RANKL Inhibition Is an Effective Adjuvant for Docetaxel in a Prostate Cancer Bone Metastases Model

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BACKGROUND. Docetaxel induces an anti-tumor response in men with advanced prostate cancer (PCa); however, the side effects associated with docetaxel treatment can be severe, resulting in discontinuation of therapy. Thus, identification of an effective adjuvant therapy to allow lower doses of docetaxel is needed. Advanced PCa is typically accompanied by skeletal metastasis. Receptor activator of NFkB ligand (RANKL) is a key pro-osteoclastic factor. Targeting RANKL decreases establishment and progression of PCa growth in bone in murine models.

METHODS. The efficacy of inhibiting RANKL, using a recombinant soluble RANK extracellular domain fused with the immunoglobulin Fc domain (RANK-Fc), was tested as an adjuvant therapy with docetaxel for PCa bone metastasis in a murine intra-tibial model.

RESULT. The combination of RANK-Fc and docetaxel reduced tumor burden in bone greater than either treatment alone.

CONCLUSION. The combination of docetaxel with a RANKL-inhibiting agent merits further investigation for treatment of advance PCa. *Prostate 68: 820–829, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; docetaxel; RANK-Fc; RANKL; bone metastasis**

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed cancer and is the third leading cause of cancer-related deaths of American men [1]. In early stages, it responds to androgen-withdrawal treatment. However, as the disease progresses, it becomes independent of hormone regulation and the cancer becomes resistant to treatments.

Docetaxel is currently one of the most effective treatments for PCa [2–5]. In combination with prednisone, docetaxel is indicated for the treatment of patients with androgen-independent (hormone-refractory), metastatic PCa [6]. Docetaxel is a member of the taxane class of chemotherapy drugs, which inhibit cancer cell growth by stopping cell division. The primary mechanism of action of taxanes is to stabilize microtubules and prevent their disassembly. Since the reorganization of microtubules is essential to cell division, disruption of this dynamic causes the cells to arrest in the G2 phase of the cell cycle [7].

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Although taxanes are initially an effective way to treat advanced PCa, their side effects can be debilitating and include localized erythema of the extremities with edema followed by desquamation, severe neuropathy, fatigue, and weakness [8]. When these symptoms occur, docetaxel doses must be reduced or stopped [8,9], leading to disease progression.

Virtually all patients with advanced PCa develop debilitating and painful bone metastases. Several factors control this bone remodeling process including parathyroid hormone-related protein (PTHrP), receptor activator of NFκB and its ligand (RANK/RANKL), osteoprotegerin (OPG), bone morphogenetic proteins (BMPs), and transforming growth factor β (TGF-β). At the bone metastatic site, osteoblasts produce RANKL which binds to its receptor, RANK, on osteoclast precursors causing them to mature and absorb bone [10–12]. Bone resorption leads to release of growth factors trapped in bone, including TGF-β. (reviewed in references [13,14]). OPG is a soluble, decoy RANKL receptor that blocks the ability of RANKL to induce osteoclastogenesis [15]. Inhibition of RANKL has been shown to inhibit establishment and progression of PCa tumors in bone in murine models [16–18]. These results indicate that RANKL inhibition may be useful for the treatment of metastatic PCa lesions in the bone.

Based on potential use of RANKL inhibition in PCa and the need to enhance docetaxel-based chemotherapy regimens, we explored the effectiveness of soluble RANK (RANK-Fc), a recombinant RANKL inhibitor, as an adjuvant to docetaxel to reduce progression of PCa in the bone. We found that the combination of RANK-Fc and docetaxel reduced tumor burden in bone greater than either treatment alone. These data suggest that inhibition of RANKL can be a useful adjuvant therapy with docetaxel for treatment of advance PCa.

MATERIALS AND METHODS

Cell Culture

C4-2B cells, isolated from bone-metastasizing LNCaP cells [19,20], were maintained in T-medium (80% DMEM-20% Ham's F12 (GIBCO), 5 μg/ml Insulin, 13.6 pg/ml triiodothyronine, 5 μg/ml transferrin, 0.25 μg/ml biotin, 25 μg/ml adenine (Sigma, St. Louis, MO), 1 × penicillin/streptomycin and 10% FBS).

RANK-Fc

The RANK-Fc used in these studies was provided by Amgen Inc. (Seattle, WA, USA) and contains the murine extracellular domain of RANK (through Pro213) fused to human immunoglobulin G1 (IgG1) Fc. The RANK:Fc protein was produced in Chinese hamster ovary (CHO) cells as previously described [17].

Animal Experiment

The effects of chemotherapy on PCa-derived cells in bone were evaluated following direct injection into one of two tibiae of male CB17 SCID mice (intratibial injection) as described previously [16]. Briefly, mouse hind limb is shaved, and the knee cap is located. Cells (5×10^5) are injected in a volume of 50 μl into the tibia percutaneously via the tibial crest into the marrow cavity. To evaluate for effects of tumor growth in soft tissues, 10^6 cells were injected to the right flank. Two weeks after injection of cells, mice were injected IP with docetaxel once a week (Taxotere, Aventis Pharma, Bridgewater, NJ). Tumors were allowed to grow for up to12 weeks at which time mice were sacrificed. Evidence of tumor-induced bone change was evaluated using radiography (Faxitron X-ray Corp., Wheeling, IL) on Week 6 and at the end of the study (Week 12). Tumor injected tibiae and contralateral tibiae without tumors were removed, fixed overnight in 10% PBS buffered formalin, and transferred and stored in 70% ethanol. All animal procedures were approved by the University of Michigan Animal Care and Use Committee.

Bone Analysis

Bone mineral densities were measured using peripheral quantitative computed tomography (pQCT) (Stratec, Dundas, Ontario, CA). pQCT scans from three slices of the proximal metaphyses region were performed to obtain trabecular bone densities. Following pQCT analysis, tibiae were decalcified in Cal-Ex II (Fisher Scientific, Hampton, NH) or EDTA, paraffin embedded, and histological sections were stained with hematoxylin and eosin (H&E). Histomorphometry was performed on H&E stained sections and tumor volume was quantitated using BioQuant Osteo II (Bioquant Image Analysis Corporation, Nashville, TN). Adjacent sections were stained for tartrate-resistant acid phosphatase (TRAP) and area of TRAP positivity was also quantified using BioQuant Osteo II (Bioquant Image Analysis Corporation, Nashville, TN). TRAP positive cells were counted along the endocortical bone surfaces. The number of TRAP positive cells is reported as osteoclasts/mm.

Tumor Measurements

The mice were monitored for body condition, body weight, and subcutaneous tumor growth. Tumor measurements using digital calipers were performed weekly starting when the tumors became palpable.

Tumor volumes were calculated using [(minimum measurement)^2 × (maximum measurement)]/2 as previously described [21] Subcutaneous tumor volumes and weights were also obtained at necropsy.

Statistics

When docetaxel dosing levels were compared, the comparison of the number of mice who developed tumors in each group was tested using a Fisher's Exact Test. Wilcoxon rank test compared subcutaneous tumor volume and weight at necropsy between 5 mg/kg and PBS only.

Tumor and bone measures were analyzed between the four groups in the RANK-Fc and docetaxel experiment. Transformations were performed to attain a centered distribution when needed. ANOVA models were used to test all pair-wise comparisons. The Tukey–Kramer adjustment was used to correct for multiple comparisons.

RESULTS

Treatment of PCa patients with docetaxel has many associated side effects. It follows that lowering the dose administered may help minimize drug-induced toxicities. However, dose reduction may be associated with reduced anti-tumor efficacy. Thus, identification of adjuvant agents to improve anti-tumor efficacy may allow dose reduction without loss of overall tumor kill. Accordingly, we tested the efficacy of a sub-optimal dose of docetaxel in combination with a RANKL inhibitor on subcutaneous PCa cell tumor growth and PCa cell growth in bone. Accordingly, we first identified a sub-optimal dose of docetaxel in our animal model. SCID mice were inoculated subcutaneously (SQ) and intratibially (IT) with C4-2B PCa cells and given escalating doses of docetaxel once a week for 10 weeks. Since 20 mg/kg in the mouse correlates with the clinically used dose for human PCa treatment [9], we chose doses lower than 20 mg/kg to determine a sub-optimal dose of docetaxel; i.e., 0, 2.5, 5, 10, and 20 mg/kg. The schematic for the treatment regimen is shown in Figure 1.

SQ tumor volumes were measured once a week and final tumor weights were obtained at necropsy 1 week after last treatment. Weekly intra-peritoneal injections of docetaxel, even as low as 5 mg/kg, decreased SQ tumor volume and tumor weight (Table I). Radiographs of the injected tibiae were taken 2 weeks post-initiation of docetaxel and during treatment to determine the progress of the lesions. The 2.5 and 5 mg/kg doses were associated with partial reduction in PCa-induced radiographic changes; whereas, the 10 and 20 mg/kg doses resulted in major reductions in radiographic changes (Fig. 2A; Table I). We confirmed this observation by histology, again finding evidence of tumor growth in tibiae in the 20, 2.5, 5, and 10 mg/kg dose groups but complete absence of tumor in the 20 mg/kg groups (Fig. 2B, Table I). H&E evidence of tumors was concordant with PSA staining of the tibiae. Taken together, these results demonstrate that docetaxel had a differential effect on SQ tumors, ablating them, versus tibial tumors, where residual disease was still evident at equal dose levels.

No toxicity was observed for any of the dosage groups; there was no change in animal weight or body condition over the course of the experiment (data not shown). Since the 5 mg/kg dose statistically decreased SQ but not IT tumor incidence and burden (Table I) and higher doses had greater anti-tumor efficacy, the 5 mg/kg dose was chosen for further analysis as the sub-optimal dose.

The next goal was to test if RANK-Fc possessed adjuvant activity with docetaxel. However, to first ensure that RANK-Fc would block RANKL in the mice, we treated non-tumor bearing mice three times per week with 250 μg/mouse RANK-Fc alone. This treatment increased the bone mineral density, as measured by pQ-CT (Fig. 3A), approximately 20% (Fig. 3B) while maintaining bone area. Taken together, these data indicate that RANK-Fc was working to diminish bone resorption as described in the literature [17].

The efficacy of 5 mg/kg docetaxel in combination with RANK-Fc, an anti-RANKL therapy, in our mouse bone metastasis model was determined. The experimental plan used is presented in Figure 3C. Briefly,

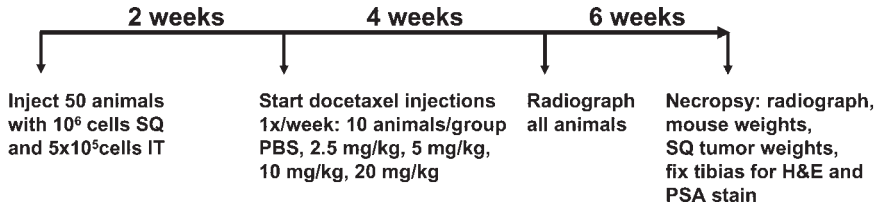

Fig. 1. Schematic of experimental procedure used to determine the sub-optimal dose of docetaxel to be used in conjunction with RANK-Fc. Briefly, 50 animals were injected with 1×10^6 C4-2B PCa cells subcutaneously and 5×10^5 C4-2B PCa cells intratibially. Two weeks later, treatment was started. Animals received one injection per week of their appointed dose of docetaxel. Radiographs of tibiae were obtained half-way through the treatment to monitor tibial lesions. The animals were necropsied 12 weeks after tumor cell injection.

TABLE I. Docetaxel Dose Response for Both SQ and IT Tumor Burden

Treatment	SQ tumor volume[a] (mm^3 by caliper)	SQ tumor weight[a] (mg)	Radiographic presence of IT tumors (% of animals)	Histological presence of IT tumors (% of animals)
PBS only	1574 (2646)	3180 (4925)	80	100
2.5 mg/kg	183 (341)	550 (1060)	70	100
5.0 mg/kg	32 (67)[a]	2 (72)[a,^]	80	100
10 mg/kg	0 (0)[^]	16 (25)[^]	30[a]	30[a]
20 mg/kg	0 (0)[^]	0 (0)[^]	0[a]	0[a]

By *t*-test using the Tukey–Kramer multiple comparisons adjustment, there were significant differences in tumor volume and tumor weights between the control group, (PBS only) and the 5.0 mg/kg, 10 mg/kg, and 20 mg/kg groups. Standard Deviations are given in parentheses.
[a]Excludes an outlier.
[^]P-value < 0.05 compared to PBS only.

mice were injected IT with tumor cells and divided into four groups (PBS/PBS, PBS/docetaxel, RANK-Fc/PBS, and RANK-Fc/docetaxel). The weekly docetaxel injections (5 mg/kg) were combined with three times per week injections of RANK-Fc (250μg/mouse; ~10 mg/kg), a dose we previously determined to be effective toward bone lesions [17].

IT tumor incidence, as measured by radiography (Fig. 3D and Table II), was not affected by docetaxel alone as compared to the control PBS group. RANK-Fc alone decreased IT tumor incidence by 50%; although it was not a statistically significant drop, and the combination of both docetaxel and RANK-Fc significantly blocked development of IT tumors versus treatment with PBS alone or docetaxel alone (Table II). In concordance with the radiographic findings, histomorphometric measurement of tumor volume revealed that docetaxel alone reduced IT tumor volume by approximately 13%, RANK-Fc alone reduced tumor volume by approximately 60%, which was a significant reduction from the PBS alone group, and the combination of docetaxel and RANK-Fc reduced tumor volume by approximately 70%, a significant reduction from both the PBS alone group and the docetaxel alone group, indicating that RANK-Fc had an adjuvant effect with docetaxel (Table II). Of note, the bone volume was

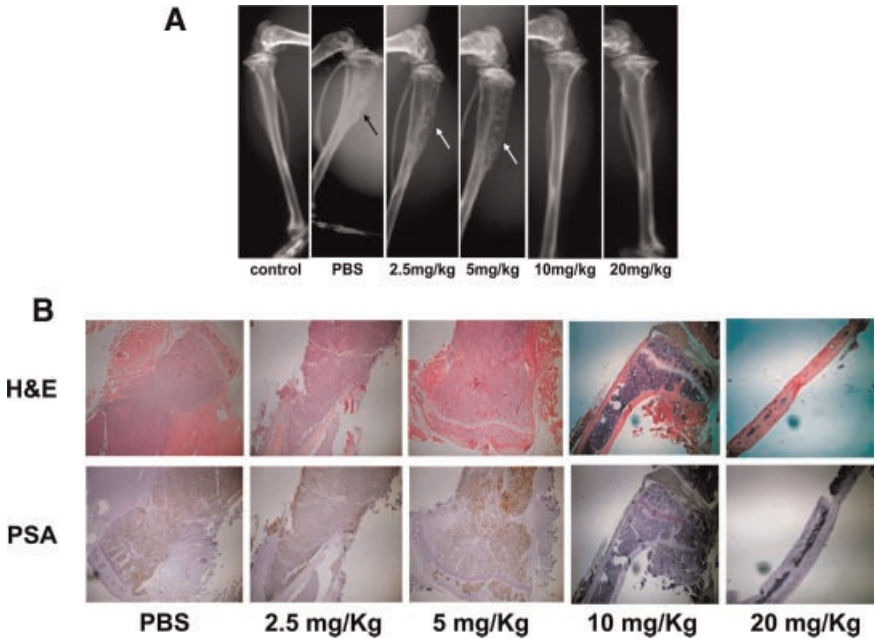

Fig. 2. Radiological and histological evidence of a tibial tumor dose response to increasing doses of docetaxel. **A**: Radiographs of representative tibiae for each dose and for a tumor free control leg (non-injected control). Arrows indicate the areas of bone destruction (saline control, 2.5, and 5 mg/kg). The bone in the 10 and 20 mg/kg treatment groups was not affected by tumor growth. **B**: H&E and PSA stains of representative tibiae. PSA was used to identify tumor in the tibiae and is indicated by the brown stain seen in the lower panels. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

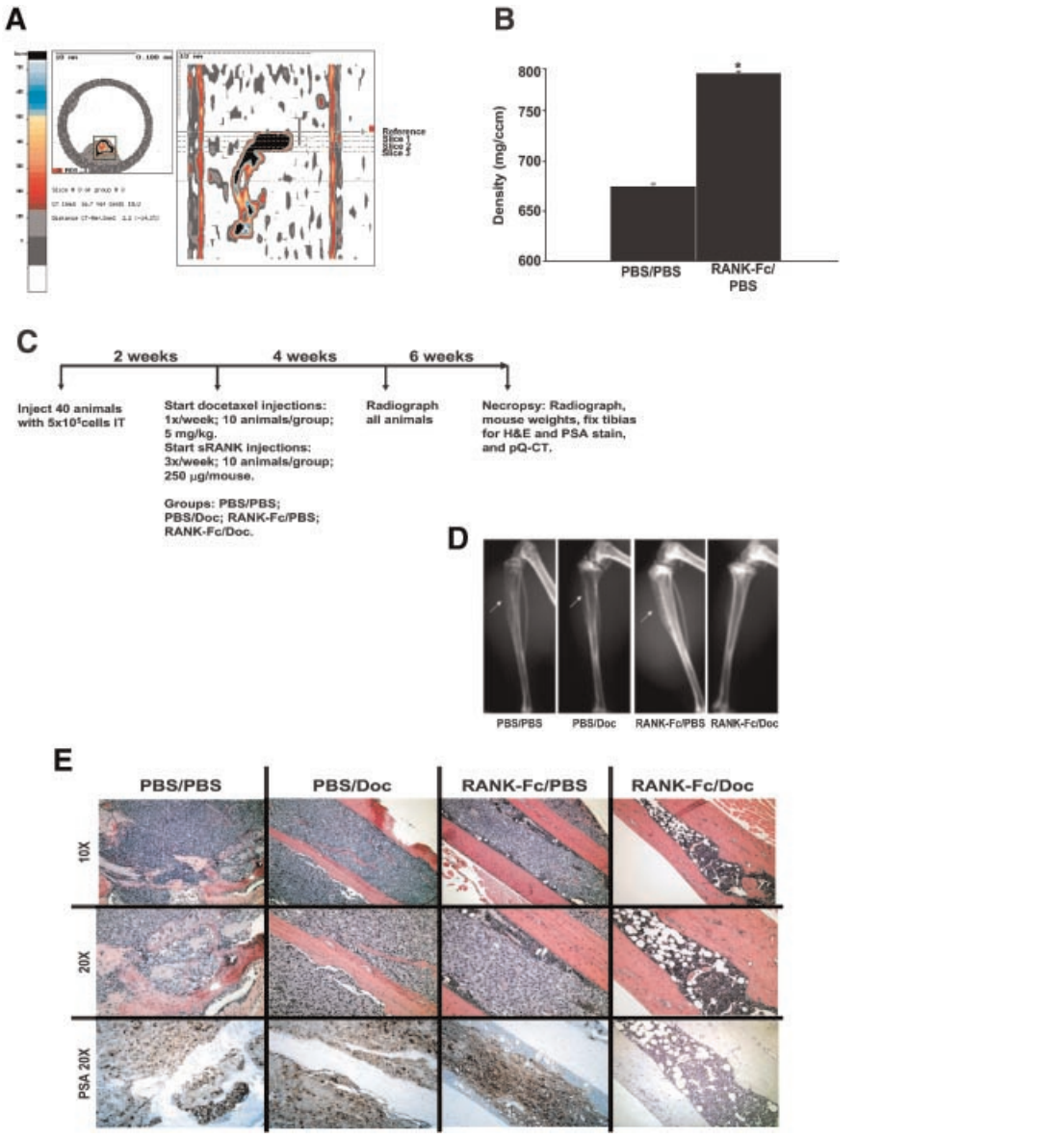

Fig. 3. RANK-Fc protects bone density. **A**: Slices that were scanned by pQ-CT. The small box in the left panel indicates the tibia. The reference slice and the slices measured are indicated on a representative tibia. **B**: Graph of the average bone density obtained by pQ-CT of tibiae from non-tumor bearing mice in the PBS/PBS or RANK-Fc/PBS group. *$P < 0.001$ versus PBS/PBS (*t*-test). **C**: Schematic of experimental procedure to determine the effect of RANK-Fc used in combination with 5 mg/kg docetaxel. Briefly, 40 animals were injected 1×10^6 C4-2B PCa cells subcutaneously and 5×10^5 C4-2B PCa cells intratibially. Two weeks later, treatment was started. Four treatment groups were formed: PBS/PBS, PBS/Doc, RANK-Fc/PBS, and RANK-Fc/Doc. RANK-Fc or PBS injections were given three times per week. Docetaxel or PBS was injected once a week IP in the opposite side than the RANK-Fc or PBS injections. Radiographs of tibiae were obtained half-way through the treatment to monitor tibial lesions. The animals were necropsied 12 weeks after tumor cell injection. Non-tumor bearing mice were also necropsied at 12 weeks after the same regimen of PBS/PBS or RANK-Fc/PBS treatment. **D**: Radiographs of representative tibiae for each treatment group. Arrows indicate tumor lesions (PBS/PBS, PBS/Doc, and RANK-Fc/PBS). RANK-Fc/Doc treated animals did not have any lesions in their tibiae. **E**: H&E and PSA stains of representative tibiae. PSA was used to identify tumor in the tibiae and is indicated by the brown stain seen in the lower panels. Doc, docetaxel. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

not significantly different between any of the groups (Table II); whereas, the bone density was significantly increased in the groups that received the RANK-Fc. The tumor volume docetaxel/RANK-Fc group was decreased compared to the PBS alone group, but the decrease was not significant (P-value = 0.052). The tumor volume to tissue volume ratios for the RANK-Fc/docetaxel group were significantly different from both the PBS alone and docetaxel alone groups (Table II). Since we were comparing tumor to bone

TABLE II. Adjuvant RANK-Fc Response for IT Tumor Burden

Treatment	Number of animals with IT tumors (radiography) (%)	Bone density (pQ-CT) (mg/cm^3)	Tumor volume (mm^3)	Bone volume (mm^3)	Tumor volume/ bone volume (histomorphometry) (%)	Bone volume/ tissue volume (histomorphometry) (%)	Tumor volume/ tissue volume (histomorphometry) (%)
PBS/PBS	80	2307.4 (462.4)	5.19 (5.1185)[a]	1.15 (0.80)	897.20 (1135.5)[a]	9.08 (5.5)[a]	76.12 (26.77)[a]
PBS/Doc	80	2694.0 (630.4)	4.559 (2.4437)	1.04 (0.73)	645.40 (493.6)	15.93 (12.60)[a]	72.71 (12.81)[a]
RANK-Fc/PBS	40	3981.3 (545.6)	2.279$^{\wedge,+}$ (1.8600)	0.919 (0.726)	340.20 (316.3)$^{\wedge}$	16.00 (10.28)	55.91 (38.45)
RANK-Fc/Doc	0$^{\wedge,+}$	3679.5$^{\wedge}$ (562.3)	1.63$^{\wedge,+}$ (0.7494)	1.14 (0.370)	159.50 (90.2)$^{\wedge,+}$	23.41 (13.21)[a,#]	36.93 (19.80)[a,^,+]

The number of IT tumors was compared using a Fisher's Exact Test. Means for the bone density were obtained using the Dunnett multiple comparisons adjustment. The means for the bone volumes, tumor volumes, and bone/tumor/tissue volume comparisons were obtained using the Bonferroni multiple comparisons adjustment. Standard deviations are given in parentheses.

[a]Excludes animals that did not have tibial tumors.

$^{\wedge}P$-value < 0.05 compared to PBS/PBS.

^{+}P-value < 0.05 compared to PBS/Doc.

$^{\#}P$-value $= 0.052$ compared to PBS/PBS.

volume ratios, we excluded measurements for all animals that did not present with tibial tumors when comparing bone, tissue, and tumor volumes. Tibiae were also stained for PSA to identify the tumor, which qualitatively demonstrated less PCa tumor with the RANK-Fc and docetaxel/RANK-Fc combination treatments compared to docetaxel alone (Fig. 3E). Taken together, these data suggest that RANK-Fc is an effective adjuvant to docetaxel for PCa bone metastases.

To determine the effect of blocking RANKL on tumor-induced osteoclastogenesis, paraffin-embedded sections of each tibia were stained for TRAP, a marker for osteoclasts (Fig. 4A). The TRAP staining was intense in the saline control and docetaxel alone samples, very faint in the RANK-Fc alone samples, and not detectable in the combination RANK-Fc/Docetaxel animals. The number of osteoclasts per IT tumor was determined, and, as a measure of osteoclast activity, the amount of osteoclast-mediated erosion was quantified. RANK-Fc alone or RANK-Fc in combination with docetaxel reduced the total number of endocortical osteoclasts (Fig. 4B) and the eroded area (Fig. 4C) by 80–90% compared to the control or to the docetaxel-alone groups. These data indicate that blocking RANKL activity decreased osteoclast numbers and activity suggesting that decreasing osteoclast activity could contribute to decreasing tumor formation in the bone in conjunction with docetaxel's anti-tumor activity.

DISCUSSION

Docetaxel is the treatment of choice against soft tissue PCa tumors; however, the side effects associated with docetaxel treatment can be severe enough to terminate the therapy. Since most advanced PCa patients have bone metastasis, therapies that target PCa bone metastasis were hypothesized to be effective as adjunct therapies to sub-optimal doses of docetaxel. Bone-targeted therapies, such as bisphosphonates, added to the chemotherapy regimen for advanced stage PCa have been effective for palliative care; however, bisphosphonates have moderate to severe toxicities associate with them and further work is needed to improve their efficacy in combination with chemotherapeutics toward bone metastases [22–24]. As an alternative to bisphosphonates for blocking osteoclast activity, we evaluated RANKL inhibition as an anti-osteoclastogenic agent for an adjuvant effect with docetaxel. We found that blocking RANKL enhanced the docetaxel's anti-tumor efficacy.

RANK ligand (RANKL) is expressed on the surface of osteoblasts and tumor cells and is sometimes released as a soluble form. RANKL binds to the receptor RANK. RANKL induces the maturation of osteoclasts, which, in turn, start resorbing bone, a step

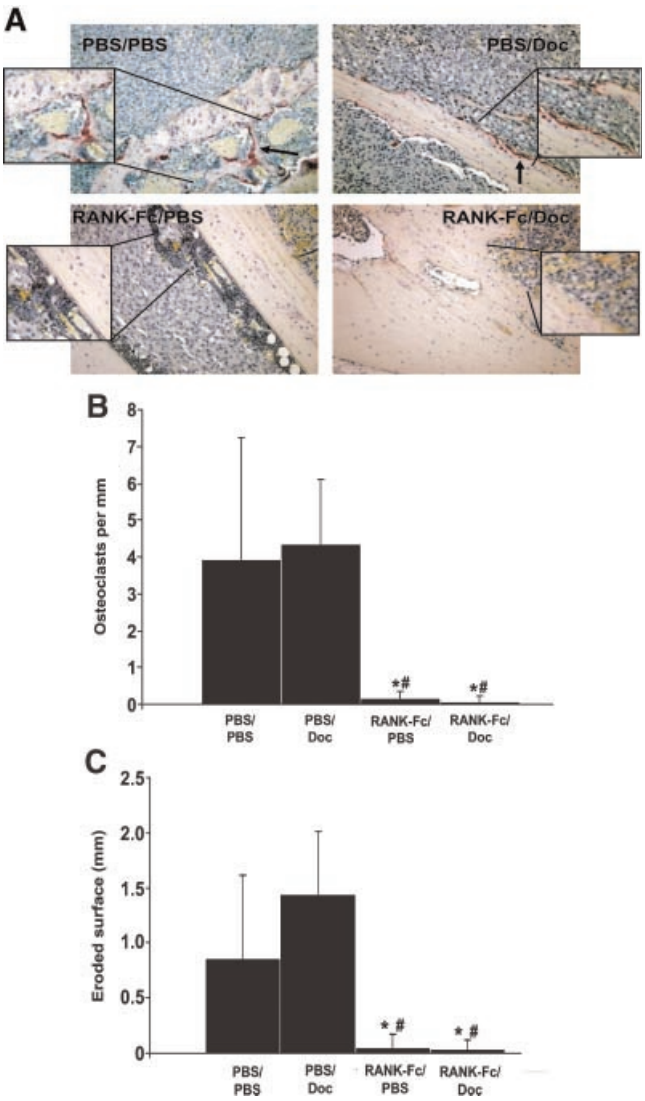

Fig. 4. RANK-Fc plus docetaxel decreases osteoclast numbers. The trabecular region was defined as the top 15% of the tibia distal to the growth plate and the osteoclasts lining the endocortical bone were quantified. **A**: Representative TRAP staining for each treatment is shown. Insets are increased magnification of TRAP staining in each sample. Purple indicates a positive TRAP stain. Arrows indicate areas of TRAP-positive staining. **B**: The number of osteoclasts per millimeter endocortical bone is shown.*$P < 0.05$ versus PBS/PBS; #$P < 0.05$ versus PBS/Doc. **C**: Amount of eroded endocortical bone surface as a measure of osteoclast actvity is shown. *$P < 0.05$ versus PBS/PBS; #<0.05 versus PBS/Doc. Doc, docetaxel. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hypothesized to be important in the seeding of PCa cells into the bone microenvironment. OPG is a soluble RANKL decoy receptor. It binds to and sequesters RANKL so it cannot activate osteoclasts. Similar to OPG, RANK-Fc is recombinant molecule consisting, in part, of the extracellular component of RANK that binds RANKL and blocks activation of osteoclasts [17].

The RANK/RANKL/OPG triad is a major part of bone remodeling. Thus, it is currently being explored as a target for bone metastasis therapy. For example, OPG-Fc has been shown in Phase-I clinical trials to reduce bone resorption as well as bisphosphonates, which are the gold standard of treatment [37–39]. Also, denosumab, a fully human monoclonal antibody to RANKL (Amgen, Inc., Thousand Oaks, CA), reduced bone resorption in osteoporosis [40] and is now in Phase II and Phase III clinical trials for treatment of bone metastases. These data suggest that targeting the RANK/RANKL/OPG triad is a selective way of reducing the effects of bone resorption in skeletal metastases.

Although docetaxel is the treatment of choice for PCa, once men develop bone metastasis, docetaxel efficacy declines. Based on their work with murine models both Brubaker et al. [25] and Quinn et al. [26] suggest that docetaxel by itself is not able to abolish osteoblastic tumors because it cannot enter the bone. However, their work suggests that docetaxel can work synergistically with the bisphosphonate zoledronic acid to suppress growth of tumors in bone. We argue that docetaxel could enter the osteoblastic environment since, in our study, a dose of 10 mg/kg greatly decreased the number and size of osteoblastic IT tumors, and a 20 mg/kg dose completely abolished IT tumors. However, lower doses of docetaxel ($\leq$5 mg/kg) induced a response on SQ tumor size but no effect on IT tumors. Taken together, these results suggest that although docetaxel can kill PCa cells in the bone, the bone provides a protective microenvironment for PCa tumors. This may be due to differences in bioavailablity of drug or actual changes in the PCa cells due to growth factors and other tumor active substances in the bone. It should be noted that Brubaker et al. [25] used LuCaP 23.1 cells for their experiments and found it was necessary to use much higher doses of docetaxel (20 mg/kg) than we found were effective. This effect could be the result of the more intense osteoblastic reaction obtained with LuCaP 23.1 cells compared to C4-2B tumors growing in tibiae. Regardless of the mechanism, our results indicate that PCa bone metastases are more resistant to docetaxel than soft tissue metastases and thus it would be beneficial to identify adjuvants to enhance docetaxel-mediated tumor killing in bone.

Kim et al. [41] have suggested that zoledronic acid cooperates with paclitaxel. Their results show only a minimal additive effect with the two drugs. The enhanced effect of zoledronic acid with paclitaxel is only seen when STI571 is also present, indicating, at least by their data, that PDGFR plays a role in bone metastasis and its inhibition is required in the case of zoledronic acid to observe an additive effect with

paclitaxel. In contrast, our report demonstrates that inhibition of osteoclast activity with RANK-Fc is sufficient to provide an adjuvant effect with docetaxel. The difference in the results may be accounted for by the drug differences; we used a different taxane than both Kim et al. [41] and Brubaker et al. [25] and RANK-Fc inhibits overall osteoclast activity via a different mechanism than bisphosphonates. Inhibition of RANKL reduces osteoclast formation; whereas, bisphosphonates promoters osteoclast apoptosis. This difference in anti-osteoclast activity could potentially account for the observed difference in adjuvant activity. Brubaker et al. [25] showed that the bone volume to tissue volume ratio increased with both zoledronic acid and with the LuCaP 23.1 tumor, suggesting that as the tumor shrank, there was less space for normal marrow to fill. Since the LuCaP 23.1 cells form a highly osteoblastic tumor, the increase in bone volume may not be attributed to either the zoledronic acid or the tumor shrinkage but rather caused by the osteoblastic activity of the tumor. We show that the bone volume to tissue volume ratio does not change significantly when RANK-Fc is used. Since we used a cell line that produces a less osteoblastic tumor than LuCap 23.1, C4-2B, we can postulate that the reduction in tissue volume as seen in the Brubaker's paper may be attributed to the osteoblastic activity of LuCaP 23.1 cells; however, we did use a different adjuvant.

Recent results from Hall et al. [27–29] suggest that in order for PCa to grow in bone, the tumor must first elicit an osteoclastic response then cause an osteoblastic response as the tumor establishes in bone, suggesting that blocking the osteoclastic response should block osteoblastic tumor establishment in bone. Therefore, we tested the effects of lower doses of docetaxel in combination with a novel therapy, RANK-Fc, an inhibitor which selectively inhibits osteoclast differentiation, activation, and survival. We found that the combination of a sub-optimal dose of docetaxel and RANK-Fc had an additive effect on diminishing PCa growth in bone.

The mechanism underlying the anti-RANKL therapy effectiveness is unclear. Specifically, RANK-Fc blocks osteoclast-mediate bone resorption in vivo, but what effect this has on the establishment of bone metastasis is still debated [28,29]. Osteoclast activity could contribute to cancer growth in bone through debulking the bone or release of growth factors from the bone microenvironment, thus, providing a favorable environment for the cancer cells to grow. This possibility is in agreement with previous reports that bone remodeling promotes cancer growth [30,31]. Therefore, blocking osteoclast activity may inhibit release of growth factors, thus, making the bone microenvironment inhospitable for tumor growth. However, since there is no significant increase in bone volume, either due to the drug or to the osteoblastic nature of the tumor, but there is a significant increase in bone density, our data suggest that an increase in bone density accompanies the decrease in tumor growth when RANK-Fc is present and that the osteoblastic nature of the C4-2B tumors does not decrease the normal tissue area. Therefore, RANK-Fc treatment promotes an anti-tumor effect in the bone and an increase in bone density. Taken together, that RANK-Fc may be a useful adjuvant for docetaxel PCa therapy.

The data presented here imply that RANKL inhibition may have a dual effect in PCa. Men with PCa that undergo hormonal therapy develop osteoporosis, likely due to the hormonal deprivation [32–34]. The observation that inhibition of RANKL increased bone density supports previous findings that RANK-Fc protects against osteoporosis [17,35,36]. The adjuvant effect with docetaxel indicates that RANK-Fc can target PCa through both inhibiting treatment-induced osteoporosis as well as tumor-induced bone destruction.

Our data suggest that RANK-Fc is a viable adjuvant therapy for docetaxel. Even though bisphosphonates are now widely used to reduce bone pain and skeletal-related events, such as bone fracture, in PCa, they can induce osteonecrosis of the jaw [42,43] as a side effect of the treatment. Also, OPG can block cancer cell apoptosis by binding to TRAIL [44–46], thus it may not be a clinically viable anti-cancer therarpy. These data taken with the data presented here indicate that antagonists which selectively bind and inhibit RANKL should be studied clinically as an option for adjuvant therapy.

In summary, the data presented here indicate that inhibition of RANKL has an additive effect with docetaxel for skeletal PCa lesions. These results suggest that RANKL inhibition should be evaluated for adjuvant activity with docetaxel in clinical trials for advanced PCa.

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