Preclinical anticancer activity of the specific endothelin A receptor antagonist ZD4054

James W. Growcott

Endothelins are a family of small peptides (ET-1, ET-2, and ET-3) that mediate various physiological processes of mitogenesis, repair, and tissue differentiation by binding to endothelin A (ET_A) and endothelin B (ET_B) cell surface receptors. Activation of the ET_A receptor by ET-1 has emerged as an important factor promoting tumor cell proliferation, survival, angiogenesis, migration, invasion, and metastasis in several tumor types including prostate, ovary, colon, cervix, breast, and lung. As activation of the ET_B receptor has an opposing effect, inducing cell death by apoptosis, a rationale exists for specific antagonism of the ET_A receptor as a treatment strategy for cancer. ZD4054 is a specific ET_A receptor antagonist currently being evaluated in hormone-resistant prostate cancer in phase III clinical trials. *In vitro*, ZD4054 reversed ET-1-mediated inhibition of apoptosis in serum-deprived rat A10 and human VLTR-16 cells in a concentration-dependent manner. ZD4054 inhibited ET-1-mediated survival signaling pathways and decreased proliferation in ovarian OVCA 433 and HEY cells and in prostate PPC-1 and LAPC-4 cells. In A673 rhabdomyosarcoma cells, ET-1-induced phosphorylation of FAK^{tyr397}, FAK^{tyr861}, and $paxillin^{tyr31}$ was reversed with ZD4054, inhibiting the invasive phenotype mediated by these adhesion factors. *In vivo*, ZD4054 led to a significant reduction in tumor growth in animals bearing ovarian tumor xenografts, and significantly inhibited tumor angiogenesis. Pretreatment with ZD4054 also significantly delayed the onset of metastatic events after intracardiac injection of bladder TSU-Pr1-B1 cells in mice. These preclinical data show the potential anticancer effects of the specific blockade of the ET_A receptor with ZD4054, supporting a program of clinical investigation. *Anti-Cancer Drugs* **20:83–88 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The endothelin axis consists of a family of three 21-amino acid signaling peptides (ET-1, ET-2, and ET-3), and two G-protein-coupled receptors, endothelin A (ET_A) and endothelin B (ET_B) [1]. All three endothelin peptides are synthesized as preprohormones; in the case of ET-1, posttranslational changes convert prepro-ET-1 into two molecules, big ET-1 and ET-1 [2].

Interaction of the endothelin peptides with their respective receptors, which are ubiquitously distributed throughout the body, mediates several physiological processes, including cellular proliferation, repair, and tissue differentiation [3]. The endothelins also play a key role in the modulation of vascular tone. Activation of the ET_A receptors in blood vessel smooth muscle induces vasoconstriction and retention of sodium, increasing blood pressure. The ET_B receptor is predominantly located on the vascular endothelium, and its activation leads to the release of nitric oxide and induces natriuresis and diuresis, thereby decreasing blood pressure [1,3,4]. In cancer, the endothelin axis has been implicated in the deregulation, growth, and proliferation of a variety of different malignancies through both autocrine and paracrine pathways [5].

Specific ET_A receptor antagonism for the treatment of cancer

It is the activation of the ET_A receptor by ET-1, in particular, that has emerged as an important factor mediating the pathophysiology of tumor cell proliferation, inhibition of apoptosis and promotion of cell survival, angiogenesis, migration, invasion, metastasis, and bone deposition in several tumor types including carcinoma of the prostate, ovary, colon, cervix, breast and lung [6–9].

Activation of the ET_B receptor, conversely, has an opposing effect, inducing cell death by apoptosis [10]. As the ET_B receptor also mediates the clearance of ET-1 [11], blockade of the ET_B receptor may increase levels of ET-1, thereby promoting tumor progression by increased activation of the ET_A receptor.

It is therefore clear that a rationale exists for antagonism of the ET_A receptor with a specific inhibitor molecule

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as a potential treatment strategy for several cancer types, blocking the procancer activity of ET-1 at the ET_A receptor, while allowing potential anticancer signaling to continue through the ET_B receptor.

ZD4054: a specific ET_A receptor antagonist

ZD4054 is a novel, small-molecule, specific ET_A receptor antagonist, currently in clinical development for the treatment of hormone-resistant prostate cancer. Encouraging results from a large phase II clinical trial have demonstrated a significant overall survival benefit of 6 months with ZD4054 10 mg/day, compared with placebo [12]. A large, multinational phase III clinical trial program investigating ZD4054 in this disease setting is currently under way. In this review, we summarize the key preclinical data obtained with ZD4054, which has provided the basis for subsequent clinical evaluation.

Chemical structure

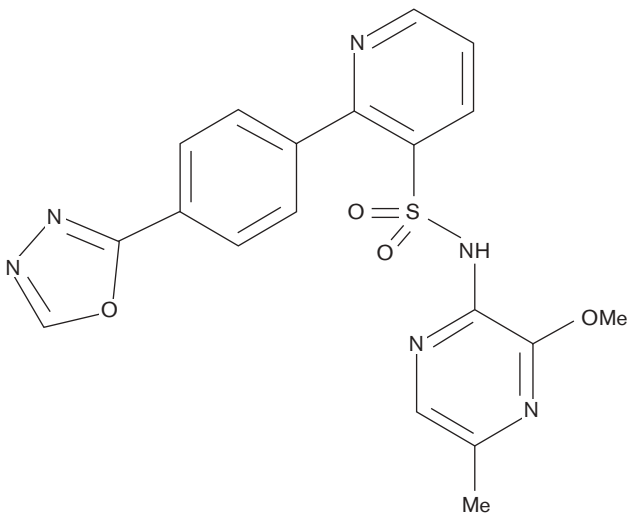
ZD4054 is *N*-(3-methoxy-5-methylpyrazin-2-yl)-2-[4-(1,3,4-oxadiazol-2-yl)phenyl] pyridine-3-sulfonamide, an orally available molecule (Fig. 1).

Receptor interaction studies

ZD4054 is an ET_A receptor antagonist with no detectable activity at the ET_B receptor [13–15]. In in-vitro multi-receptor binding assays, ZD4054 has been shown to specifically and competitively compete with ^{125}I-ET-1 binding to cloned human ET_A receptors, with an IC_{50} value of 21 nmol/l [15]. ZD4054 showed no binding to the ET_B receptor at concentrations up to 100 μmol/l.

Moreover, *in vivo* in the pithed rat model, ZD4054 produced dose-related antagonism of the pressor response to big ET-1, whereas the selective ET_B agonist, BQ3020, produced a depressor response, which was not significantly affected by the presence of ZD4054 [16]. These data effectively show the specificity of ZD4054 for the ET_A receptor and its lack of any detectable activity at the ET_B receptor.

Fig. 1



The chemical structure of ZD4054 [*N*-(3-methoxy-5-methylpyrazin-2-yl)-2-[4-(1,3,4-oxadiazol-2-yl)phenyl] pyridine-3-sulfonamide].

The ET_A receptor specificity of ZD4054 was confirmed in the clinical setting, where administration of ZD4054 in healthy volunteers had no effect on the plasma concentrations of ET-1, a biomarker of ET_B receptor antagonism [15].

The effects of ZD4054 on cellular function *in vitro*

Apoptosis

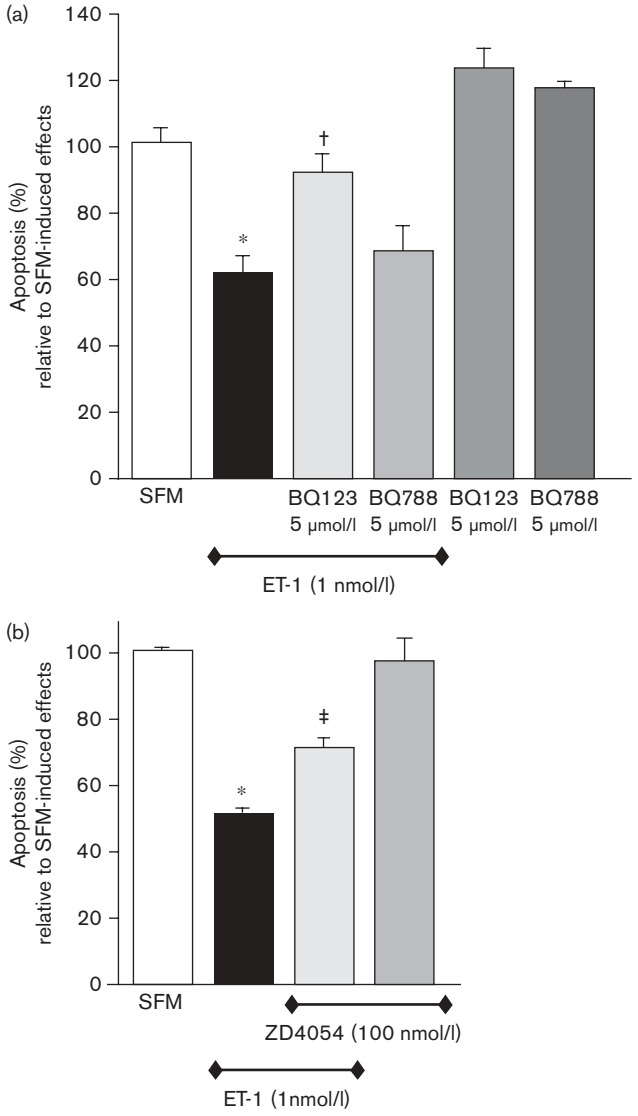
Serum withdrawal in rat A10 and human VLTR-16 smooth muscle cells induces apoptosis, an effect that is efficiently inhibited by the addition of ET-1 [17]. In human VLTR-16 cells, this antiapoptotic effect of ET-1 was attenuated and reversed by the selective ET_A receptor antagonist peptide BQ123, whereas the selective ET_B receptor antagonist peptide BQ788 had no effect (Fig. 2a). In the same model system, ZD4054 dose dependently reversed ET-1-mediated inhibition of apoptosis induced by serum deprivation (Fig. 2b) [17]. These results indicate that the ET_A receptor mediates an antiapoptotic signal, which can be attenuated by inhibition of the ET_A receptor, as shown by the administration of either BQ123 or ZD4054.

ZD4054 inhibition of ET-1-mediated survival signals has also been demonstrated in ovarian OVCA 433 and HEY cells, and in prostate PPC-1 and LAPC-4 cells [18,19]. In ovarian cancer cells, ZD4054 treatment was associated with the activation of caspase-dependent pathways and inhibition of Bcl-2 [18], whereas in prostate cancer cells, ZD4054 treatment inhibited ET-1-induced activation of PI3-kinase and AKT, resulting in enhanced cytotoxic-drug-induced apoptosis [19].

Investigation of the role of ET_B receptor signaling in tumor evolution is not straightforward, as the majority of cultured tumor cell lines available has limited expression of the ET_B receptor, most likely because of its role in mediating apoptosis and the drive of the tumor to survive 'at all costs'. The mixed ET_A/ET_B receptor profile of the A673 rhabdomyosarcoma tumor cell line, however, presents a useful in-vitro model system for evaluating the effects of ET_B as well as ET_A receptor signaling and inhibition.

As with rat A10 and human VLTR cells, serum withdrawal from A673 cells results in apoptosis. Incubation with the selective ET_B receptor antagonist BQ788 produced a concentration-dependent reduction in apoptosis in serum-deprived A673 cells, whereas the ET_A receptor

Fig. 2



The effects of ET-1 and ET_A and ET_B receptor inhibitors on apoptosis in serum-deprived human VLTR-16 cells. (a) ET_A receptor inhibition with BQ123, but not ET_B receptor inhibition with BQ788, reverses ET-1-mediated inhibition of apoptosis induced by serum deprivation. (b) ZD4054 dose dependently reverses ET-1-mediated inhibition of apoptosis induced by serum deprivation. Each histogram represents the mean percent apoptosis relative to serum-free media (SFM)-induced effects obtained from at least three independent experiments, and bars are SEM. *$P<0.05$ compared with SFM; $^{\dagger}P<0.01$ and $^{\ddagger}P<0.05$ compared with ET-1. Adapted from Curtis *et al.*, 2004 [17].

antagonists ZD4054 and BQ123 had no effect [20]. These data show that apoptosis induced by serum deprivation in A673 rhabdomyosarcoma cells is indeed mediated by ET-1 acting at the ET_B receptor. Interestingly, in this model the selective ET_A receptor antagonist, atrasentan, exhibited concentration-dependent inhibition of apoptosis similar to that seen with BQ788, which is likely to reflect supplementary activity of atrasentan at the ET_B receptor when incubated at high concentrations. Although high concentrations of both atrasentan (0.001–100 nmol/l) and ZD4054 (0.1–10 000 nmol/l) were used in this assay, the estimated functional affinity of atrasentan for the ET_B receptor has been demonstrated to be within this concentration range, with a pA_2 value of 6.45 [21,22], whereas ZD4054 has not shown any measurable affinity for the ET_B receptor up to 100 μmol/l [15,20]. The lack of any negative impact on apoptosis with ZD4054 has also been shown in ovarian OVCA 433 and HEY cells [18], and in prostate PPC-1 and LAPC-4 cells [19]. Indeed, these studies showed that apoptosis could, in fact, be enhanced when ZD4054 was combined with either paclitaxel [18] or docetaxel [19].

Collectively, these data confirm that ET-1 acts as an inhibitor of apoptosis through the ET_A receptor, and that ZD4054 can reverse this effect, thereby increasing apoptosis. ET_B receptors, when they are functionally intact (as they appear to be in A673 cells), induce apoptosis; inhibition of the ET_B receptor therefore results in a reduction in apoptosis. Hence, in the setting of targeted anticancer therapy, blockade of functional ET_B receptors may be deleterious, whereas the use of a 'specific' ET_A receptor antagonist is warranted.

Proliferation

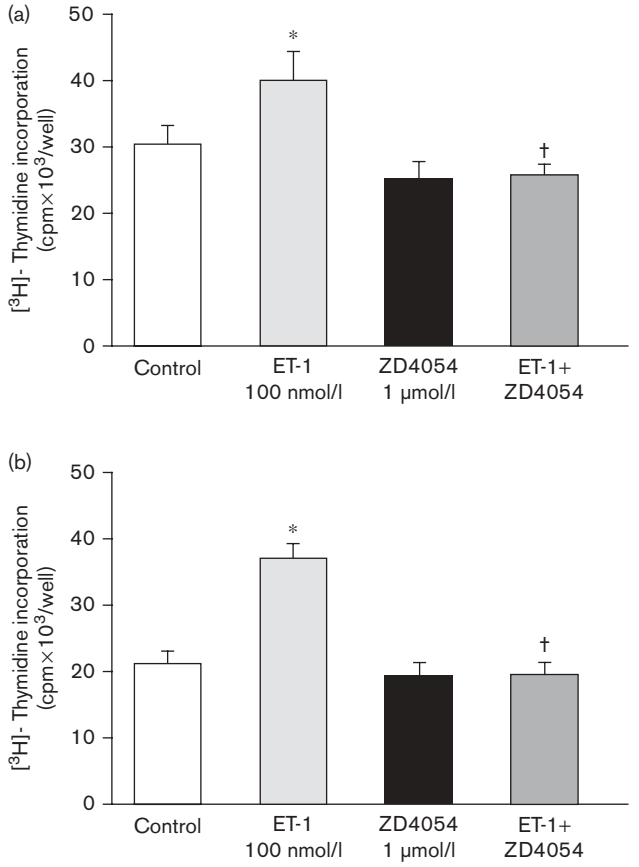
ET-1 induces proliferation in serum-deprived human ovarian cancer cell lines OVCA 433 and HEY, an effect that was inhibited by ZD4054 (Fig. 3) [23,24]. In this system, a combination of ZD4054 with gefitinib led to a complete blockade of epidermal growth factor receptor phosphorylation, mitogen-activated protein kinase (MAPK) phosphorylation, and AKT phosphorylation and further increased the reduction in proliferation when compared with the effects of monotherapy [24]. Similarly, in prostate PPC-1 and LAPC-4 cells, increased cytotoxicity was observed when ZD4054 was combined with paclitaxel, docetaxel, or doxorubicin [19].

In the human immature osteoblast cell line HBC-171, ET-1 induced proliferation of serum-deprived cells, an effect associated with phosphorylation of p44/42 MAPK [17]. This ET-1-induced proliferation and MAPK phosphorylation was inhibited by ZD4054 (Fig. 4). Similarly, in murine MC-3T3.E1/J1 osteoblasts, ET-1 induced a concentration-dependent increase in p44/42 MAPK phosphorylation that was competitively antagonized by ZD4054 [25].

Invasion and migration

In A673 rhabdomyosarcoma cells, ET-1-induced phosphorylation of FAK^{tyr397}, FAK^{tyr861}, and $paxillin^{tyr31}$ was inhibited by ZD4054 [26]. These phosphorylation events were also inhibited by the selective ET_A receptor antagonist BQ123 but not by the selective ET_B receptor

Fig. 3



ZD4054 inhibition of ET-1-mediated proliferation in serum-deprived human OVCA 433 and HEY ovarian carcinoma cells. Serum-deprived (a) OVCA 433 and (b) HEY cells were treated with ET-1 100 nmol/l alone or in combination with ZD4054 1 μmol/l. Cell proliferation was analyzed after 24 h. Each histogram represents the mean of sextuplicate determinations of three independent experiments, and bars are SD. *$P<0.0001$ compared with control; †$P<0.005$ compared with ET-1. Adapted from Rosano *et al.*, 2007 [24].

antagonist BQ788. Phosphorylation of FAK^{tyr397}, FAK^{tyr861}, and $paxillin^{tyr31}$ induces an invasive phenotype, causing the A673 cells to invade through a matrigel matrix. This invasion was inhibited by the presence of ZD4054 [26]. Combining ZD4054 with the Src inhibitor AZD0530 resulted in a synergistic inhibition of this invasive phenotype [27], implying a linkage between these signaling pathways. In MDA-MB-468 and MCF-7 breast cancer cells, ZD4054 significantly reduced cellular migration and invasion, respectively [28]. In aromatase-overexpressing MCF-7aro cells, although monotherapy with ZD4054 or the aromatase inhibitors letrozole or anastrozole had minimal effects on cellular migration, combining ZD4054 with either of the two aromatase inhibitors was associated with significant reductions in cellular migration. Furthermore, in MCF-7 cells, the combination of ZD4054 with the estrogen receptor downregulator fulvestrant (Faslodex, AstraZeneca, Macclesfield, Cheshire, UK) was associated with a significant reduction in both cellular migration and invasion, with effects exceeding those observed with ZD4054 [28].

This wealth of in-vitro data point to a role for the endothelin axis in the pathogenesis of an invasive, proliferative tumor cell phenotype in several different cancer settings. The ET_A receptor seems to play a pivotal role in driving the ET-1-induced invasive phenotype and inhibition of apoptosis, effects that ZD4054 is capable of inhibiting or reducing. Importantly, the combination of ZD4054 with other therapies is associated with an increased benefit, adding further weight to the hypothesis that different signaling pathways contribute to the invasive phenotype.

The effects of ZD4054 on tumor biology *in vivo*

Primary tumor growth and proliferation

The antiproliferative effects of ZD4054 10 mg/kg/day either as monotherapy or in combination with gefitinib 125 mg/kg/day translated into a significant reduction in tumor growth in animals bearing ovarian HEY tumor xenografts [24]. In addition, Ki67 staining – an indicator of the cellular proliferation rate – in tissue samples taken from the tumors of animals treated with ZD4054 was reduced compared with untreated animals. Staining was further reduced when ZD4054 was combined with gefitinib, compared with ZD4054 monotherapy [24].

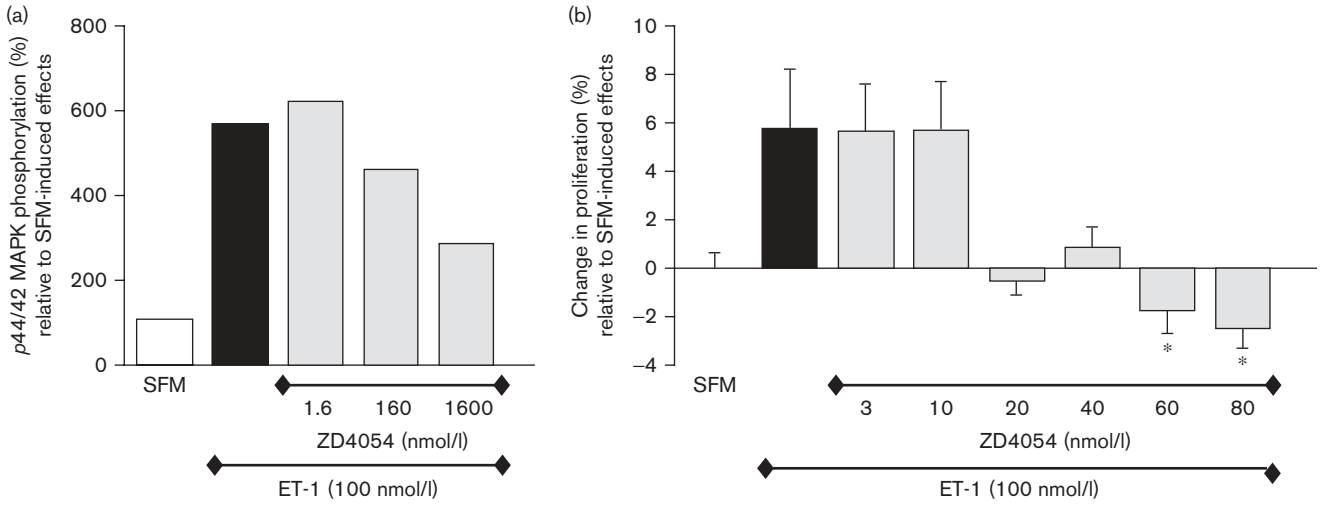
Angiogenesis

In an intradermal model of tumor blood flow in animals bearing either prostate or colorectal tumor xenografts, ZD4054 was shown to significantly reduce blood vessel formation [29]. In animals bearing HEY ovarian carcinoma xenografts, microvessel density (MVD) assessment using CD31 labeling revealed that ZD4054 significantly reduced new vessel formation, and that this effect was enhanced when ZD4054 was combined with gefitinib [24]. Vascular endothelial growth factor levels were also reduced in line with the MVD profile. ET-1 has been shown to be intimately involved in various stages of neovascularization [30], and elevated expression of ET-1 correlates with MVD and vascular endothelial growth factor expression [31]. ZD4054, by virtue of its ability to block the effects of ET-1 at the ET_A receptor, has demonstrated potential for reducing new vessel formation in the tumor setting.

Metastasis

In a murine model where intracardiac injection of bladder TSU-Pr1-B1 cells results in bone metastasis, pretreatment with ZD4054 significantly delayed the onset of these events [32]. Indeed, combining ZD4054 with the bisphosphonate pamidronate completely prevented the formation of any bone metastasis in this

Fig. 4



Effects on proliferation and p44/42 mitogen-activated protein kinase phosphorylation in serum-deprived human HBC-171 cells. ZD4054 dose dependently inhibited (a) ET-1-induced p44/42 MAPK phosphorylation and (b) ET-1-induced proliferation. Each histogram represents the mean percent relative change in viable cells [compared with the effect seen in serum-free media (SFM)] obtained from at least three independent experiments, and bars are SEM. *$P<0.05$ compared with SFM. Adapted from Curtis *et al.*, 2005 [25].

model. Interestingly, these effects do not seem to be confined to bone, as significant reductions in soft tissue metastases were also observed with combinations of these two agents [33], although the mechanism behind this reduction in soft tissue metastasis with the combination of ZD4054 and pamidronate is yet to be elucidated. Whether the presence of the bisphosphonate prevents primary metastatic cells located in bone from re-invading soft tissue, or whether ZD4054 itself prevents this phenomenon or inhibits primary cancer cells from invading and embedding into soft tissue, is not yet determined, and further investigation will be required to rationalize these observations. Circulating levels of ET-1 are known to be elevated in prostate cancer patients with osteoblastic metastases [34], and the ET-1/ET_A receptor autocrine pathway is overexpressed in primary and metastatic ovarian carcinomas [35]. The observations of the effect of ZD4054 on metastases *in vivo* supported the endothelin axis playing a key role in driving metastatic disease.

Conclusion

ZD4054 is a novel, potent, and specific small-molecule ET_A receptor antagonist. Taken together, data from the in-vitro and in-vivo studies described here show that ZD4054 is able to inhibit tumor cell survival and proliferation, and clearly demonstrate the potential anticancer effects of specific blockade of the ET_A receptor. Along with reducing proliferation, angiogenesis, and metastatic spread, ZD4054 has been shown to increase apoptosis, with no detectable activity at the ET_B receptor, the inhibition of which would be deleterious in the clinical setting. Moreover, the effects of ET_A receptor blockade with ZD4054 seem to be increased when this agent is combined with other therapies, including cytotoxic agents (e.g. docetaxel and paclitaxel), epidermal growth factor receptor-tyrosine kinase inhibitors (e.g. gefitinib), aromatase inhibitors (e.g. letrozole and anastrozole), Src inibitors (e.g. AZD0530), and bisphosphonates (e.g. pamidronate). Although the mechanisms underlying these additive or synergistic effects are still unclear, evidence suggests that several cell signaling pathways are linked with regard to their protumor activity; indeed, such crosstalk has the potential to lead to even greater beneficial effects when therapies targeting multiple receptors and signaling pathways are combined with ZD4054. It is also possible that ET-1 modulates the resistance pathways evoked by treatment with cytotoxic therapies, and that by blocking the ET_A receptor, the potential for resistance to such treatments may be diminished.

The sum of preclinical data presented here provides a strong foundation and rationale for specific ET_A receptor inhibition with ZD4054 as a promising therapeutic approach in cancer. After promising results of a large phase II clinical trial, in which ZD4054 was associated with a survival benefit of 6 months in patients with hormone-refractory prostate cancer [12], a multinational phase III program has recently been initiated in this disease setting.

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References

1 Kedzierski RM, Yanagisawa M. Endothelin system: the double-edged sword in health and disease. *Annu Rev Pharmacol Toxicol* 2001; **41**:851–876.
2 Turner AJ, Murphy LJ. Molecular pharmacology of endothelin converting enzymes. *Biochem Pharmacol* 1996; **51**:91–102.
3 Goldie RG. Endothelins in health and disease: an overview. *Clin Exp Pharmacol Physiol* 1999; **26**:145–148.
4 Agapitov AV, Haynes WG. Role of endothelin in cardiovascular disease. *J Renin Angiotensin Aldosterone Syst* 2002; **3**:1–15.
5 Bagnato A, Spinella F. Emerging role of endothelin-1 in tumor angiogenesis. *Trends Endocrinol Metab* 2003; **14**:44–50.
6 Bagnato A, Natali PG. Endothelin receptors as novel targets in tumor therapy. *J Transl Med* 2004; **2**:16.
7 Nelson J, Bagnato A, Battistini B, Nisen P. The endothelin axis: emerging role in cancer. *Nat Rev Cancer* 2003; **3**:110–116.
8 Levin ER. Endothelins. *N Engl J Med* 1995; **333**:356–363.
9 Masaki T. The endothelin family: an overview. *J Cardiovasc Pharmacol* 2000; **35**:S3–S5.
10 Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T. Endothelin-induced apoptosis of A375 human melanoma cells. *J Biol Chem* 1998; **273**:12584–12592.
11 Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun* 1994; **199**:1461–1465.
12 James ND, Caty A, Borre M, Zonnenberg BA, Beuzeboc P, Morris T, *et al*. Safety and efficacy of the specific Endothelin-A receptor antagonist ZD4054 in patients with hormone-resistant prostate cancer and bone metastases who were pain free or mildly symptomatic: A double-blind, placebo-controlled, randomised, Phase 2 trial. *Eur Urol, In press doi:10.1016/j.eururo.2008. 11.002.*
13 Bradbury RH, Bath C, Butlin RJ, Dennis M, Heys C, Hunt SJ, *et al*. New non-peptide endothelin-A receptor antagonists: synthesis, biological properties, and structure-activity relationships of 5-(dimethylamino)-*N*-pyridyl-,-*N*-pyrimidinyl-,-*N*-pyridazinyl-, and -*N*-pyrazinyl-1-naphthalenesulfonamides. *J Med Chem* 1997; **40**:996–1004.
14 Stensland B, Roberts RJ. *N*-(3-Methoxy-5-methylpyrazin-2-yl)-2-[4-(1,3,4-oxadiazol-2-yl)phenyl]pyridine-3-sulfonamide (ZD4054 Form 1). *Acta Crystallogr Section E Structure Rep* 2004; **60**:o1817–o1819.
15 Morris CD, Rose A, Curwen J, Hughes AM, Wilson DJ, Webb DJ. Specific inhibition of the endothelin A receptor with ZD4054: clinical and pre-clinical evidence. *Br J Cancer* 2005; **92**:2148–2152.
16 Curwen JO, Wilson C. ZD4054: a specific endothelin A receptor antagonist with potential utility in prostate cancer and metastatic bone disease. *Eur J Cancer* 2002; **38**:S102.
17 Curtis N, Howard Z, Brooks N, Curwen J. ZD4054 specifically inhibits endothelin A receptor-mediated anti-apoptotic effects, but not endothelin B receptor-mediated pro-apoptotic effects. *Eur J Cancer Suppl* 2004; **2**:27.
18 Rosanò L, Di Castro V, Spinella F, Nicotra MR, Natali PG, Bagnato A. ZD4054, a specific antagonist of the endothelin A receptor, inhibits tumor growth and enhances paclitaxel activity in human ovarian carcinoma in vitro and in vivo. *Mol Cancer Ther* 2007; **6**:2003–2011.
19 Pflug BR, McHugh K, D'Antonio JM, Bocola-Mavar E, Curwen J, Growcott JW, *et al*. Defining the basis of an operational model for enhanced efficacy of combination therapy using an endothelin receptor antagonist and chemotherapeutic agents [abstract]. *Mol Cancer Ther* 2007; **6**:A287.
20 Growcott JW, Hickinson M, Curtis N, Curwen J. Phenotypic in vitro differentiation of the specific endothelin A receptor antagonist, ZD4054, from the selective endothelin antagonist, atrasentan [abstract]. *Mol Cancer Ther* 2007; **6**:B269.
21 Opgenorth TJ, Adler AL, Calzadilla SV, Chiou WJ, Dayton BD, Dixon DB, *et al*. Pharmacological characterization of A-127722: an orally active and highly potent ETA-selective receptor antagonist. *J Pharmacol Exp Ther* 1996; **276**:473–481.
22 Wessale JL, Adler AL, Novosad EI, Calzadilla SV, Dayton BD, Marsh KC, *et al*. Pharmacology of endothelin receptor antagonists ABT-627, ABT-546, A-182086 and A-192621: ex vivo and in vivo studies. *Clin Sci (Lond)* 2002; **103** (**Suppl 48**):112S–117S.
23 Rosano L, Di Castro D, Spinella F, Natali PG, Bagnato A. Combined targeting of the endothelin A receptor and the epidermal growth factor receptor in ovarian cancer shows enhanced antiproliferative effects [abstract]. *Proc Am Assoc Cancer Res* 2006; **47**:1509.
24 Rosanò L, Di Castro V, Spinella F, Tortora G, Nicotra MR, Natali PG, *et al*. Combined targeting of endothelin A receptor and epidermal growth factor receptor in ovarian cancer shows enhanced antitumor activity. *Cancer Res* 2007; **67**:6351–6359.
25 Curtis N, Anderson E, Brooks N, Curwen J. ZD4054 blocks ET-1-stimulated phosphorylation of p44/42 mitogen-activated kinase and proliferation of osteoblast cells [abstract]. *Proc Am Assoc Cancer Res* 2005; **46**:1512.
26 Growcott J, Hickinson M, Curwen J, Isherwood B, Carragher N, Curtis N. Anti-invasive activity of the specific ET_A receptor antagonist, ZD4054, in A673 rhabdomyosarcoma cells. 10th International Conference on Endothelin (ET-10) Bergamo, Italy, 16–19 September 2007; abst P-116.
27 Hickinson M, Curtis N, Green TP, Growcott J, Curwen J, Isherwood B, *et al*. Enhanced in vitro anti-invasive activity in A673 rhabdomyosarcoma cells of the specific endothelin-A receptor (ET_A) antagonist ZD4054 when combined with the novel Src inhibitor AZD0530 [abstract]. *Proc Am Assoc Cancer Res* 2008; **49**:1487.
28 Wülfing P, Smollich M, Gotte M, Fischgrabe J, Radke I, Chen S, *et al*. ZD4054, a selective endothelin A receptor antagonist, reduces breast cancer cell migration and invasion and exhibits additive effects with aromatase inhibitors and fulvestrant [abstract]. *Mol Cancer Ther* 2007; **6**:A275.
29 Curwen J, Hughes G, Hickinson M, Curtis N, Growcott JW, Isherwood B, *et al*. The impact of ZD4054, a specific endothelin A receptor antagonist, on tumor blood supply, invasion, and the bone microenvironment [abstract]. *Mol Cancer Ther* 2007; **6**:A272.
30 Salani D, Di Castro V, Nicotra MR, Rosano L, Tecce R, Venuti A, *et al*. Role of endothelin-1 in neovascularization of ovarian carcinoma. *Am J Pathol* 2000; **157**:1537–1547.
31 Salani D, Taraboletti G, Rosano L, Di CV, Borsotti P, Giavazzi R, *et al*. Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. *Am J Pathol* 2000; **157**:1703–1711.
32 Williams ED, Thompson EW, Sreedharan D, Brooks N, Curwen J, Growcott JW. The combination of a specific endothelin A receptor antagonist ZD4054 and submaximal bisphosphonate pamidronate prevents bone metastasis. *Eur J Cancer Suppl* 2006; **4**:15.
33 Williams ED, Thompson EW, Sreedharan D, Brooks N, Curwen J, Growcott JW. The combination of the specific endothelin A receptor antagonist ZD4054 and submaximal bisphosphonate pamidronate prevents soft-tissue metastasis [abstract]. *Mol Cancer Ther* 2007; **6**:A271.
34 Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, *et al*. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. *Nat Med* 1995; **1**:944–949.
35 Bagnato A, Salani D, Di Castro V, Wu-Wong JR, Tecce R, Nicotra R, *et al*. Expression of endothelin 1 and endothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. *Cancer Res* 1999; **59**:720–727.