cells without or with increased platelet-derived growth factor D (PDGF D) expression. Tumor-bearing mice were treated by daily gavage administration of cediranib and/or weekly i.p. injection of docetaxel for 7 weeks. Tibiae were monitored by *in vivo/ex vivo* x-rays and histomorphometry analysis was performed to estimate tumor volume and tumor-associated trabecular bone growth.

RESULTS—Cediranib reduced intraosseous growth of prostate tumors as well as tumor-associated bone responses. When compared to the standard chemotherapeutic agent docetaxel, cediranib exhibited a stronger inhibition of tumor-associated bone response. The efficacy of cediranib was further enhanced when the drug was co-administered with docetaxel. Importantly, the therapeutic benefits of cediranib and docetaxel are more prominent in intraosseous prostate tumors overexpressing PDGF D.

CONCLUSION—These novel findings support the utilization of cediranib, either alone or in combination with docetaxel, to treat bone metastatic prostate cancer exhibiting PDGF D expression.

Keywords

PDGF D; Prostate Cancer; AZD2171; Metastasis; Markers

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INTRODUCTION

Prostate cancer (PCa) is the leading cause of cancer-related death among American men with the main cause of mortality being metastatic lesions (1,2). Approximately 90% of PCa patients with hematogenous metastases develop metastatic deposits in the bone (3). The presence of tumor cells within the bone disrupts the delicate balance maintained in this milieu leading to anemia, pathological bone fractures, and spinal cord compression lowering the patient's quality of life. Currently, patients with bone metastatic PCa are mainly treated with docetaxel and prednisone; however, these patients eventually fail to respond to those treatments (4). In spite of the tremendous progress in the development of novel therapeutic agents targeting cancer cells, treatments for PCa bone metastatic lesions have been slow in coming (4). This may be attributed to the management of this disease as a homogenous disorder where patients receive the same or similar treatment. With the availability of new target therapies, clinicians and patients need guidance regarding personalized treatment options, especially in light of the varied rate of disease progression in metastatic PCa and its associated heterogeneous genetic alterations. An area of intense research is within the family of tyrosine kinase inhibitors (TKI) leading to the developments of drugs such as sunitinib, which have shown some promise reducing PSA levels as well as metastatic lesions (5). However, the development of novel agents have proven a challenge in the clinic due to adverse events.

The platelet-derived growth factor (PDGF) family plays a critical role in the progression of multiple tumors, including PCa (6–9). This family is composed of four members, A, B, C, and D, which form homodimers or the heterodimeric AB complex to bind their cognate receptors, α -PDGFR and β -PDGFR (7,10). Increasing evidence demonstrated tumor-derived PDGF ligands activate PDGF receptors in surrounding stromal cells, mediating tumor-stromal interactions critical for tumor cell invasion and metastasis (6,11). Of the two PDGF family receptors, β -PDGFR is shown to mediate potent transforming signals (7), and is upregulated in 88% of localized and 80% of bone metastatic prostate cancer (12). In fact, β -PDGFR is among a 5-gene signature predicting the clinical progression of prostate cancer (13). Furthermore, inhibition of β -PDGFR was shown to reduce intraosseous growth of PCa *in vivo* (2,14), suggesting a critical role of β -PDGFR in PCa bone metastasis. While the search for PDGF B, once thought to be the sole ligand for β -PDGFR, has been unsuccessful (15,16), our recent study found that a newly characterized family member, PDGF D, is a ligand for β -PDGFR in PCa (17). In fact, PDGF D is significantly correlated with tumor stage and Gleason score. PDGF D demonstrates transforming and angiogenic potential through β -PDGFR activation by both autocrine and paracrine mechanisms as well as indirectly through the upregulation of the vascular endothelial growth factor (VEGF)/VEGFR axis (18). In fact, overexpression of this ligand mediates prostate cancer tumorigenesis and supports stromal cell recruitment *in vivo* (19).

The goal of the present study is to test the efficacy and general toxicity of a newly developed TKI, cediranib (otherwise known as AZD2171), in an animal model of intraosseous tumor growth of PCa with constitutively activated signaling network initiated by platelet-derived growth factor (PDGF) D overexpression. Here, we report the effects of activation of the PDGF D/ β -PDGFR axis in intraosseous growth of prostate cancer cells as well as in tumor-associated bone reactions. In addition, we demonstrate the therapeutic value of cediranib, a TKI initially developed to target the VEGFR/PDGFR family, as a monotherapy or in combination with docetaxel.

MATERIALS AND METHODS

Generation of PDGF D overexpressing prostate cancer cells

DU145 human prostate cancer cells were obtained from ATCC and grown in RPMI supplemented with 5% fetal bovine serum (Invitrogen, Carlsbad, CA), 2mM glutamine, 100U/ml penicillin, and 100mg/ml streptomycin (Invitrogen). Stable PDGF D overexpression was accomplished using a pcDNA3.1-PDGF D: His vector, described previously (19). Briefly, cells at subconfluence were transfected with pcDNA3.1 empty vector or pcDNA3.1-PDGF D: His using Lipofectamine 2000 (Invitrogen). Cells were selected with 200 μ g/mL Geneticin (G418) and resulting pooled population referred to as vector or PDGF D DU145, respectively. PDGF D expression was confirmed through RT-PCR as well as Western blotting of conditioned media (CM) as previously described (20).

Drug acquisition and preparation

Cediranib (also known as AZD2171) was obtained from AstraZeneca and prepared per manufacturer's protocol in an aqueous polysorbate 80 solution (21). Docetaxel (Taxotere, Sanofi-Aventis, Bridgewater, NJ) was obtained from the Karmanos Cancer Center through Dr. Elisabeth Heath and reconstituted per manufacturer's instructions in 1.3% ethanol in distilled water.

Intratribial injection and drug delivery

Intraosseous tumor growth was performed as previously described (22). Briefly, vector or PDGF D DU145 cells were injected at 2×10^5 cells/10 μ L of serum-free medium into the proximal tibiae of 5-week old male C.B.-17 SCID mice (Taconic Farms, Germantown, NY). Mice were imaged with a mammography unit every 2 weeks for 8 weeks. Nine weeks post injection, mice were sacrificed and tibiae collected for *ex vivo* imaging and histology.

For the preclinical drug study, mice were injected with vector or PDGF D DU145 cells, imaged at 2 weeks post injection to confirm bone reaction, then randomly divided into 4 groups as follows: (Group 1, control treatment) each vector and PDGF D DU145 tumor bearing mice received one i.p. injection of 1.3% ethanol in distilled water per week and daily gavage administration of polysorbate 80 distilled water; (Group 2, docetaxel treatment) each vector and PDGF D DU145 tumor bearing mice received one i.p. injection of 8mg/kg docetaxel per week; (Group 3, cediranib treatment) each vector and PDGF D DU145 tumor bearing mice received one i.p. injection of 1.3% ethanol in distilled water per week and daily gavage administration of 5mg/kg cediranib; (Group 4, docetaxel plus cediranib treatment) each vector and PDGF D DU145 tumor bearing mice received one i.p. injection of 8mg/kg docetaxel per week and daily gavage administration of 5mg/kg cediranib. Body weight was monitored weekly, and radiography was performed at weeks 2, 4, and 8 post-injection. At the end of the trial period (nine weeks post injection), mice were sacrificed and tibia resected. *Ex vivo* x-rays were performed on each tibia to evaluate osteoblastic or osteolytic responses. Livers of trial mice were also harvested and utilized to determine drug toxicity based on liver weight/body weight ratio (23).

Histomorphometry

Tibiae were fixed with 4% paraformaldehyde for 24 hours, decalcified in 10% EDTA for 10–14 days, and paraffin-embedded. The bone tissues were sectioned longitudinally across the bone marrow cavity with a thickness of 5- μ m and stained with hematoxylin and eosin (H&E). Digital photomicrographs were captured under 5X magnification using a Zeiss Axioplan2 microscope (Zeiss, Göttingen, Germany) equipped with a software-controlled digital camera (AxioVision; Zeiss), and the images were merged to display a panoramic panel of the entire sagittal section of the tibia. The percentage area occupied by tumor cells,

and tumor-associated trabecular, and cortical bone in the histological section was calculated by the software based on the measurement of the corresponding areas in pixels², as described previously (22).

Trichrome Staining

Serial tibia sections were stained using Sigma Accustain Trichrome Staining kit following manufacturer's protocol. Deparaffinized bone sections were stained step-wise with hematoxylin, bieberich scarlet acid fuchsin, phosphomolybdic/phosphotungstic acid, and aniline blue. The bieberich scarlet acid fuchsin stains the cytoplasm red, and the aniline blue stains collagen blue.

Assessment of treatment response

Tumor and bone treatment response was determined using *in vivo* and *ex vivo* x-rays as well as histomorphometric analysis of H&E stained sections. Tumor-mediated bone response over time was determined through x-rays at the aforementioned time points, and presence/size of tumor and bone response was validated using histomorphometric analysis. The guidelines utilized are as follows: No tumor specifies no visible bone response was observed by x-rays throughout the treatment period, and H&E sections also did not show any evidence of tumor cells; Progression signifies increase in bone response throughout the treatment period and evidence of tumor cells in H&E sections; Stabilization represents bone response shown by x-ray that does not change throughout the treatment period and evidence of tumor cells in H&E sections; Regression indicates bone response that was initially observed but subsided throughout the treatment period and evidence of small tumor nests in H&E sections.

Immunohistochemistry

Serial sections were utilized for PDGF D immunohistochemistry (IHC) using a custom anti-PDGF D polyclonal antibody (8D2, 1:50 dilution). Tissue slides were deparaffinized then serially rehydrated in ethanol and water. Steaming was performed for 20 minutes for antigen retrieval. Sections were then washed with PBS and blocked with CAS-Block (Invitrogen). Slides were incubated with primary antibody for 2 hours at room temperature followed by washing. The ABC Vectastain and DAB reagent (Vector Labs, Burlingame, CA) were used per manufacturer's protocol to develop. Once counterstained with Harris' hematoxylin (Sigma, St. Louis, MO), sections were dehydrated and mounted with Permount (Sigma).

Statistical analysis

Statistical significance was determined using unpaired Student's t-test when comparing two groups. ANOVA with Tukey-Kramer post-testing was utilized when comparing multiple groups. Differences were considered significant when p-value <0.05.

RESULTS

Development of an *in vivo* intraosseous tumor model with PDGF D overexpression

To establish an animal model for assessing the significance of PDGF D in intraosseous PCa growth and PCa-mediated bone reaction, we utilized the DU145 cell line which expresses low levels of PDGF D and exhibits a mixed osteoblastic/osteolytic reaction *in vivo* (22,24). DU145 cells were transfected with empty vector or full length PDGF D (referred to as vector and PDGF D DU145, respectively) and PDGF D overexpression in PDGF D DU145 cells was confirmed by RT-PCR and immunoblot analysis (Fig. 1A). To analyze intraosseous growth, vector or PDGF D DU145 cells were injected into the proximal tibiae of male SCID mice and tumor formation was monitored indirectly through *in vivo*

radiographic imaging. Two weeks post injection, radiographic analyses showed visible signs of bone reactions as shown in Figure 1B. At 9 weeks, the injected tibiae were resected, and the subsequent *ex vivo* imaging displayed both lytic and sclerotic lesions. Interestingly, PDGF D DU145 tumor-bearing tibiae showed marked increases in osteosclerotic lesions, similar to those observed in human PCa bone metastases. To ascertain that PDGF D DU145 tumors maintained PDGF D expression *in vivo*, we performed immunohistochemistry in tibial sections and observed that overexpression remained throughout the experiment (Fig. 1C). Since new bone formation in the proximal tibia is through trabecular bone generation, we utilized histomorphometry to monitor tumor-associated trabecular bone growth. As shown in Figure 1D, PDGF D upregulation significantly enhanced trabecular bone formation consistent with the *ex vivo* imaging analyses. These findings were confirmed by H&E histology and Trichrome staining (Fig. 1E). We did not observe a difference in cortical bone formation (data not shown). These results establish an *in vivo* model for intraosseous PCa with increased PDGF D expression.

Cediranib and docetaxel treatment *in vivo*

Cediranib is a newly characterized PDGFR/VEGFR inhibitor that has shown promising preclinical results in many types of primary or metastatic tumors (25). To test the efficacy of cediranib as a monotherapy or in combination with docetaxel in controlling intraosseous PCa growth and/or protection of the bone integrity, especially when tumors express high levels of PDGF D, we utilized our intratibial-injection model of vector and PDGF D DU145 described above. Two groups, 44 in each group, were injected with vector or PDGF D DU145 cells; at two weeks post injection tumor bone response was verified via radiographic imaging. Mice in each group were randomized into four treatment groups: Group 1) vehicle control treatment; 2) docetaxel treatment; 3) cediranib treatment; 4) combined treatment with docetaxel and cediranib (referred to as doce/ced) (Fig. 2A). Docetaxel was administered weekly via i.p. injection, and cediranib was delivered daily through oral gavage, as summarized in Figure 2B. Bone response was monitored via radiographic imaging at weeks 2, 4, 8, and 9 (marked with “X” in Fig. 2B). While 80% of tibiae injected with vector DU145 cells showed visible signs of bone reactions by x-ray analysis, all tibiae injected with PDGF D DU145 cells showed noticeable changes in bone morphology (Fig. 2C). *Ex vivo* imaging at the end of the trial (week 9) demonstrated that both vector and PDGF D DU145 tumors exhibited mixed osteolytic and osteoblastic responses. Consistent with initial analysis shown in Figure 1, PDGF D expression resulted in more prominent osteosclerotic lesions (Supplemental Fig. 1–4). Importantly, radiographic analysis indicated reduced bone reactions in tumor-bearing tibiae upon drug treatments, especially cediranib (Fig. 2C). It should be emphasized that cediranib was effective in reversing PDGF D-mediated bone reactions. These results suggest potential benefits of cediranib in protecting bone integrity in PCa bone metastasis.

Tumor growth and bone reaction are abrogated by cediranib and cediranib/docetaxel treatment

In an effort to evaluate the efficacy of cediranib and/or docetaxel over the treatment period, we monitored the radiographic images at different time points. As described in the “Materials and Methods” section, injected tibiae were categorized into 4 groups (no tumor, progression, stabilization, and regression) based on both bone x-ray images and H&E sections. As summarized in Figure 3A, docetaxel alone had minimal therapeutic value when compared to the vehicle treatment. In contrast, cediranib treatment, especially in combination with docetaxel, resulted in stabilization or regression of disease (Fig. 3).

To analyze the therapeutic effects of cediranib at the cellular level in our intratibial model, we also utilized bone histomorphometry and quantitated the area occupied by different

components of the bone in histological sections (26). We first analyzed tumor cell area and observed ~60% increase in PDGF D DU145 tumor area compared to vector DU145 tumors (vector vs. PDGF D in vehicle treatment groups in Fig. 4A), although this difference was statistically insignificant. Whereas drug treatments had no significant effect on vector DU145 tumor cell area, cediranib treatment, especially in combination with docetaxel, markedly reduced PDGF D DU145 tumor volume (Fig. 4A), demonstrating the effectiveness of cediranib in controlling intraosseous growth of PCa with elevated PDGF D signaling.

Human prostate cancer bone lesions present mixed osteoblastic and osteolytic lesions with a net osteoblastic phenotype causing skeletal complications (27). When we examined bone response through trabecular bone levels (osteoblastic reaction) in our animal model, tumor-associated trabecular bone growth was drastically enhanced in response to PDGF D overexpression (Fig. 4B), corroborating our findings in Figure 1. While drug treatments had little effect on vector DU145 tumor-associated trabecular bone formation, the same treatments, especially with cediranib, significantly diminished trabecular bone levels in PDGF D tumors (Fig. 4B).

Adverse effects of drug treatment

Since cediranib has been shown to interfere with different physiological functions (21), we wanted to ascertain any toxicities that our trial drugs may present. Thus, we monitored weight changes throughout the trial period and observed no statistical difference between vehicle and experimental drug treatment, with the exception of the cediranib treated arm in the PDGF D DU145 injected group (Fig. 5A, B). Since cediranib is an orally administered therapeutic, we tested the potential metabolic toxicity by calculating liver/body weight ratio (Fig. 5C) and observed no significant difference in liver/body weight ratio across treatment arms. We surmise that the observed weight difference shown in Fig. 5B may be due to chronic gavage administration leading to a decrease in food intake. Previously, Wedge et al. demonstrated endochondral ossification retardation by cediranib (21). To this end, we monitored epiphyseal growth plate ossification and observed no significant difference across treatment arms (Supplemental Fig. 5). Taken together, our study suggests a therapeutic potential of cediranib for the protection of bone integrity in patients with PCa bone metastases without noticeable adverse side effects.

DISCUSSION

The major cause of morbidity and mortality in cancer patients is tumor metastasis. In prostate cancer, 24% of diagnosed cases present with metastatic lesions, and current treatment options for this late stage disease are very limited (14). Androgen ablation and docetaxel have been a staple treatment for metastatic disease; however, patients become refractory to these therapeutics over time. As a result, more efficacious and personalized therapeutics are needed to target molecules involved in heterogeneous metastatic disease progression. Cediranib (also known as AZD2171), a newly characterized small molecular inhibitor of VEGFR and PDGFR, has shown promising results in preclinical and clinical trials (25). Cediranib inhibits both α - and β -PDGFRs, but with greater efficacy against β -PDGFR at concentrations similar to VEGFRs (21). While cediranib is ineffective in the treatment of leukemia, it has been shown to be effective in the treatment of solid tumors (28,29). In glioblastoma, cediranib led to improved radiographic response as well as progression free survival when combined with standard therapy (30,31). Importantly, cediranib led to a reduction of the dose of standard therapy, resulting in lower toxic side effects, and also enhanced the efficacy of standard cytotoxic drugs leading to partial response or stabilization of disease in colorectal, ovarian and non-small cell lung cancers (32–35).

In this manuscript, we established an animal model to test the therapeutic efficacy of cediranib in treating bone metastatic prostate cancer with signaling network initiated by PDGF. Studies demonstrated that tumor-derived PDGF promotes tumor progression through activation of its cognate receptor by both autocrine and paracrine mechanisms (19,36–38). In prostate cancer, we and others showed elevated β -PDGFR expression/activation and its implication in intraosseous growth of prostate cancer cells (2,12,14). The traditional β -PDGFR ligand, PDGF B, has not been detected in prostate cancer suggesting an alternative growth factor responsible for the oncogenic activity of β -PDGFR (15,16). Recently, we identified PDGF D as a ligand for β -PDGFR in prostate cancer and demonstrated an association of PDGF D expression with tumor stage and Gleason grade (17), which was validated through the Oncomine database across multiple clinical sample sets (<http://www.oncomine.org>). In a subcutaneous injection model in SCID mice, PDGF D expression accelerated early onset of prostate tumor growth and drastically enhanced prostate carcinoma cell invasion and their interactions with surrounding stromal cells (19). In this study, we established an *in vivo* model for intraosseous PCa growth with increased PDGF D signaling, which demonstrated the role of PDGF D in inducing osteoblastic and osteolytic responses with a net osteoblastic phenotype, similar to the bone reactions seen in human PCa bone metastases (24). These findings are consistent with previously suggested roles for PDGF signaling in bone formation by regulating commitment of stromal mesenchymal cells to differentiate into osteoprogenitor cells and inducing proliferation and migration of osteoblasts (39–41). In fact, we found that tumor-derived PDGF D induces osteoblast migration and differentiation *in vitro*, supporting a role for this growth factor in bone pathophysiology (Najy et al., unpublished). Although our recent finding demonstrated that PDGF D induces osteoclastogenesis, a critical step for initiation of bone remodeling (Huang et. al., manuscript in press), in the present study, DU145 tumor-mediated osteoclastic reaction was observed independent of PDGF D expression (data not shown).

With a clinically relevant *in vivo* model established in this study, we report the therapeutic potential of cediranib alone or in combination with docetaxel on bone metastatic prostate cancer. A main concern with TKIs is their relatively frequent adverse effects ranging from cardiotoxicity to hypertension halting clinical trials (42,43). Thus, we monitored the health of our experimental animals for signs of toxicity and observed no significant adverse effects except in the PDGF D DU145 group treated with cediranib. Although we see a slower rate in weight change, these mice were not cachectic, and their liver/body weight ratio was normal. We believe the observed difference may be a result of chronic gavage administration. Overall, we observed minimal drug toxicity on the mice throughout the trial, which corroborates previous reports (44). When assessing responses using radiographic and histological data, we observed that docetaxel alone had modest effect in both vector and PDGF D DU145 injected mice. These findings agree with previous clinical data showing taxanes not being curative of prostate cancer bone lesions (45,46). In contrast, cediranib and docetaxel/cediranib treatment demonstrated disease stabilization and/or regression, which was corroborated by bone histomorphometry. These positive effects are more pronounced in the PDGF D DU145 groups suggesting that tumor-derived PDGF D-initiated signaling networks are effective targets of cediranib. For instance, PDGF D signaling is known to upregulate the VEGF/VEGFR axis (18) and cediranib is a potent inhibitor of VEGFRs. Previous work by Wedge et. al. demonstrated dramatic effects (84% and 98% tumor inhibition) on PC-3 tumors *in vivo* using 3 and 6 mg/kg of cediranib, respectively (21). PC-3 cells naturally express PDGF D and the sensitivity exhibited by these cells to the inhibition of the PDGF D cognate receptor, β -PDGFR, further supported our hypotheses that PDGF D plays a crucial role in prostate cancer and cediranib effectively targets this signaling pathway. Increased sensitivity to cediranib in tumors expressing PDGF D may also be associated with the effects of PDGF D on drug delivery. In fact, PDGF D has been shown to play a role in modulating interstitial fluid pressure through pericyte recruitment and blood

vessel stabilization (38). As a result PDGF D may facilitate the delivery of the administered drugs, leading to disease regression.

Taken together, we propose that cediranib, especially in combination with other chemotherapeutic agents such as docetaxel, has a substantial anti-tumor activity in prostate cancer bone metastasis. Consistent with our findings, cediranib has shown promise in advanced prostate cancer (47). These benefits are enhanced when combined with docetaxel, where some patients displayed lower PSA levels and loss of metastatic lesions (47,48). However, not all patients in these trials benefited from treatments as they may not possess the proper molecular profile targeted by the drug. Therefore, it has become necessary to stratify patients according to prognosis, which will guide clinicians and patients for more efficacious treatment options with less general toxicity. In this study, we demonstrated that PDGF D overexpressing bone lesions were more responsive to cediranib and docetaxel/cediranib suggesting PDGF D as a potential biomarker for treatment selection. PDGF D is stable in biological fluids and has been detected in the sera of cancer patients (49,50). Thus, we propose PDGF D screening may prove to be a useful clinical tool in personalizing treatment of metastatic prostate cancer for treatment with cediranib alone or in combination with docetaxel for the control of intraosseous tumor growth and protection of bone integrity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. PDGF D upregulation in prostate cancer cells supports osteoblastic response in mouse tibia

A) RT-PCR analysis of PDGF D mRNA and immunoblot analysis of PDGF D protein using conditioned media (CM) from vector and PDGF D DU145 cells **B)** *In vivo* radiographic images of tibiae 2 weeks post-injection (left) and *ex vivo* radiographic images of tibiae taken 9 weeks post-injection of vector and PDGF D DU145 cells. **C)** Intraosseous tumor PDGF D expression was monitored in representative sections utilizing PDGF D immunohistochemistry. Images were captured at magnifications 40X. **D)** Bone histomorphometric analysis of tibiae injected with vector and PDGF D DU145 cells. Percentage of the whole-tissue cross section occupied by bone tissue in tibiae. Values are mean $\pm$ SD. * $p < 0.05$. **E)** Sections of intraosseous vector and PDGF D DU145 tumors were analyzed by H&E (20X) and trichrome (20X). C.B., cortical bone; T, tumor; T.B., trabecular bone.

Figure 2. Design of the preclinical trial and radiographic analysis of tumor-induced bone reactions

Treatment arms (A) and experimental outline (B) of the docetaxel/cediranib preclinical trial. Weekly i.p. of docetaxel (triangle) and/or daily oral gavage of cediranib (solid black bar) were administered during the seven week treatment period. *In vivo* x-rays were performed at 2, 4, and 8 weeks post injection (X) followed by *ex vivo* imaging at week 9. C) Bone response was analyzed through *ex vivo* radiographic imaging.

Figure 3. Cediranib or docetaxel/cediranib results in intraosseous tumor regression
A) Disease progression/regression was monitored throughout the preclinical trial using *in vivo* (weeks 2, 6 and 8 post injection) and *ex vivo* (week 9 post injection) x-ray imaging in addition to H&E histology. B) Representative x-ray images of disease development at weeks 2, 6, 8 and 9 post injection, categorized as i) no tumor burden; ii) progression; iii) stabilization; or iv) regression.

Figure 4. Cediranib or docetaxel/cediranib combination abrogate PDGF D enhanced tumor load and bone response

Bone histomorphometry was utilized to quantitate tumor area (**A**) as well as trabecular bone area (**B**). Values are mean $\pm$ SD. * $p < 0.05$, comparing PDGF D vehicle vs. docetaxel/cediranib. ** $p < 0.05$, comparing vector vs. PDGF D vehicle groups. € $p < 0.05$, comparing PDGF D vehicle vs. docetaxel. ‡ $p < 0.05$, comparing PDGF D vehicle vs. cediranib. ¶ $p < 0.05$, comparing PDGF D vehicle vs. docetaxel/cediranib. £ $p < 0.05$, PDGF D docetaxel vs. cediranib. ¤ $p < 0.05$, comparing PDGF D docetaxel vs. docetaxel/cediranib.

Figure 5. Drug toxicity in treated mice

Body weight of vector (A) and PDGF D (B) DU145 injected mice was monitored and plotted as percentage weight change over time. C) Livers were resected, weighed, and the liver/body weight ratio was plotted. Values are mean $\pm$ SD. * $p < 0.05$, cediranib compared to vehicle treatment.