Targeting the α Receptor for Platelet-Derived Growth Factor as a Primary or Combination Therapy in a Preclinical Model of Prostate Cancer Skeletal Metastasis

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Abstract

Purpose: Platelet-derived growth factor α (PDGFRα) is highly expressed in primary prostate cancer and associated skeletal metastases. Here, we tested whether targeting this receptor could impair metastatic colonization and progression, as well as prolong survival, either as primary or as combination therapy.

Experimental Design: We used a preclinical animal model of metastasis in which PC3-ML human prostate cancer cells are inoculated directly in the blood circulation. First, the humanized, monoclonal antibody IMC-3G3 was administered to mice bearing established skeletal metastases. Second, we targeted the stromal PDGFRα with IMC-1E10, an antibody specific for the murine receptor. Third, IMC-3G3 and the bisphosphonate zoledronic acid (ZA), administered separately or in combination, were tested on the progression of skeletal lesions and overall survival. In addition, the ability of IMC-3G3 and ZA to impair initial colonization of the bone marrow by prostate cancer cells was investigated.

Results: The blockade of PDGFRα on prostate cancer cells by IMC-3G3 reduces the size of established skeletal metastases, whereas the IMC-1E10 antibody directed against the stromal PDGFRα fails to inhibit metastatic progression. IMC-3G3 and ZA, either separately or in combination, significantly slow tumor growth and seem to prolong survival. Lastly, the blockade of PDGFRα by IMC-3G3 inhibits the initial phase of bone colonization, whereas ZA is ineffective at this stage.

Conclusion: This study presents compelling evidence that targeting PDGFRα with IMC-3G3 delays the progression of early metastatic foci and reduces the size of more established lesions. In addition, IMC-3G3, either alone or in combination with ZA, prolongs survival in animal models. *Clin Cancer Res; 16(20); 5002–10. ©2010 AACR.*

Although skeletal metastases occur with a significantly high rate in patients affected by advanced prostate carcinoma (1, 2), a limited range of options is currently available for the treatment of metastatic bone lesions (3, 4). Equally frustrating is the evidence that the burden deriving from skeletal dissemination represents the main cause of death for these patients, but the current standard of care for advanced prostate cancer produces merely palliative effects (5). Skeletal metastases cause bone resorption and chronic pain and predispose patients to skeletal-related events such as pathologic fractures and spinal cord compression. This skeletal morbidity is presently treated with the administration of bisphosphonates in prophylactic and adjuvant settings (6). Bisphosphonates are inorganic pyr-

ophosphates that inhibit the enzymatic activities of osteoclasts, the cells responsible for the degradation of bone matrix (7, 8). These compounds are effective in alleviating bone loss and pain, as well as reducing the occurrence of skeletal-related events (9, 10). However, bisphosphonates are not without side effects, including renal toxicity, hypocalcemia, and osteonecrosis of the jaw, which may prevent their long-term administration in bone-metastatic prostate cancer patients (11, 12).

In addition to the management of symptoms produced by skeletal involvement and the prevention of skeletal-related event, the use of bisphosphonates in the clinic to target osteoclasts is also aimed to directly inhibit cancer cell growth and survival. This approach is based on evidence that the degradation of bone matrix releases a variety of trophic factors capable of supporting the disseminated cancer cells in their metastatic growth. The resulting increase in tumor mass will recruit and activate a progressively higher number of osteoclasts, thereby generating a self-sustained vicious cycle (13–15). Unfortunately, results from clinical trials have not been encouraging and seem to indicate that, although successful in reducing bone loss and pain, bisphosphonates are ineffective in arresting disease progression or improving survival in patients with metastatic prostate

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Translational Relevance

Skeletal metastases are responsible for significant morbidity and represent the main cause of death in patients with advanced prostate adenocarcinoma. The treatment options currently available for these patients are mostly palliative.

Platelet-derived growth factor α is responsible for conferring bone-metastatic potential to prostate cancer cells. Our studies provide preclinical evidence that the blockade of this receptor is a new and very effective approach to treat skeletal secondary tumors. We targeted platelet-derived growth factor α with IMC-3G3, a humanized monoclonal antibody that is entering phase II clinical trials for advanced solid tumors, including prostate cancer. We showed that this antibody has a broad spectrum of activity because it is effective on initial and established skeletal lesions. In addition, alone and in combination with the bisphosphonate zoledronic acid, IMC-3G3 significant prolongs survival. This work will expedite the path toward the clinical use of this antibody as a new treatment for skeletal dissemination of prostate adenocarcinoma.

negatively affect the survival of PDGFRα-expressing prostate cancer cells by internalizing the receptor and thereby impeding its transactivation and consequent downstream signaling. Indeed, we have previously observed a strong antimetastatic effect when using IMC-3G3 prophylactically in a preclinical model of bone-metastatic prostate cancer. In this context, the work presented here expands our previous study by addressing any antimetastatic effect of IMC-3G3 administered in a curative protocol, as well as the role played by the simultaneous targeting of PDGFRα on cancer and stromal cells. We also report that IMC-3G3, unlike bisphosphonates, is extremely effective in reducing metastatic growth during the first week following the arrival of cancer cells to the skeleton. Finally, we show that the administration of IMC-3G3, alone or in combination with a bisphosphonate, prolong the survival of mice bearing skeletal metastases from human prostate cancer cells.

Materials and Methods

Reagents

The IMC-3G3 and IMC-1E10 antibodies were provided by ImClone, Eli Lilly. The bisphosphonate zoledronic acid (ZA) was obtained from Sigma.

Cell lines and cell culture

NIH-3T3 cells were obtained from American Type Culture Collection, whereas the highly bone-metastatic PC3-ML subline was derived from the parental PC-3 cells (American Type Culture Collection) as previously described (25). Cells were cultured at 37°C and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum (Hyclone) and 0.1% gentamicin (Invitrogen). The PC3-ML cells were engineered to stably express enhanced variant of green fluorescent protein (GFP) by using a lentiviral vector from America Pharma Source (17).

In vitro experimental protocol

Cells were washed twice with sterile PBS and then starved from serum for 4 hours before being incubated for 30 minutes with either IMC-1E10 or IMC-3G3 (20 μg/mL) and successively exposed to PDGF-AA (30 ng/mL).

SDS-PAGE and Western blotting

Cell lysates were obtained and SDS-PAGE and Western Blot analysis were done as previously described (24), with few modifications. Membranes were blotted with antibodies targeting phosphor-Akt (Ser-473; Cell Signaling Technology), PDGFRα (R&D Systems), and total Akt (Cell Signaling). Primary antibody binding was detected using a horseradish peroxidase-conjugated secondary antibody (Pierce). Chemiluminescent signals were obtained using SuperSignal West Femto reagents (Pierce) and were detected with the Fluorochem 8900 imaging system and relative software (Alpha Innotech). Densitometry analysis was done using the UN-SCAN IT software (Silk Scientific).

cancer (6, 16). This scenario is likely determined by the fact that disseminated cancer cells may benefit from additional trophic support that does not derive from bone matrix degradation but is readily available in the bone marrow environment and, therefore, not necessarily dependent on osteoclast activity. Indeed, our previous studies have shown that isolated cancer cells and small foci detected shortly after their initial colonization of the bone are either independent of or much less reliant on spatial interactions with osteoclasts than are larger, more advanced lesions (17). Thus, it seems plausible that other compounds used in combination with bisphosphonates, such as those that target survival and/or proliferative pathways in cancer cells, may result in pronounced curative effects.

The α receptor for platelet-derived growth factor (PDGFRα) is a receptor tyrosine kinase that has been correlated with advanced prostate carcinoma and was found associated with skeletal metastases by others (18–20) and us (17, 21). The activation of this receptor in prostate cancer cells is responsible for the recruitment of the PI3K/Akt cell survival pathway. To target the human PDGFRα, we used the humanized monoclonal antibody IMC-3G3 (ImClone, Eli Lilly). This antibody was developed for the treatment of several pathologic conditions and is currently in phase II clinical trials (22). We found that IMC-3G3 suppresses PDGFRα-mediated activation and downstream Akt stimulation by inducing receptor internalization (23). Interestingly, we have also shown that the soluble fraction of human bone marrow is able to transactivate human PDGFRα through a ligand- and dimerization-independent mechanism (23, 24). Thus, IMC-3G3 could

Animal model of metastasis

Five-week-old, male, immunocompromised mice (CB17-SCRF) were obtained from Taconic and were housed in a germ-free barrier. At 6 weeks of age, mice were anesthetized with 100 mg/kg ketamine and 20 mg/kg xylazine and successively inoculated in the left cardiac ventricle with PC3-ML cells (5×10^4 in a volume of 100 μL of serum-free DMEM/F12). Although consistently producing skeletal tumors in tibiae and femora of >80% of inoculated mice, PC3-ML cells do not metastasize to any soft-tissue organ with the exception of small tumors produced in the adrenal glands and, very seldom, the liver. Mice were sacrificed at specified time points following inoculation and tissues prepared as described below. For the survival studies, mice were euthanized when in a moribund state as defined by evident and prolonged shivering, extreme prostration, labored breathing, >20% of body weight loss, and/or unresponsiveness to external stimulation. All experiments were conducted in accordance with NIH guidelines for the humane use of animals. All protocols involving the use of animals were approved by the Drexel University College of Medicine Committee for the Use and Care of Animals.

Administration of antibodies for PDGFRα

Animals received a loading dose of 214 mg/kg immediately after inoculation with PC3-ML cells, followed by a maintenance dose of 60 mg/kg every 72 hours thereafter. Doses and administration schedule were selected based on pharmacokinetic and tumor-growth studies (26). Control mice were maintained on an identical dosing schedule and received similar injection volumes of either saline or human immunoglobulins of the IgG_1 subclass as the IMC-3G3 and IMC-1E10 antibodies (26).

Tissue preparation

Tibiae and femora were collected and fixed in 4% formaldehyde solution for 24 hours and then transferred into fresh formaldehyde for additional 24 hours. Bones were decalcified in 0.5 mol/L EDTA for 7 days, followed by incubation in 30% sucrose for cryoprotection or 1% formaldehyde for long-term storage. Tissues were maintained at 4°C during all aforementioned steps and frozen in optimal cutting temperature (OCT) medium (Electron Microscopy Sciences) by placement over dry ice-chilled 2-methylbutane (Fisher). Serial sections of 80 μm in thickness were obtained using a Microm HM550 cryostat (Mikron).

Fluorescence stereomicroscopy and morphometric analysis of metastases

Bright-field and fluorescent images of skeletal metastases were acquired using a SZX12 Olympus stereomicroscope coupled to an Olympus DT70 CCD color camera. Digital images were analyzed with ImageJ software (http://rsb.info.nih.gov/ij/) and calibrated for measurement by obtaining a pixel to millimeter ratio. Morphometric evaluation of skeletal tumors was conducted by analysis of serial cryosections, in which the largest representative tumor section for each metastasis was identified. A freehand tool was used to outline the border of each metastatic lesion, and the area was computed using the ImageJ "measure area" function.

Tartrate-resistant acid phosphatase staining

Slides were incubated at 37°C for 5 minutes in a solution containing naphthol 7-Bromo-3-hydroxy-2-naphthoic-*o*-anisidide phosphate and ethylene glycol monoethyl ether (Sigma). Slides were then transferred to a solution containing sodium nitrite and pararosaniline chloride (Sigma) for ~3 minutes.

Statistics

We analyzed number and size of skeletal metastases between groups using a two-tailed Student's *t* test. Statistical significance between multiple groups was conducted using a one-way ANOVA, followed by Tukey's multiple comparison test. A value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Previous studies from our group have shown that bone-metastatic human prostate cancer cells express high levels of PDGFRα (21). The exposure of these cells to the soluble fraction of human bone marrow induces phosphorylation of PDGFRα and its downstream activation of the PI3K/Akt survival pathway (23). The antagonism of human PDGFRα achieved with the IMC-3G3 antibody attenuates the signaling events *in vitro* and reduces the number and size of skeletal metastases in preclinical animal models (17). These studies show a prophylactic action of the IMC-3G3 antibody on skeletal metastases; here, we sought to determine whether PDGFRα inhibition could also produce a curative effect on metastatic bone lesions that had sufficient time to establish and progress before IMC-3G3 administration. To this end, we used a model of experimental metastasis that uses PC3-ML human prostate cancer cells previously isolated from the PC3 parental cell line for their high bone-metastatic potential (25). These cells were engineered to stably express an enhanced variant of GFP and directly inoculated in the blood circulation of immunocompromised severe combined immunodeficient mice through the left cardiac ventricle. Metastatic tumors ranging from microscopic foci to macroscopic lesions were identified and measured as previously described (17).

A first set of experiments was conducted with mice inoculated with PC3-ML cells and left untreated for either 7 or 14 days, thus providing a time interval for metastatic tumor growth. Following this first period, treatment with IMC-3G3 began and continued until sacrifice at 4 weeks postinoculation. The dosing protocol consisted of a loading dose followed by maintenance doses administered three times weekly through an i.p. route (17). Control mice were maintained on an identical dosing schedule and received either saline or human immunoglobulins of the same IgG_1 subclass as the IMC-3G3 antibody

(26), with both controls providing identical results. At the end of the study, mice were euthanized and the area of metastatic lesions was measured by fluorescence microscopy of frozen tissue sections. Animals that were administered IMC-3G3 according to these two different curative protocols presented with skeletal lesions in the tibia and femur that seemed significantly reduced in size from those observed in mice from control groups (Fig. 1A). In addition, delaying administration of IMC-3G3 for 1 or 2 weeks following tumor cell inoculation resulted in nearly identical growth inhibition (Fig. 1B). This strongly suggests that prostate cancer cells that express PDGFRα still depend on this receptor for their growth and survival when tumor foci occupy an average area of the bone marrow $<35 \pm 6 \times 10^3$ μm^2 as we determined earlier (17). Interestingly, only at this point in time the bone lesions in our model became spatially associated with the surrounding osteoclasts. In fact, a comparable lack of osteoclast involvement during

the initial stages of bone colonization has been also observed in humans (27). Thus, our data seem to point out to a role for PDGFRα in sustaining metastatic progression in the bone before the onset of osteolysis and induction of the vicious cycle.

Multiple studies have shown that reciprocal interactions between cancer cells and the surrounding stroma play a critical role in tumor growth (28–30). For instance, it has been shown that stromal PDGFRα maintains tumor growth and local angiogenesis when stimulated by PDGF-AA, PDGF-CC, or vascular endothelial growth factor-A secreted by cancer cells (31, 32). Based on this evidence, we sought to establish the contribution of the PDGFRα expressed by stromal cells (i.e., osteoblasts and cells of the mesenchymal compartment of the bone marrow) on the skeletal colonization and metastatic progression of PC3-ML cells. The IMC-3G3 antibody has been shown to be highly selective for human PDGFRα (26). We confirmed this specificity
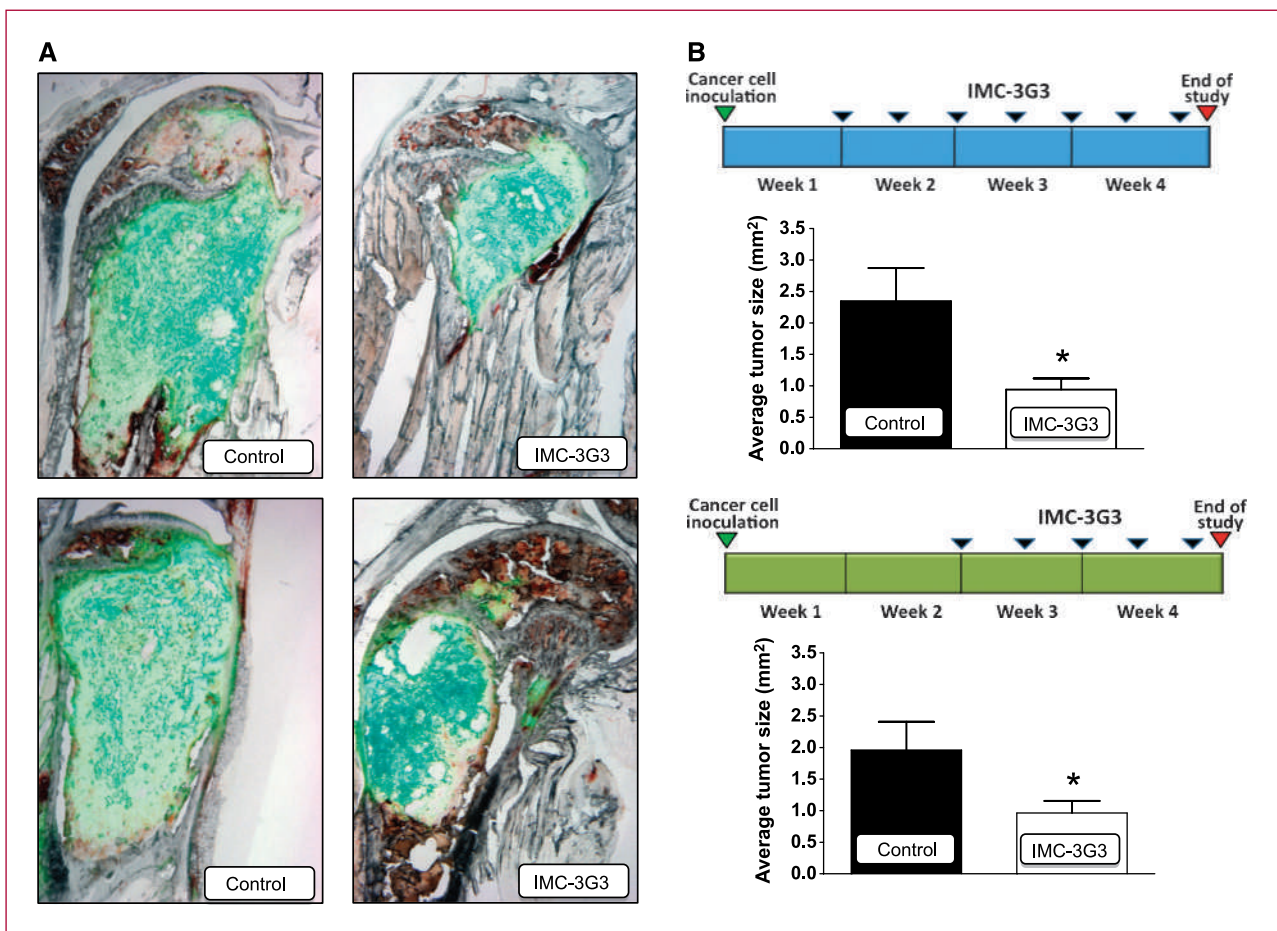

Fig. 1. The monoclonal antibody for human PDGFRα IMC-3G3 reduces the size of established skeletal metastases. Six-week-old male CB17-SCRF mice were inoculated with PC3-ML cells expressing an enhanced variant of GFP through the left cardiac ventricle. Mice had withheld treatment for either 1 or 2 weeks, after which they were maintained on IMC-3G3 for the remainder of the experiment. After 4 weeks, mice were euthanized, their tibiae and femora were cryosectioned, and metastatic skeletal lesions were identified by fluorescence stereomicroscopy and measured. Mice that received IMC-3G3 showed a significant reduction in the average size of bone tumors in 1-week (A) and 2-week (B) treatment-withheld conditions as compared with controls. One-week group, controls (n = 8) and IMC-3G3 (n = 7); 2-week group, controls (n = 10) and IMC-3G3 (n = 8). Two different experiments were conducted for each experimental group. *, P ≤ 0.05.

by exposing NIH-3T3 mouse fibroblasts to PDGF-AA upon pretreatment with IMC-3G3 and noticed that the phosphorylation of Akt was unaffected as compared with control cells (Fig. 2A). Therefore, to effectively target the stroma, we used IMC-1E10, a humanized antibody selected for binding to murine PDGFRα and otherwise sharing an identical structure with IMC-3G3 (33). The species selectivity of this antibody was confirmed by the complete inhibition of Akt phosphorylation observed in NIH-3T3 cells stimulated with PDGF-AA (Fig. 2A).

In the ensuing studies, mice were inoculated with PC3-ML cells and randomly assigned to three experimental groups, which received saline solution (control group), the IMC-1E10 antibody alone, or IMC-1E10 and IMC-3G3 antibodies in combination. Mice were treated according to a prophylactic protocol we described previously (i.e., loading dose administered immediately following cell inoculation) and maintained on a three times weekly dosing schedule until sacrifice after 4 weeks (17). We show that solely targeting stromal PDGFRα by administration of IMC-1E10 had no effect on reducing the size of metastatic lesions in either the tibiae or femora of treated mice (Fig. 2B). On the other hand, administration of IMC-1E10 in combination with IMC-3G3 retained the ability of IMC-3G3 alone to impair metastatic growth (Fig. 2B). Despite that the activity of 1E10 on stromal PDGFRα was not directly assessed, these results suggest that targeting PDGFRα of the stromal cellular compartment is ineffective in decreasing bone metastatic growth and also fails to provide a synergistic effect when coupled with inhibition of the PDGFRα expressed by metastatic prostate cells. Notably, we found that the 4-week antagonism of stromal PDGFRα did not cause toxicity because animals maintained a healthy weight throughout the experiments and failed to show signs of overt organ damage at the necropsy. These preclinical findings are supported by the recent completion of phase I clinical trials reporting the absence of significant adverse effects by the prolonged administration of IMC-3G3 (22). The absence of toxicity observed in these trials may be explained by the minor physiologic role exerted by PDGFRα in fully developed organisms. Although PDGFRα plays a crucial role in embryonic development, in the adult, its function is mostly overshadowed by that of PDGFRβ in angiogenesis, wound healing, tissue homeostasis, and other systemic effects (34–36). In fact, there is evidence from genetic replacement experiments that PDGFRβ signaling can fully compensate for PDGFRα signaling, but not vice versa (37). The predominant function of PDGFRβ in human physiology strongly supports the rationale of safely targeting PDGFRα to counteract cancer cells that depend on this receptor for growth and survival (22). This seems particularly relevant to the field of prostate cancer because the therapeutic options currently available in the clinic for patients with skeletal metastatic disease are extremely limited, present a limited therapeutic window, and are only minimally effective.

Thus, the next series of experiments aimed to compare IMC-3G3 with a current standard of care for patients pre-
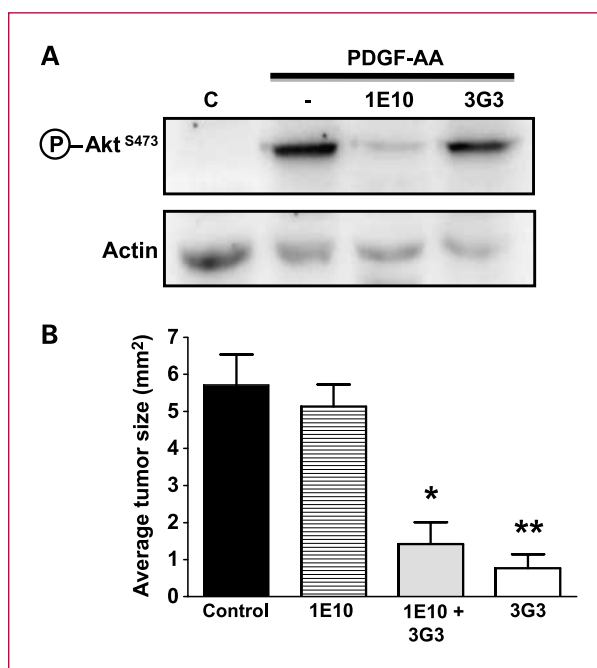

Fig. 2. Targeting of stromal PDGFRα by the mouse-specific IMC-1E10 antibody fails to reduce the size of skeletal metastases. Specificity of the IMC-1E10 antibody (20 μg/mL) for the mouse PDGFRα was confirmed by the blockade of Akt phosphorylation induced by PDGF-AA (30 ng/mL) in NIH-3T3 murine fibroblasts. In contrast, in the same cells treated with the human-specific IMC-3G3 antibody, the extent of Akt phosphorylation was unchanged as expected (A). Mice were inoculated with PC3-ML cells through the left cardiac ventricle to induce skeletal metastases and treated with either IMC-1E10, IMC-3G3, or a combination of the two antibodies. B, after 4 weeks, mice were sacrificed and the size of metastases in tibiae and femora was measured. Animals that received IMC-1E10 showed no decrease in tumor size versus controls, whereas mice treated with IM-3G3 either alone or in combination with IMC-1E10 showed a significant reduction in the size of skeletal tumor foci. Five animals were used for each experimental group. *, $P < 0.01$; **, $P < 0.001$. C, control.

senting with dissemination of prostate cancer to the bone. The presence of significant pain in these patients is caused by alterations in bone turnover deriving from the considerable recruitment and activation of osteoclasts at the site of metastasis (1, 38, 39). The resorption of mineralized bone matrix by these cells followed by deregulated deposition of sclerotic bone by osteoblasts is responsible for the mixed lytic-blastic appearance of metastatic lesions from prostate cancer (40). These histopathologic alterations almost invariably correspond to systemic clinical conditions such as hypercalcemia of variable degrees, as well as skeletal-related event such as spinal nerve compression and pathologic bone fractures (41). Because of the resulting morbidity and reduced quality of life, the administration of inhibitors of osteoclast activity is currently standard of care for patients with bone-metastatic dissemination, including that from prostate cancer (7). ZA is a bisphosphonate with a potent analgesic effect, which has shown significant activity in delaying the time to skeletal-related event in clinical trials

conducted with prostate cancer patients (16). Therefore, we decided to investigate whether metastatic bone lesions would respond to the combined administration of ZA and IMC-3G3. For these studies, mice were assigned to groups receiving ZA or IMC-3G3 alone, as well as ZA plus IMC-3G3 combination therapy, and compared with control groups. IMC-3G3 was dosed as reported above, whereas ZA was administered by weekly s.c. injections of 100 μg/kg. At the end of the 4-week study, tissues obtained from sacrificed mice were examined for staining of tartrate-resistant acid phosphatase, a widely used histologic indicator of osteoclast activity (42, 43). As expected, mice that received weekly injections of ZA showed a marked reduction in the number and activity of osteoclasts at the tumor-stroma border (Fig. 3A). We also found that mice receiving either ZA or IMC-3G3 alone had a significant decrease in the size of skeletal lesions. However, there was no synergistic reduction of tumor size when the two therapies were combined (Fig. 3B).

Although ZA and IMC-3G3 were equally effective in delaying the growth of metastatic prostate cancer cells in the bone, we decided to ascertain whether these similar outcomes were the result of different mechanisms of action. This possibility seemed to be supported by the initial evidence presented above that blockade of PDGFRα by IMC-3G3 is particularly effective previous the onset of osteoclast-mediated degradation of the bone matrix, which is the main event inhibited by ZA. To address this point, mice inoculated with PC3-ML cells received either ZA or IMC-3G3 and were sacrificed after only 1 week of bone-metastatic tumor growth. We found that, although both groups of mice had comparable numbers of bone metastases, the lesions in IMC-3G3 treated mice were significantly smaller than the tumors detected in animals treated with ZA, which were indistinguishable from those measured in controls (Fig. 4). These results provide compelling evidence that the antagonism of PDGFRα, achieved in this study with IMC-3G3, effectively impairs the initial colonization of the skeleton by prostate cancer cells. This phase of metastatic progression seems refractory to ZA, which relies on the inhibition of osteoclasts and therefore targets later-stage tumors.

Fig. 3. Inhibitory effect on skeletal metastases exerted by ZA alone or in combination with IMC-3G3. Mice inoculated with PC3-ML cells were treated with a weekly administration of ZA or with IMC-3G3 three times weekly, either separately or in combination. After 4 weeks, mice were sacrificed and inspected for skeletal metastases. Inhibition of osteoclast activity by ZA was confirmed by the uniform reduction of tartrate-resistant acid phosphatase staining, showing fewer and less active osteoclasts recruited to the tumor periphery (A). Animals that received either ZA or IMC-3G3 administered separately showed comparable reduction in the size of metastatic bone tumors. The concurrent inhibition of osteoclast activity by ZA and PDGFRα by IMC-3G3, administered as a combination therapy, did not produce any synergistic effect on tumor growth (B). Five animals were used for each experimental group. *, $P \leq 0.0001$. M, metastatic lesion.
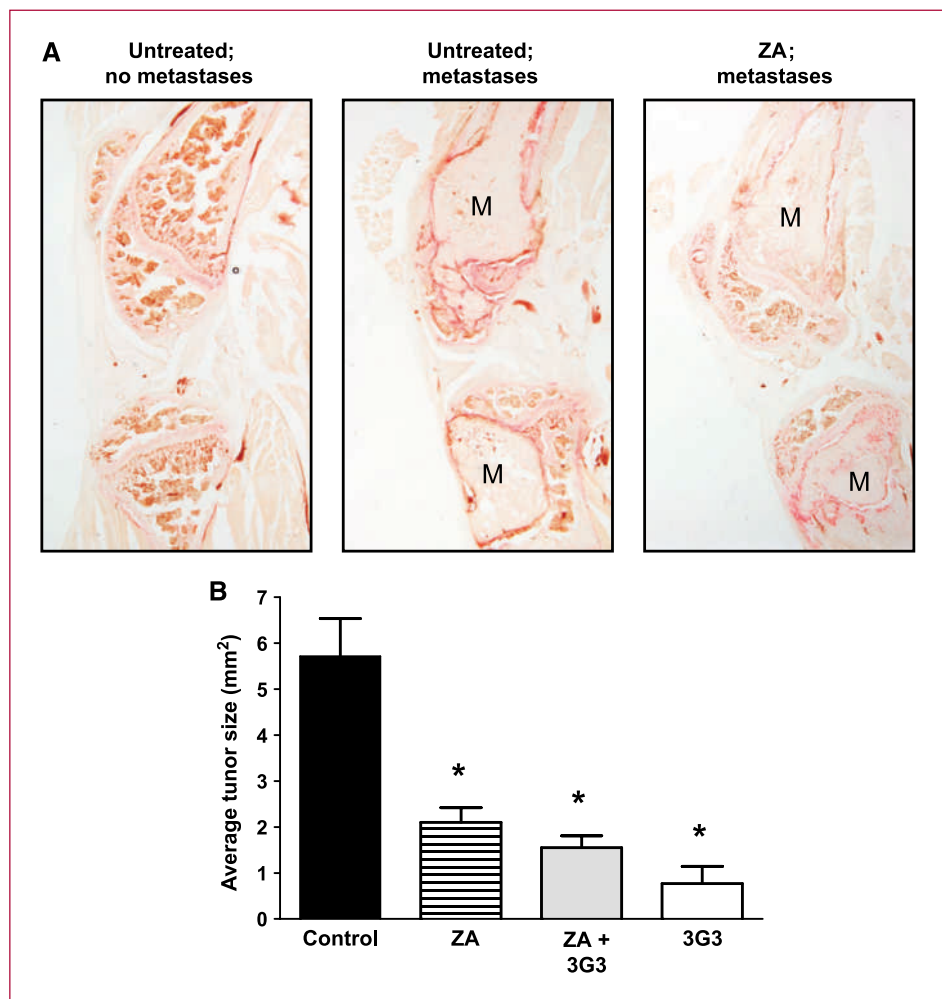
A conclusion that could be drawn from these experiments is that targeting either early metastases with IMC-3G3 or more advanced lesions with ZA leads to a similar outcome in terms of controlling the size of bone metastatic tumors from prostate cancer cells. However, preclinical models of metastatic breast cancer (44) and clinical trials conducted with advanced prostate cancer patients indicate the inability of bisphosphonates to improve overall survival from bone metastatic disease (16). Recent studies with animal models using radiologic and tumor imaging approaches show that reducing the extent of bone destruction and invasion may not fully prevent metastatic breast cancer cells from growing, for example, by infiltrating surrounding soft tissues (44). Based on the evidence that IMC-3G3 reduces metastatic progression by directly impairing cancer cell growth without affecting osteoclasts or bone degradation, we tested the effect on survival of

IMC-3G3 and ZA administered either separately or as a combination therapy.

To carry out these studies, mice were inoculated with PC3-ML cells and randomly assigned to one of four groups: control, IMC-3G3, ZA, or IMC-3G3 plus ZA. as described above, mice received IMC-3G3 three times weekly following the initial loading dose, whereas ZA was administered weekly. Animals were kept in the study until moribund. Necroscopic examination did not detect visceral metastases by PC3-ML cells. The only exception was represented by enlarged adrenal glands, frequently bilaterally, consistent with what we have previously reported (45). These small adrenal tumors were only detected by fluorescence stereomicroscopy, always found contained within the gland parenchyma, and therefore very unlikely to cause lethal tumor burden. As shown in Fig. 5, mice treated with ZA alone showed an increase in survival as compared
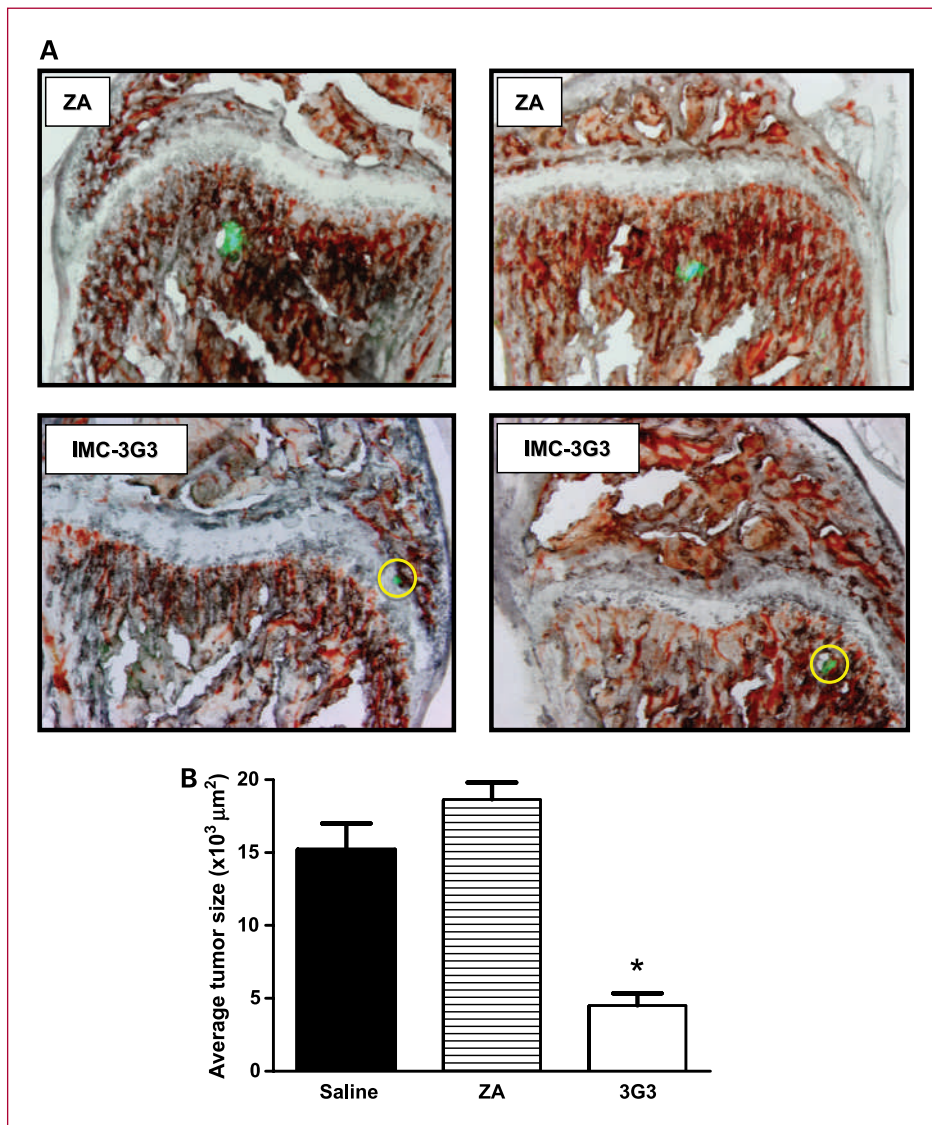

Fig. 4. Blockade of PDGFRα by IMC-3G3 inhibits the early stages of bone-metastatic tumor growth. Animals were injected through an intracardiac route with PC3-ML cells stably expressing enhanced variant of GFP, treated with either ZA or IMC-3G3, and sacrificed 1 week later. Early bone-metastatic lesions were identified by fluorescence stereomicroscopy (A). Tumor foci in the two groups of animals were compared with those detected in control saline-treated animals. The treatment with IMC-3G3 significantly reduced tumor size, whereas the lesions detected in mice that received ZA were comparable in size to those measured in control animals (B). Control, 5 mice and 13 metastases analyzed; ZA, 10 mice and 22 metastases analyzed; IMC-3G3, 10 mice and 26 metastases analyzed. *, $P < 0.0001$. Circles indicate small metastatic foci.
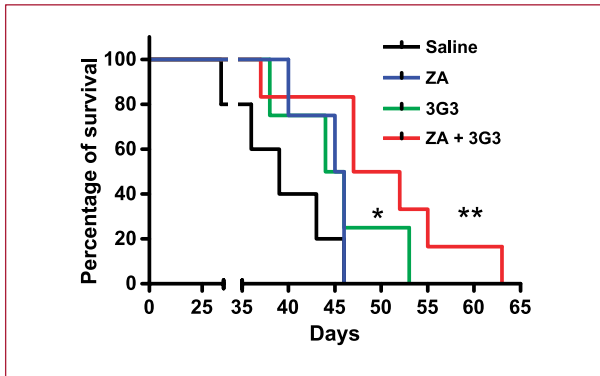
Fig. 5. The IMC-3G3 antibody, alone or in combination with ZA, prolongs survival in animals with skeletal metastases. Mice were inoculated with PC3-ML cells and maintained on a treatment consisting of either IMC-3G3 or ZA administered separately or as a combination. Controls received equal volumes of saline solution. Animals were sacrificed when moribund. The Kaplan-Meier graph shows that IMC-3G3 alone and in combination with ZA was able to prolong survival. Between 4 and 6 animals were used for each group. *, $P < 0.05$; **, $P < 0.01$.

with the control group, although this effect did not reach statistical significance most likely because of the small number of mice used. However, the main conclusion of this set of experiments is that IMC-3G3 used alone prolonged survival and this effect was even more pronounced when this antibody was administered in combination with ZA. Taken together, these results clearly indicate that the blockade of PDGFRα through administration of IMC-3G3 delays the growth of preexisting skeletal metastases from prostate cancer cells and significantly extends survival in animals with bone-metastatic dissemination. These effects were exerted independently from the inhibition of osteolysis, which is the main mechanism of action of bisphosphonates such as ZA. Remarkably, the administration of ZA resulted in an enhanced survival benefit when combined with the direct antitumor action of IMC-3G3 on prostate cancer cells. This indicates that the mobilization of growth factors from the bone matrix by osteolytic events is likely to play a role in supporting prostate cancer growth at the skeletal level. A strategy aimed to contain bone degradation combined with a direct interference of PDGFRα functioning can significantly prolong survival in preclinical models of metastatic prostate cancer.

References

1. Mundy G. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002;2:584–93.
2. Buijs JT, van der Pluijm G. Osteotropic cancers: from primary tumor to bone. Cancer Lett 2009;273:177–93.
3. Denmeade SR, Isaacs JT. A history of prostate cancer treatment. Nat Rev Cancer 2002;2:389–96.
4. Bagi CM. Targeting of therapeutic agents to bone to treat metastatic cancer. Adv Drug Deliv Rev 2005;57:995–1010.
5. Mohammad KS, Fournier PG, Guise TA, Chirgwin JM. Agents targeting prostate cancer bone metastasis. Anticancer Agents Med Chem 2009;9:1079–88.
6. Gnant M. Bisphosphonates in the prevention of disease recurrence: current results and ongoing trials. Curr Cancer Drug Targets 2009;9: 824–33.
7. Saad F, Schulman CC. Role of bisphosphonates in prostate cancer. Eur Urol 2004;45:26–34.
8. Russell RG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 2008;19:733–59.
9. Hatoum HT, Lin SJ, Smith MR, et al. Zoledronic acid and skeletal complications in patients with solid tumors and bone metastases: analysis of a national medical claims database. Cancer 2008;113: 1438–45.
10. Costa L, Major PP. Effect of bisphosphonates on pain and quality of life in patients with bone metastases. Nat Clin Pract Oncol 2009;6:163–74.
11. Body JJ, Mancini I. Bisphosphonates for cancer patients: why, how, and when? Support Care Cancer 2002;10:399–407.
12. Abrahamsen B. Bisphosphonate adverse effects, lessons from large databases. Curr Opin Rheumatol 2010;22:404–9.
13. Roodman GD. Mechanisms of bone metastasis. N Engl J Med 2004; 350:1655–64.
14. Guise T, Mohammad K, Clines G, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res 2006;12:6213–6s.
15. Kingsley LA, Fournier PG, Chirgwin JM, Guise T. Molecular biology of bone metastasis. Mol Cancer Ther 2007;6:2609–17.
16. Saad F, Gleason DM, Murray R, et al. A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. J Natl Cancer Inst 2002;94:1458–68.
17. Russell M, Jamieson W, Dolloff N, Fatatis A. The α-receptor for platelet-derived growth factor as a target for antibody-mediated inhibition of skeletal metastases from prostate cancer cells. Oncogene 2009;28: 412–21.
18. Fudge K, Wang CY, Stearns ME. Immunohistochemistry analysis of platelet-derived growth factor A and B chains and platelet-derived growth factor α and β receptor expression in benign prostatic hyperplasias and Gleason-graded human prostate adenocarcinomas. Mod Pathol 1994;7:549–54.
19. Chott A, Sun Z, Morganstern D, et al. Tyrosine kinases expressed in vivo by human prostate cancer bone marrow metastases and

loss of the type 1 insulin-like growth factor receptor. Am J Pathol 1999;155:1271–9.

20. Hofer M, Fecko A, Shen R, et al. Expression of the platelet-derived growth factor receptor in prostate cancer and treatment implications with tyrosine kinase inhibitors. Neoplasia 2004;6:503–12.

21. Dolloff N, Shulby S, Nelson A, et al. Bone-metastatic potential of human prostate cancer cells correlates with Akt/PKB activation by α platelet-derived growth factor receptor. Oncogene 2005;24: 6848–54.

22. Shah G, Loizos N, Youssoufian H, et al. Rationale for the development of IMC-3G3, a fully human immunoglobulin G subclass 1 monoclonal antibody targeting the platelet-derived growth factor receptor α. Cancer 2010;116:1018–26.

23. Dolloff N, Russell MR, Loizos N, Fatatis A. Human bone marrow activates the Akt pathway in metastatic prostate cells through transactivation of the α-platelet-derived growth factor receptor. Cancer Res 2007;67:555–62.

24. Russell MR, Liu Q, Lei H, et al. The α-receptor for platelet-derived growth factor confers bone-metastatic potential to prostate cancer cells by ligand- and dimerization-independent mechanisms. Cancer Res 2010;70:4195–203.

25. Wang M, Stearns ME. Isolation and characterization of PC-3 human prostatic tumor sublines which preferentially metastasize to select organs in S.C.I D. mice. Differentiation 1991;48:115–25.

26. Loizos N, Xu Y, Huber J, et al. Targeting the platelet-derived growth factor receptor α with a neutralizing human monoclonal antibody inhibits the growth of tumor xenografts: implications as a potential therapeutic target. Mol Cancer Ther 2005;4:369–79.

27. Clarke NW, Hart CA, Brown MD. Molecular mechanisms of metastasis in prostate cancer. Asian J Androl 2009;11:57–67.

28. Loberg R, Gayed B, Olson K, Pienta KJ. A paradigm for the treatment of prostate cancer bone metastases based on an understanding of tumor cell-microenvironment interactions. J Cell Biochem 2005;96:439–46.

29. Gassmann P, Haier J. The tumor cell-host organ interface in the early onset of metastatic organ colonisation. Clin Exp Metastasis 2008;25: 171–81.

30. Lunt SJ, Chaudary N, Hill RP. The tumor microenvironment and metastatic disease. Clin Exp Metastasis 2009;26:19–34.

31. Anderberg C, Li H, Fredriksson L, et al. Paracrine signaling by platelet-derived growth factor-CC promotes tumor growth by recruitment of cancer-associated fibroblasts. Cancer Res 2009;69:369–78.

32. Ball SG, Shuttleworth CA, Kielty CM. Vascular endothelial growth factor can signal through platelet-derived growth factor receptors. J Cell Biol 2007;177:489–500.

33. Shen J, Vil MD, Jimenez X, et al. Single variable domain-IgG fusion. A novel recombinant approach to Fc domain-containing bispecific antibodies. J Biol Chem 2006;281:10706–14.

34. Heldin CH. Structural and functional studies on platelet-derived growth factor. EMBO J 1992;11:4251–9.

35. Heldin CH, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999;79:1283–316.

36. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev 2008;22:1276–312.

37. Klinghoffer RA, Mueting-Nelsen PF, Faerman A, et al. The two PDGF receptors maintain conserved signaling in vivo despite divergent embryological functions. Mol Cell 2001;7:343–54.

38. Orr FW, Lee J, Duivenvoorden WC, Singh G. Pathophysiologic interactions in skeletal metastasis. Cancer 2000;88:2912–8.

39. Mantyh P, Clohisy D, Koltzenburg M, Hunt S. Molecular mechanisms of cancer pain. Nat Rev Cancer 2002;2:201–9.

40. Yin J, Pollock CB, Kelly K. Mechanisms of cancer metastasis to the bone. Cell Res 2005;15:57–62.

41. Coleman RE, Guise T, Lipton A, et al. Advancing treatment for metastatic bone cancer: consensus recommendations from the Second Cambridge Conference. Clin Cancer Res 2008;14:6387–95.

42. Gomes R, Buttke P, Paul E, Sikes R. Osteosclerotic prostate cancer metastasis to murine bone are enhanced with increased bone formation. Clin Exp Metastasis 2009;26:641–51.

43. Thiolloy S, Halpern J, Holt GE, et al. Osteoclast-derived matrix metalloproteinase-7, but not matrix metalloproteinase-9, contributes to tumor-induced osteolysis. Cancer Res 2009;69:6747–55.

44. van der Pluijm G, Que I, Sijmons B, et al. Interference with the microenvironmental support impairs the de novo formation of bone metastases in vivo. Cancer Res 2005;65:7682–90.

45. D'Ambrosio J, Fatatis A. Osteoblasts modulate Ca2+ signaling in bone-metastatic prostate and breast cancer cells. Clin Exp Metastasis 2009;26:955–64.