Endothelin Receptor Antagonists: Rationale, Clinical Development, and Role in Prostate Cancer Therapeutics

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The endothelins (ETs), which include ET-1, ET-2, ET-3, and their receptors ET-A and ET-B, play a major role in tumor growth, proliferation, apoptosis, angiogenesis, and bone metastasis. Atrasentan is a novel and selective inhibitor of ET-1 and ET-A. In vitro and in vivo data show that this oral agent is capable of inhibiting tumor cells in vitro. More recently, this agent was studied in several phase I trials with refractory carcinoma patients. Subsequently, phase II and III clinical trials evaluating atrasentan in patients with hormone-refractory prostate carcinoma have suggested that targeting this pathway may be a new therapeutic strategy in the treatment of solid malignancies, specifically, prostate cancer.

Introduction

The endothelins (ETs) are a family of three separate 21–amino acid peptides (ET-1, ET-2, and ET-3) produced by endothelial cells and originally described as potent vasoconstrictors [1••,2]. Recent studies, however, demonstrate that ETs are also involved in cell proliferation, cell migration, apoptosis, and angiogenesis [2]. ETs exert their effects by binding a specific cell surface receptor (ET-A, ET-B). Both ETs and their receptors have been implicated in cancer progression through an autocrine and paracrine pathway [3,4]. Although all three ETs appear to have similar functions, ET-1 is the most common circulating form of ET. The main function of ET-1 appears to be the promotion of cell proliferation, matrix/bone remodeling, inhibition of apoptosis, and metastases [2]. Thus, ET-1 has emerged as an important growth factor in a wide number of solid malignancies, including prostate, renal, colon, ovarian, and breast carcinoma. The understanding that the ET-1 mediates tumor progression in different epithelial malignancies led to identification of the ET pathway as a rational, targeted approach to inhibit the effects of endothelins in cancer. Many antagonists of this pathway have shown promising results in animal and in vitro models of cardiovascular disease and renal disease and in different malignancies [5]. Atrasentan, an orally bioavailable endothelin inhibitor that selectively binds and blocks the effects of ET-A, is perhaps the most active ET antagonist in cancer therapeutics.

The Endothelin System

Endothelins exert their effects by binding to two different cell surface ET receptors, ET-A and ET-B. This is done through the G-protein pathway [1••,2,3] Additionally, specific cells are involved in the production of the individual ET subtypes. ET-1 is mainly produced by endothelial cells. ET-2 is produced predominantly within the kidney and intestine, with smaller amounts produced in the myocardium, placenta, and uterus, whereas ET-3 is mainly a substrate from the central nervous system. The selectivity of each type of cell surface receptor is dependent upon the particular endothelin. Whereas ET-A has affinity for only ET-1 and ET-2, ET-B has affinity for all three endothelin subtypes [1••,2–6].

The physiologic actions of the endothelin family are variable among the different subtypes (Table 1) [1••]. Although both receptor types display similar actions when stimulated, ET activation influences cellular proliferation, inhibition of apoptosis, and bony matrix remodeling in the setting of metastasis. This is similar to the physiologic cascade that results from ET-B activation, though to a much lesser degree. The main effect of ET-B, however, is in the regulation of cellular apoptosis, angiogenesis, and clearance of ET-1. The activation of ET-A by ET-1 and ET-2 proceeds by activation of phospholipase C (PLC), protein tyrosine kinase (PTK), and RAS protein by way of G protein activation [7••,8]. Ultimately, downstream activation of the RAF/MEK/MAPK pathway occurs, resulting in nuclear transcription of several pro-

Table 1. Function and activity of endothelins

Function	ET-A	ET-B
Vasoconstriction	+++	+
Bronchoconstriction	+++	-
Mitogenesis	+++	+
Acute/neuropathic pain	+++	-
Anti-apoptosis	+++	-
Angiogenesis	+++	+++
ET clearance	-	+++
Vasodilation	-	+++

tooncogenes (*c-FOS*, *c-MYC*, *c-JUN*), which in turn leads to cell growth and proliferation. This complex activation scheme is known as the endothelin axis.

Endothelins and Cancer Progression

ET-1 has been implicated in several of the molecular pathways that lead to tumor proliferation. These include angiogenesis, tumor invasion, tumor cell proliferation, and apoptosis [8–22]. ET-1 has demonstrated the ability to induce neovascularization via ET-receptor stimulation [16,17]. Overexpression of ET-1 and its receptor is directly correlated with increased microvessel density (MVD) and vascular endothelial growth factor (VEGF) overexpression. Activation of ET-A also facilitates the migration of vascular smooth muscle cells and pericytes, an important step in neovascularization. Furthermore, ET-1 stimulates VEGF secretion in a dose-dependent fashion, facilitating endothelial cell proliferation. Similar to clear renal cell carcinoma, patients with ovarian carcinoma have increased VEGF production that results from increased levels of hypoxia-inducible factor-1α (HIF-1α) [23]. In fact, degradation of HIF-1α appears to be reduced when ovarian carcinoma cells are treated with an ET-1 antagonist. In addition, in vitro data suggest that under normoxic conditions VEGF transcription mediated through HIF-1α is also regulated by ET-1. Furthermore, ET-1 can be unregulated during hypoxia by amplifying HIF-1α stability and promoting overexpression of VEGF [19,20].

The ET-A receptor also functions as a survival factor after activation by ET-1. In vitro, ET receptor stimulation mediates survival on epithelial cells, smooth muscle cells, and fibroblasts. This apoptotic suppression is through the MAPK as well as through the Bcl-2 and phosphatidylinositol 3-kinase (PI3K) mediated Akt pathways. Recognition of these phenomena was further demonstrated in rat colon cancer cell lines exhibiting resistance to the FasL (Fas ligand)–induced apoptosis [12]. Normally, the FasL binding regulates for cell death via caspase activation. These cell lines were subsequently susceptible after the administration of bosentan, a mixed ET-A/ET-B antagonist [23].

It is well known that changes in tumor microenvironment often allow for tumor invasion. Binding of ET-1 to the ET-A receptor induces tumor invasion in ovarian carcinoma models by promoting the secretion of metastasis-related proteinases such as urokinase type plasminogen activator system (uPAR) and the matrix metalloproteinases (MMPs). Co-induction of the uPAR system, by continuous secretion of MMPs and uPAR by ET-1, results in a higher invasive potential of ovarian tumor cells. Decreased production of MMPs after administration of the ET-1 inhibitor (BQ123) leads to a decrease in cell migration and tumor invasion [24,25]. Similarly, ET-1 appears to promote the production of growth factors that are linked to the development of bone metastases. ET-1 influences the interaction between bone and tumor, which may also contribute to the clinical benefit observed in prostate cancer patients receiving atrasentan [26]. ET-1 stimulates phosphate transport, a process important for the initiation of bone matrix calcification, in osteoblast-like MC3TC cells by ET-A–mediated activation on protein kinase C (PCK). In addition to having an increased number of ET receptors, osteoblasts also respond to ET-1 by increasing the synthesis of proteins such as osteopontin and osteocalcin. These osteoblastic proteins, coupled with an increase of serum levels of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, facilitate the derangement of bone remodeling. In an osteoblastic murine model, overexpression of ET-1 and a selective ET-A inhibitor decreased new bone formation. Several reports also demonstrate that selective ET-A receptor inhibitors can also inhibit the development of bone metastases in human cancer cell lines and in murine breast carcinoma models [27,28].

Clinical Development of Atrasentan

ET-1/ET-A overexpression has been identified in several human cancer cell lines in vitro. Likewise, dysregulation of the ET axis has been also found in ovarian, breast, human papilloma virus (HPV)-associated cervical, and lung cancer (Table 2) [29–37]. In breast carcinoma, overexpression of ET-1, ET-A, and ET-B is associated with pathologic features that predict poor outcome, including overexpression of VEGF [38–40]. Similarly, VEGF, MMP-2, and cyclooxygenase (COX)-2 overexpression in ovarian carcinoma animal models appear to be promoted by the ET-1/ET-A pathway [39]. As with the ovarian carcinoma data, using atrasentan as a selective ET-A inhibitor in HPV-infected cervical carcinoma cells, the production of proangiogenesis and molecules that promote tumor invasion is reduced [40,41]. When used in combination with paclitaxel in HPV-positive cervical cancer cell lines, atrasentan displayed additive effects. Several other in vitro studies suggest that the ET pathway also mediates tumor invasion of melanoma and sarcoma cells as well as lung and colorectal carcinoma [29–37]. ET-1 is

Table 2. Role of ET-1 in selected malignancies

Type of cancer	ET-1 action ~ ET-1 receptor status	Name of receptor antagonist
Breast carcinoma	Decreased tumor differentiation ~ AT-B overexpression	BQ123/BQ788
Cervical carcinoma	Promotion of proliferation ~ ET-A overexpression in HPV-positive cells	Atrasentan/BQ123
Colon carcinoma	Inhibition of apoptosis ~ overexpression of ET-A and ET-B receptors	Bosentan
Kaposi's sarcoma	Induction of cell proliferation and migration ~ ET-A and ET-B overexpression	BQ123/BQ788
Melanoma	Promotion of proliferation and invasion ~ ET-A overexpression and downregulation of ET-B	BQ788
Ovarian carcinoma	Cellular proliferation, survival, and invasion ~ ET-A overexpression	Atrasentan/BQ123/BQ788
Prostate carcinoma	Promotion of growth and inhibition of apoptosis ~ ET-A overexpression, methylation of ET-B gene/downregulation	Atrasentan

HPV—human papillomavirus.

also normally secreted by prostate epithelial cells; nine ET-1 concentrations have been found to be significantly elevated in patients with metastatic hormone-resistant prostate carcinoma (HRPC) [9,26,42–44]. Expression of ET-1 correlates with stage and grade in human prostatic cell lines. Increased ET-1 levels in prostate cancer appear to be related to the loss of the expression and activity of the enzyme responsible for its cleavage, often seen in patients with HRPC. In fact, ET-A expression is associated with disease progression in prostate cancer.

On the basis of the potential of a selective inhibitor to block the ET-1/ET-A pathway in multiple tumor types, several studies evaluating the toxicity and efficacy of atrasentan have been reported [45••–50••]. Initial safety studies using healthy volunteers demonstrated minimal and reversible side effects at a dosage of 40 mg/day. Subsequently, three phase I studies have been conducted [45••–47••]. In one study, the vast majority of patients had refractory carcinomas [45••], whereas in the other two trials, the majority of the patients enrolled had HRPC [46••,47••]. The phase I dose-escalation trial by Carducci et al. [45••] evaluated the safety and pharmacokinetics of atrasentan administered in a daily dosage for 4 weeks. A total of 31 patients were enrolled (14 prostate and 17 other carcinomas). The most common adverse events were rhinitis, asthenia, and peripheral edema. All of the toxicities were reversible on drug discontinuation and responded to symptomatic treatment. Headache was the dose-limiting adverse event; the maximum tolerated dosage in this study was 60 mg/d. The pharmacokinetics of atrasentan across the 10- to 75-mg dose range were dose proportional. Atrasentan reached plasma steady-state concentrations within 7 days, and its terminal elimination half-life was 24 hours. In the same way Ryan et al. [46••] examined the safety, tolerability, and pharmacokinetics of this selective ET-A inhibitor. A total of 35 patients (19 female, 16 male) with refractory malignancies were enrolled (12 gastrointestinal tract, 6 lung, 5 kidney, 2 sarcoma, 2 cervical, and 7 other). No objective responses were observed in the study. As reported in the previous study, the most common reversible side effects encountered were headaches (60%), rhinitis (49%), and peripheral edema (31%). No significant differences in toxicity or pharmacokinetics between male and female subjects was observed. A second phase I trial conducted by the group in the Netherlands [47••] included a total of 39 patients, of which 30 had HRPC. The observed toxicities, compared with the previous two studies ,were similar. Interestingly, 68% of HRPC patients obtained a prostate-specific antigen (PSA) decline of more than 50%. No correlation between dose and PSA response was observed.

Based on the preclinical findings implicating ET-1 in the pathogenesis of bone metastasis in prostate cancer patients and the provocative PSA data generated from the previously described phase I studies, a randomized phase II, placebo-controlled trial of atrasentan in HRPC patients was conducted. [48••]. Two hundred eighty-eight HRPC patients were randomly assigned to receive placebo (n=104); atrasentan, 2.5 mg (n=95); or atrasentan, 10 mg (n=89). The primary endpoint of the study was time to progression (TTP), defined by objective evidence of bone or soft tissue disease; the requirement of opioid analgesic for new disease-related pain; and the occurrence of new disease-related symptoms that require medical intervention. Secondary endpoints included time to PSA progression (TTP-PSA), changes in bone biomarkers, and quality of life.

In the intention-to-treat analysis, median TTP was longer in the high-dose atrasentan arm compared with the placebo group (183 days vs 137 days, respectively; P=0.29). Median TTP was also longer when low-dose atrasentan was compared with placebo (178 days vs 137 days; P=0.29). In the evaluable patients (n=244) median TTP was also statistically superior in the group receiving atrasentan, 10 mg, compared with the placebo group

(196 days vs 129 days; $P=0.21$). Similar results were observed with the lower atrasentan dose ($P=0.35$). Delay in PSA progression also favored the high-dose atrasentan group (155 days vs 71 days respectively, $P=0.002$). Quality of life, as measured by Functional Assessment of Cancer Therapy–Prostate (FACT-P) or European Organization for Research and Treatment of Cancer (EORTC) scores was not adversely affected by treatment with atrasentan. As observed in previous studies, atrasentan was well tolerated, with minimal side effects that included headache, peripheral edema, and rhinitis. When evaluating bone biomarkers, baseline elevation in markers of bone deposition and resorption (alkaline phosphatase, bone alkaline phosphatase, N-telopeptides, C-telopetides, and deoxypyridinoline) were reduced in a dose-dependent manner after patients received treatment with atrasentan.

The demonstration that atrasentan can delay disease progression in prostate cancer patients led to the design of two phase III trials. The first trial [49••] randomly assigned asymptomatic metastatic HRPC patients to receive either placebo or 10 mg of atrasentan. Contrary to the randomized phase II study, this study required objective documentation of disease progression. Thus, patients underwent restaging scans every 3 months while on therapy. More than 800 HRPC patients were randomized to either placebo ($n=401$) or atrasentan, 10 mg/day ($n=408$). In the intention-to-treat analysis no significant differences were observed between placebo and atrasentan ($P=0.09$; hazard ratio [HR] 1.14, 95% CI, 0.98–1.34). When only the evaluable subjects were analyzed ($n=329$ placebo and $n=342$ active drug) TTP was superior in the atrasentan arm (log-rank $P=0.007$; HR 1.26, 95% CI, 1.06–1.50). PSA progression, quality of life issues, and bone biomarkers favored the atrasentan arm. Volgezang et al. [50••] recently reported the results of a meta-analysis of all atrasentan clinical trials in HRPC patients. TTP, time to bone pain (TTBP), TTP-PSA, and time to bone alkaline phosphatase progression (TTP-AP) were analyzed. In this series more than 1000 HRPC patients were randomly assigned to receive either atrasentan, 10 mg/day ($n=497$) or placebo ($n=505$). When compared with placebo patients, patients receiving atrasentan had a significant delay in TTP ($P=0.045$), TTBP ($p=0.025$), TTP-PSA ($P=0.002$), and TTP-AP ($P<0.001$). These results, combined with the phase I and II data presented here, indicate that atrasentan, a novel cytostatic agent, may provide significant clinical benefit to patients with metastatic HRPC. With the current availability of effective systemic chemotherapy and the recent approval of docetaxel/prednisone for the treatment of HRPC patients, a rational approach could be the combination of active cytotoxic chemotherapy to this type of cytostatic agent. Such studies have been designed and are underway. One of the studies is evaluating the activity of docetaxel and atrasentan in HRPC patients, and the second is designed to assess the synergistic effects of atrasentan and zoledronic acid in the prevention of skeletal-related events in the same cohort of patients. A randomized phase III Southwestern Oncology Group (SWOG) trial evaluating the combination of atrasentan and docetaxel will soon be open. This study will address whether the addition of atrasentan to active chemotherapy improves overall survival in HRPC patients.

Two other studies designed to evaluate the activity of atrasentan in early prostate cancer are also underway. The first, a randomized double-blind placebo-controlled trial, is designed to determine the effects of atrasentan, 10 mg/day, on time to disease progression in men with biochemical failure only–disease. This study has completed accrual, and final analysis in currently underway. The second study is also a phase II double-blind, placebo-controlled trial. The subjects are men with rising PSA after primary definitive therapy with radical prostatectomy who have a PSA doubling time of less than 12 months after prostatectomy.

Conclusions

The endothelin axis represents a novel target for cancer therapy. Preclinical models and completed phase I–III studies have demonstrated that agents designed to interrupt this axis can play an effective clinical role in management of patients with various solid malignancies. Ongoing clinical trials in HRPC and lung cancer will define the clinical activity of these selective antagonists when they are given in combination with cytotoxic therapy. Given the role of the ET pathway in osteoblast activity, further work to explore the role of atrasentan in the development of bone metastases is warranted. Although many questions concerning atrasentan as a single agent remain, in the era of targeted therapy, it is clear that a novel approach to cancer therapy could be to treat patients with multiple selective inhibitors alone or in combination with standard cytotoxic therapy. Therefore, combination of ET-1/ET-A antagonists with other growth factor inhibitors, or even with cytotoxic agents, may result in better responses compared with those obtained with atrasentan as a single agent. These combinations are an attractive therapeutic strategy that requires clinical investigation in future clinical trials.

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