Targeting Bone Metastasis in Prostate Cancer with Endothelin Receptor Antagonists

Michael A. Carducci and Antonio Jimeno

Abstract

Recent advances in the understanding of prostate cancer biology and its progression to bone metastasis have led to the development of drugs directed against precise molecular alterations in the prostate tumor cell and host cells in the normal bone environment such as osteoclasts and osteoblasts. Endothelins (ETs) and their receptors have emerged as a potential target in prostate cancer bone metastasis. By activating the ETA receptor, ET-1 is pathogenically involved in facilitating several aspects of prostate cancer progression, including proliferation, invasion, escape from apoptosis, invasion, and new bone formation, processes that are general to many malignancies. Notwithstanding, there are a number of features specifically driven by the ET axis in prostate cancer, such as creating and perpetuating a unique interaction between the metastatic prostate cancer cell and the bone microenvironment (osteoblast, osteoclast, and stroma) or altering the equilibrium in pain modulation. These features have led to the preferential clinical evaluation of atrasentan (ABT-627) as a biological therapy in prostate carcinoma, first in hormone-refractory prostate cancer. Biological activity of atrasentan in patients with prostate cancer has been shown by the suppression of biochemical markers of prostate cancer progression in bone, and clinical activity is evidenced by a consistent trend demonstrating a delay in time to disease progression when compared with placebo, especially in patients with bone metastases. Further studies of atrasentan and other selective ET-1 antagonists (ZD4054) are ongoing.

ETs and Their Receptors

The ETs constitute a family of three 21-amino-acid peptides (ET-1, ET-2, and ET-2) that are synthesized as propeptides and are transformed to their active forms by sequential endopeptidase- and ET-converting enzyme-mediated cleavage. ET-1 is the most common circulating form of ET and has a median half-life of 7 minutes (2). It is cleared by a double mechanism that consists in ET receptor B (ETB)–mediated uptake and degradation by neutral endopeptidase (3). Each of the three ETs has a unique pattern of distribution; ET-1 is not organ specific and is expressed primarily by endothelial cells, whereas ET-2 is mainly present in the intestine and kidney, and ET-3 is mainly localized in the brain and to a lesser extent in gastrointestinal stromal cells and lung epithelial cells (4). As illustrated in Fig. 1, ETs exert their effect by binding to two different membrane receptors, ETA and ETB. ETB has a similar affinity for the three peptides and functions primarily as a decoy receptor and modulator of ET-1 (5, 6). ETA preferentially binds ET-1 and is considered the major effector of the ET axis. Therefore, it has been the main focus of research and also the main target in the development of pharmacologic strategies to modulate the ET axis.

On activation by ET-1, ETA interacts with and activates a G-protein that triggers a parallel activation of several signal-transducing pathways. These include phospholipase C activity with a consequent increase in intracellular Ca2+ levels, protein kinase C (7), epidermal growth factor receptor (8), phosphatidylinositol-3-kinase (9), and ras/raf/mitogen-activated protein kinase pathways (10, 11). This cascade of events ultimately induces nuclear transcription of several proto-oncogenes.
ETB expression is reduced or abolished, presumably by a combination of acid and neutral endopeptidase activity, are decreased (17, 18). The clearing mechanisms, such as ETB binding capacity and stage, emphasize the relevance of ETB in maintaining the signal transduction homeostasis.

ET-1 is one of the most potent endogenous vasoconstrictors known, and it may be involved in the long-term regulation of vascular tone (12). ET-1 production is stimulated by cytokines (interleukin-1), growth factors (tumor necrosis factor–α, transforming growth factor–β, and platelet-derived growth factor), and major signals of cardiovascular stress, such as vasoactive agents (angiogenesis II, norepinephrine, vasopressin, and bradykinin), thrombin, mechanical stress, and hypoxia. Prostacyclin, nitric oxide, and atrial natriuretic peptide are the predominant effectors of negative feedback loops (13). ET-1 is secreted by a wide array of normal cells, including endothelial cells; vascular smooth myocytes; and bronchial, mammary, endometrial, and prostatic epithelial cells. It exerts a mitogenic stimulus on vascular and bronchial myocytes, fibroblasts, melanocytes, osteoblasts, mesangial cells, and endometrial cells, among others.

ET-1 is produced by several epithelial tumors as well, where it acts as an autocrine and/or paracrine growth factor (11). Extensive research has revealed that the ET axis is relevant in cancer development and progression at several stages. Most of these studies have been developed in prostate and ovarian cancer models where several findings are overlapping, indicating that they are not model dependent and constitute general and consistent mechanisms for the cancer cell to acquire beneficial features (14, 15).

In the healthy prostate, ET-1 is produced by epithelial cells, and the highest body concentrations of ET-1 are found in the seminal fluid, with concentrations 500-fold higher than those found in plasma (16). ETB receptors are present in the prostate gland, and their expression is directly proportional to tumor grade and stage in prostate carcinoma (16). In prostate cancer specimens, ET-1 clearance mechanisms, such as ETB binding capacity and neutral endopeptidase activity, are decreased (17, 18). ETB expression is reduced or abolished, presumably by a promoter methylation–mediated mechanism; the functionality of the remaining receptors is also abnormal, due to an absence of active ET-1–binding sites (19). The other major mechanism of ET-1 clearance, neutral endopeptidase–mediated degradation, is also diminished in the prostate cancer environment (20).

ET-1 is associated with the osteoblastic nature of breast and prostate cancer bone metastasis, because it is the basis of a unique interaction between the in-transit metastatic cell and the local environment. It was postulated that ET-1 production by metastatic prostate and breast cancer cells located in the bone is stimulated by osteoblast- and endothelial cell–secreted interleukin-1, tumor necrosis factor–α, and transforming growth factor–β. ET-1 in turn, closing this paracrine loop, would stimulate mitotic activity in osteoblasts, decreasing both osteoclastic bone resorption and motility (21). This was shown in a series of experiments where coculture of human prostate cancer cell lines with bone slices increased the level of ET-1 mRNA; osteoclastic bone resorption was significantly blocked by the presence of both prostate cancer cells and ET-1 in a dose-dependent manner. This inhibition was neutralized by a specifically directed anti-ET-1 antibody (22). In another series of experiments, tumor-produced ET-1 stimulated new bone formation in vitro and osteoblastic metastases in vivo via ETB (23). These findings explain in part the characteristic blastic nature of prostate and breast cancer bone metastases. It may well be that more than a true osseous tropism of prostate cancer cells, this ET-1–secreting capability and the resulting environment favoring disorganized bone growth explain the preponderance of bone disease and its devastating clinical consequences in prostate cancer patients. In prostate and breast cancer models, selective ETB inhibition, but not ETB modulation, abrogated ET-1–induced osteoblastic response (23, 24).

ET-1 is thought to play a pathogenic role in pain elicitation and control. ET-1 is found in high concentrations in dorsal root ganglion neurons and ETB receptors are found on small- to medium-sized root ganglion neurons and their axons (25). ET-1 enhances pain states in various models of acute chemical- and inflammation-induced pain and in chronic pain types such as neuropathic pain, where selective ETB inhibition shows preclinical efficacy (26). The key relevance of ET-1 and the effectiveness of ETB inhibition have also been shown in acute pain models, both irritant induced and prostate cancer related (27). In the latter experiments, ET-1 axis inhibition both with small-molecule inhibitors resulted in significant pain control. ET-1–mediated enhancement of pain responses in a prostate cancer inoculation–induced pain model is mediated through ETB, and this effect is reversed by selective ETB inhibition (28). Recently, a compensatory analgesic action has been proposed, which is initiated by ET-1 on binding to ETB and is mediated through the release of β-endorphin (29). Thus, ETB selective inhibition might theoretically abrogate ET-1–initiated nociceptive response while preserving the analgesic effect.

By activating ETB, ET-1 is pathogenically involved in facilitating several aspects of prostate cancer progression, including proliferation, escape from apoptosis, invasion, and new vessel formation, processes that are general to many malignancies (30–36). Notwithstanding, there are a number of features specifically driven by the ET axis in prostate cancer, such as creating and perpetuating a unique interaction between the metastatic prostate cancer cell and the bone microenvironment (osteoblast, osteoclast, and stroma) or altering the equilibrium in pain modulation. These features have led to our current understanding.
the preferential clinical evaluation of a selective ETA receptor antagonist, in particular, atrasentan (ABT-627), as a biological therapy in prostate carcinoma, first in hormone-refractory prostate cancer (HRPC) patients.

**Atrasentan: An ET<sub>A</sub> Receptor Antagonist**

Atrasentan (ABT-627) exerts its activity by selective inhibition of the ET<sub>A</sub> receptor. Atrasentan decreases the binding affinity of ET-1 without affecting the receptor density, indicating that it is a potent, competitive inhibitor of ET-1 binding, with 1,800-fold superior selectivity for ET<sub>A</sub> compared with ET<sub>B</sub>. Atrasentan is rapidly absorbed, with a time of occurrence for maximum drug concentration of ~1.5 hours. The half-life ranges from 21 to 24 hours. The free (not protein-bound) plasma concentrations achieved at doses of 2.5 mg and above exceeded the human ET<sub>A</sub> Ki (0.034 nmol/L) for atrasentan and corresponded to biologically active concentrations achieved in vivo in animal studies (37–39).

**Atrasentan safety profile.** In the three reported continuous-dosing phase 1 studies in cancer patients, the most common adverse events of atrasentan were rhinitis, headache, asthenia, and peripheral edema; they were reversible on drug discontinuation and responded to symptom-specific treatment (37–39). Reversible hemodilution was apparent in laboratory findings, as was weight gain. In both U.S.-based trials, the maximum-tolerated dose was 60 mg/d, and headache (37) and hypotension and hypotension (39) were the dose-limiting adverse events at 75 mg/d in those trials. The toxicity profile documented during the clinical development of atrasentan phases 1 to 3 has shown a high level of consistency and seems to be mainly related to ET<sub>A</sub> inhibition, which results in fluid retention and/or vasodilation.

Taking the preliminary results of the completed phase 3 trial as a reference, the main (incidence ≥5%) adverse events associated with atrasentan compared with placebo were peripheral edema (40% versus 12%, P < 0.001), rhinitis (36% versus 14%, P < 0.001), dyspnea (9% versus 4%, P = 0.005), headache (21% versus 14%, P = 0.013), infection (13% versus 8%, P = 0.019), dry mouth (6% versus 2%, P = 0.009), and edema (3% versus 2%, P = 0.033). More than 96% of these events were rated as grade 1 or 2, and few resulted in discontinued use of the study drug. More than a third of the atrasentan recipients reporting peripheral edema experienced resolution of peripheral edema during the treatment period, both with and without diuretics. Although less prevalent, there was an increased incidence of heart failure in the atrasentan group (4% versus 1%, P = 0.002). Subjects who developed heart failure tended to be older (mean age, 78 years), to have larger metastatic tumor burden, and to present with preexisting cardiovascular disorders; some patients resumed atrasentan therapy after treatment with diuretics and/or angiotsin-converting enzyme inhibitors. Bone pain was reported more frequently in the placebo group (49% versus 54%), although not significantly (40).

**Atrasentan clinical effects.** Early hints of clinical activity were documented in the initial phase 1 trials, as evidenced by a decrease in cancer-related pain in 5 of 15 subjects and decreases in prostate-specific antigen levels in 5 of 11 subjects with HRPC (37). The disease-specific activity of atrasentan was evaluated in a double-blind, randomized, placebo-controlled phase 2 trial conducted in 288 asymptomatic patients with HRPC and evidence of metastatic disease. Patients were randomly assigned to one of three study groups consisting of daily oral placebo, 2.5 mg atrasentan, or 10 mg atrasentan (41). Median time to progression (TTP) in intent-to-treat patients (n = 288) was nonsignificantly longer in the 10 mg atrasentan group compared with the placebo group (183 vs. 137 days, respectively; P = 0.13). A subset analysis was planned in patients who met classification criteria defined before breaking the study blind; this analysis excluded patients not meeting disease classification (n = 15), receiving <50% of the doses of study drug or fewer than 20 doses (n = 10), noncomplying (n = 8), with insufficient antiandrogen withdrawal (n = 7), or taking excluded medications (n = 4). Median TTP in this evaluable patient population (n = 244) was significantly prolonged in patients treated with atrasentan 10 mg, from 129 to 196 days (P = 0.021) compared with placebo. For both intent-to-treat and per-protocol evaluable populations in the 10 mg atrasentan group, median time to prostate-specific antigen progression was twice that of the placebo group (155 versus 71 days; P = 0.002). Patients who received placebo continued to have significant increases from baseline in serum lactate dehydrogenase, a marker of disease burden; elevations in lactate dehydrogenase were uniformly attenuated by atrasentan in the intent-to-treat population (41).

In a separate pharmacodynamic analysis of biological endpoint points in this trial, changes in bone deposition markers [total alkaline phosphatase (TAP) and bone alkaline phosphate (BAP)] and bone resorption markers (N-telopeptides, C-telopeptides, and deoxyxypyrindoline) were assessed (42). Baseline 1.5- to 2.7-fold elevations above respective upper limits of normal in markers of bone deposition and resorption were shown. Subjects receiving placebo experienced a 58% elevation in mean TAP and a 99% elevation in mean BAP, whereas subjects receiving 10 mg atrasentan maintained stable mean TAP and BAP values compared with baseline. TAP, BAP, and deoxyxypyrindoline mean changes from baseline were consistently lower in patients receiving 10 mg atrasentan compared with placebo (all P < 0.05). N-telopeptides and C-telopeptides showed nonsignificant decreases in treated subjects. Changes in clinical bone scan studies paralleled bone marker changes, adding to the robustness of these findings.

In a randomized, double-blind, placebo-controlled phase 3 study of 10 mg atrasentan that enrolled 809 (401 placebo, 408 atrasentan) subjects with metastatic HRPC, TTP (a composite of radiographic and clinical measures; time to BAP progression (>50% increase from nadir); quality of life; and changes from baseline to final value in prostate-specific antigen, BAP, and TAP were compared (40). This study was closed early by an Independent Data Safety Monitoring Committee on review of the unexpectedly large number of early events that suggested the trial results would not be different from control outcomes. Once all of the events were accounted for, on intent-to-treat analysis, atrasentan compared with placebo nonsignificantly delayed TTP (log-rank = 0.091; hazard ratio, 1.14; 95% confidence interval, 0.98-1.34). As mentioned, the rate of progression was unexpectedly rapid, with >50% of subjects achieving end point within 100 days, most through bone scan progression. To evaluate the differences on mean TTP, a G<sup>1.1</sup> analysis was used per protocol (40). Although the study statistics were based on the progression rates in the earlier
phase 2 study, the phase 2 study did not mandate bone scans at a 12-week frequency. The mandated bone scans showed early radiographic progression in the absence of clinical symptoms in most patients. The significance of early radiographic progression without clinical symptoms remains controversial.

On evaluation of secondary end points, atrasentan delayed time to BAP progression (254 versus 505 days; log-rank $P < 0.001$; hazard ratio, 1.78; 95% confidence interval, 1.34–2.37) and significantly preserved prostate cancer–specific quality of life measured by the FACT-P prostate cancer subscore ($P = 0.032$). Time to prostate-specific antigen progression was longer in patients taking atrasentan, but not significantly (log-rank $P = 0.056$). The atrasentan group showed smaller mean increases from baseline to final value than the placebo group in prostate-specific antigen ($P = 0.025$), BAP ($P = 0.001$), and TAP ($P < 0.001$). A protocol-specific analysis was conducted on a subset of 671 patients (329 placebo, 342 atrasentan) who met classification criteria defined before breaking the study blind, showing that atrasentan significantly delayed TTP (log-rank $P = 0.007$; hazard ratio, 1.26; 95% confidence interval, 1.06–1.50) and time to BAP progression (log-rank $P < 0.001$) and resulted in smaller mean changes from baseline to final in the three biological markers (40).

To assess the hypothesis that atrasentan efficacy would be more evident in men who had metastases confined to bone at baseline [474 (59%) of 809 patients], an exploratory analysis was conducted. Atrasentan-treated subjects in this subgroup experienced a significant delay in time to disease progression ($P = 0.002$), further emphasizing the targeted effect of this drug on the ET$_{A}$ receptor in the bone microenvironment (43).

Based on the large unmet need for therapies for this patient population, the biological rationale of the ET$_{A}$ receptor in prostate cancer, as well as consistent trends in the clinical trial results, a solicitation for approval of atrasentan for patients with metastatic HRPC was reviewed by the Food and Drug Administration Oncologic Drugs Advisory Committee. The committee did not recommend approval of atrasentan, citing that more definitive data of clinical benefit were required from additional studies in the metastatic hormone refractory patient population and that the data was more hypothesis generating than definitive. Further studies targeting documented bone metastasis in men with prostate cancer patients was recommended.

Other ET receptor antagonists: ZD4054. ZD4054 is an orally active, specific ET$_{A}$ antagonist in clinical development. In receptor-binding studies, ZD4054 specifically bound to ET$_{A}$ with high affinity and no binding with ET$_{B}$ was detected. More specific clinical data on this agent are limited. Phase 1 and 2 studies are under way with this compound in prostate cancer with little data on efficacy reported to date (44).

Conclusions

The ET-1/ET$_{A}$ receptor is a pathway that is dysregulated in prostate cancer and that is involved in key tumorigenic cellular events, such as proliferation, invasion, escape from programmed cell death, new vessel formation, abnormal osteogenesis, and alteration of nociceptive stimuli. Atrasentan is a novel agent that targets the ET-1/ET$_{A}$ pathway. Biological activity in patients with prostate cancer has been shown by the suppression of biochemical markers of prostate cancer progression in bone, and clinical activity is evidenced by a consistent trend demonstrating a delay in time to disease progression when compared with placebo, especially in patients with bone metastases.

The ET paradigm shows that the interaction of the tumor cell with the microenvironment at the different organ systems ultimately dictates the biological pattern of disease progression. It may well be that more than a true osseous tropism of prostate cancer cells, their ET$_{1}$–secreting capability, and the favorable environment that results create a welcoming atmosphere that promotes tumor growth not only by direct, active invasion but also by cooperation with the host organ. This collaboration together with the disorganized bone growth help explain the preponderance of bone disease with its devastating clinical consequences in prostate and breast cancer patients. Therefore, it may be worthwhile and pathogenically relevant to aim therapy at rationally modulating the host response (e.g., osteoblastic reaction) rather than just targeting the triggering factor (prostate tumor cells).

Because the ET axis seems to be a reasonable target with a growing literature demonstrating its central role in osteoblastic bone metastasis as well as the findings of the exploratory analysis of the phase 3 clinical trial data demonstrating the greatest clinical benefit was seen in patients with bone metastasis, ET receptor antagonists theoretically seem better suited to act in the early phases of the cell-bone interaction. Therefore, it is particularly appealing to assess atrasentan in patients with a low disease burden. The first evidence of metastatic spread in prostate cancer patients is the occurrence of bone disease, and atrasentan is under investigation in a maturing phase 3 study in >900 men with nonmetastatic HRPC to test the hypothesis that it delays the onset of metastatic disease. This would test the biological principle that the disruption of the initial cancer cell-bone microenvironment interaction would prevent or delay the initiation of the mutual stimulation processes. Results from this study are anticipated in 2006. In addition, phase 3 studies of atrasentan in combination with docetaxel are planned in advanced-stage prostate cancer patients with bone metastasis through the Southwest Oncology Group. The future of this agent in terms of further studies and plans for approval depend on the results of these studies and continued industrial commitment to this pathway.

References

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