

Serveur Académique Lausannois SERVAL [serval.unil.ch](http://serval.unil.ch)

## Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

**Title:** Endothelin-Receptor Antagonists beyond Pulmonary Arterial Hypertension: Cancer and Fibrosis.

**Authors:** Aubert JD, Juillerat-Jeanneret L

**Journal:** Journal of medicinal chemistry

**Year:** 2016 Sep 22

**Issue:** 59

**Volume:** 18

**Pages:** 8168-88

**DOI:** 10.1021/acs.jmedchem.5b01781

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

# Endothelin-receptor antagonists beyond pulmonary arterial hypertension: cancer and fibrosis

*John-David Aubert<sup>†\*</sup> and Lucienne Juillerat-Jeanneret<sup>‡\*</sup>*

<sup>†</sup>John-David Aubert, Pneumology Division and Transplantation Center, Centre Hospitalier  
Universitaire Vaudois (CHUV), Lausanne, Switzerland

<sup>‡</sup>Lucienne Juillerat-Jeanneret: PhD, University Institute of Pathology and Transplantation  
Center, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne  
(UNIL), Lausanne, Switzerland

**KEYWORDS** cancer - cardiovascular diseases - clinical trials - endothelin-receptor antagonists - fibrosis - pulmonary arterial hypertension – kidney diseases

## **ABSTRACT**

The endothelin axis, and in particular the two endothelin receptors, ET<sub>A</sub> and ET<sub>B</sub>, are targets for therapeutic intervention in human diseases. Endothelin-receptor antagonists are in clinical use to treat pulmonary arterial hypertension and have been under clinical investigation for the treatment of several other diseases, such as systemic hypertension, cancer, vasospasm and fibrogenic diseases. In this Perspective, we review the molecules that have been evaluated in human clinical trials for the treatment of pulmonary arterial hypertension, as well as other cardiovascular diseases, cancer and fibrosis. We will also discuss the therapeutic consequences of receptor selectivity with regard to ET<sub>A</sub>-selective, ET<sub>B</sub>-selective or dual ET<sub>A</sub>/ET<sub>B</sub> antagonists. We will also consider which chemical characteristics are relevant to clinical use and the properties of molecules necessary for efficacy in treating diseases against which known molecules displayed suboptimal efficacy.

## INTRODUCTION

### **The endothelin axis**

The endothelin (ET) axis is mainly recognized for its action as a potent vasoconstrictor system involved in the regulation of vascular tone, but it has pleiotropic other functions able to mediate fundamental cellular processes such as cell proliferation and apoptosis. Thus, beside its normal functions the endothelin axis has also been involved in human hypertensive and cardiovascular pathologies and in fibrogenic, inflammatory and oncologic diseases, and has putative roles in other pathologies including septic shock, atherosclerosis, heart failure, renal insufficiency and cerebrovascular conditions associated with subarachnoid hemorrhage.

The endothelins (ET-1, ET-2 and ET-3) constitute a family of 21 amino-acid (aa) peptides synthesized as longer precursor polypeptides of 212 aa (ppETs). These peptides are further proteolytically cleaved by a signal peptidase to produce pro-ET-1, and further activated by a subtilisin-like convertase/furin-like protease that release the 38 aa-long pro-ETs (big ETs). Big ETs are then subsequently cleaved by the more specific membrane-bound endothelin converting enzyme-1 (ECE-1)<sup>1</sup> to yield the 21-aa active ET peptides. Following their proteolytic processing, the ET peptides can be either constitutively secreted or can be stored in intracellular granules, then secreted according to a regulated pathway in response to a stimulus.<sup>2</sup> Following activation and secretion, ET peptides act on two distinct high-affinity receptor subtypes, ET<sub>A</sub> and ET<sub>B</sub>, which are located on target cell membranes.<sup>3</sup>

The cell and tissue expression of the components of the endothelin axis are numerous. The vascular endothelium is an abundant source of the components of the endothelin axis; however, they may also be expressed by leukocytes, smooth muscle cells, mesangial cells, cardiac myocytes, or astrocytes. The most representative peptide of the axis, endothelin (ET)-1, can be induced in endothelial cells by many factors including mechanical stimulation, various hormones and pro-inflammatory cytokines. Its production is inhibited by nitric oxide

(NO), cyclic nucleotides, prostacyclin and atrial natriuretic peptide (ANP). ET-1 also stimulates cardiac contraction and the growth of cardiac myocytes, regulates the release of vasoactive substances, and stimulates smooth muscle cell mitogenesis. ET-1 may control inflammatory responses by promoting the adhesion and migration of neutrophils and by stimulating the production of pro-inflammatory cytokines. It has also been implicated in cancer progression, regulating the proliferation and migration of tumor cells and acting as a pro-angiogenic factor and an inducer of stromal reaction.

In this Perspective, we will first describe the components of the endothelin axis, then, we will discuss attempts made to therapeutically control the functions of the endothelin axis in diseases. We will also discuss and evaluate in more detail the therapeutic interest of endothelin-receptor antagonists in three pathological conditions of proliferative fibrogenic disorders: 1) pulmonary arterial hypertension (PAH), for which endothelin-receptor antagonists are in clinical use; 2) cancer, for which clinical trials have been launched but for which no antagonist has yet reached clinical use; and finally 3) fibrogenic diseases of the kidney, for which such antagonists are being considered for therapy. Fibrogenic disorders involve the recruitment of inflammatory/immune cells, which ultimately drive the activation and proliferation of fibroblasts and the replacement of tissue cells by these activated (myo)fibroblasts. These three conditions imply an enhanced proliferative component involving different but complementary cells. Not surprisingly, as ET-1 and its associated components, are cell-proliferation, anti-apoptotic, pro-survival and contractile factors, they have been evaluated as therapeutic targets for these potential therapeutic possibilities. Only drugs able to inhibit the binding of ETs to their cognate ET<sub>A</sub> and/or ET<sub>B</sub> receptors, the “sentan” family of endothelin-receptor antagonists, have reached clinical evaluation and use and will be described in detail in this Perspective.

## **The components of the endothelin axis**

### *The endothelin peptides: ET-1, ET-2 and ET-3*

The most-studied peptide from the ET family, endothelin-1 (ET-1), was discovered in 1988<sup>4</sup> as a potent and long-lasting vasoconstrictor peptide, much more potent than angiotensin II. The 21-aa active ET peptides are characterized by a single alpha helix, two essential disulfide bridges, Cys3-Cys11 and Cys1-Cys15, and six conserved amino acid residues at the C-terminus. ET-1 has been the most studied peptide of the family; however, the other peptides of the endothelin family, ET-2 and ET-3 may also be of therapeutic interest.<sup>5</sup> A role for ET-2 is emerging in ovarian development, the cardiovascular system, immunology and cancer. In human, ET-2 differs from ET-1 by only two amino acids and has the same affinity as ET-1 for ET<sub>A</sub> and ET<sub>B</sub> receptors, unlike ET-3. Until recently, it was assumed that ET-2 mimics the functions of ET-1; however, recent evidence has pointed to a specific ET-2 pathway as a key regulator of ovarian contractile physiology, with roles as well in heart failure, immunology and cancer. ET-3 differs from ET-1 by six amino acids and has differential affinities for ET<sub>A</sub> and ET<sub>B</sub>, being highly selective for ET<sub>B</sub>. ET-3 is the main ET peptide in the central nervous system. In cancer, ET-2 and ET-3 may counteract the functions of ET-1, but are also frequently silenced. In human, the genes encoding for ET-1, ET-2 and ET-3 are located on chromosomes 6, 1 and 20, respectively. Epigenetic inactivation by hypermethylation of ET-2 and ET-3 promoters in colon cancer was postulated to favor ET-1-mediated tumor progression., suggesting that ET-2 and ET-3 are natural antagonists of ET-1 and that re-expression of ET-2 and ET-3 may be a complementary approach to receptor antagonists.<sup>6</sup> The activation of ET precursors by ECEs and chymase may also allow the selective activation of one of the pathways.

### *The endothelin activating enzymes: furins and endothelin-converting enzymes*

Furins are a family of intracellular serine proteases belonging to the subtilisin-like

proprotein convertase family. The members of this family process latent precursor proteins at paired basic amino-acid sequences (Arg/Lys-Arg) into their biologically active products. These endoproteases can cleave many precursor proteins, including ppETs, and are involved in several human disorders.<sup>7</sup> Furins are mainly located within the Golgi/trans-Golgi secretory pathway. The catalytic mechanism in enzymes of the serine protease family involves a catalytic triad consisting of three essential amino acids: one histidine, one serine and one aspartic acid. In the enzymatic mechanism, covalent intermediates are generated. Inhibitors of furins, which include  $\alpha$ 1-antitrypsin, polyarginine compounds, decanoyl-Arg-Val-Lys-Arg-chloromethylketone, as well as a few synthetic molecules, are under consideration as therapeutic agents.<sup>8</sup> However, to the best of our knowledge, no therapeutic attempts have been made to control the endothelin axis with furin inhibitors.

Two metalloprotease endothelin-converting enzymes (ECEs) have been described.<sup>1,9-12</sup> ECE-1 (EC 3.4.24.71) hydrolyzes the Trp11-Val12 bond of human bigET-1 at neutral pH and is expressed as four isoforms, ECE-1<sub>a-d</sub>, from one gene through the use of different promoters. ECE-2 is active at acidic pH, but as little information exists concerning its physiological importance in human diseases, it will not be discussed in this Perspective. The effect of inhibition of ECE-1 has been evaluated only in animal models of cardiovascular disorders and *in vitro* in cancer<sup>13</sup> and will not be reviewed in detail here because no human trials for therapeutic purposes have been published with these inhibitors.<sup>14</sup> However, some observations may be of interest. Depending on the particular ECE-1 isoform or on external factors, ECE-1 may be secreted or expressed at the cell surface (ECE-1<sub>a</sub> and ECE-1<sub>c</sub>) and thus may be directly accessible to inhibitors. Alternatively, ECE-1 may remain intracellular (ECE-1<sub>b</sub> and ECE-1<sub>d</sub>), thus necessitating that inhibitors traverse the cell membrane. NEP 24.11/nepilysin may also activate and degrade big-ET-1, but with a much lower efficiency than ECE-1.<sup>15</sup> Some information also suggests that mast-cell-derived chymase cleaves the

Tyr<sup>31</sup>-Gly<sup>32</sup> bond of big-ET-1 to yield ET-1 (1-31). This peptide is further processed into the active ET- (1-21) by either ECE-1, the main activator of big-ET-1, or NEP24.11, as both thiorphan (a selective NEP 24.11 inhibitor) and phosphoramidon (a dual ECE-1/NEP24.11 inhibitor) abolish ET-1 (1-31) processing.<sup>16</sup> The enzymes of this latter pathway and their inhibitors have not been evaluated in much detail for therapeutic purposes, and this alternate pathway will not be discussed further here. However, while pure ECE-1 inhibitors have not been tested in the clinic, dual ECE-1/ACE inhibitors have been evaluated in clinical trials.<sup>17,18</sup>

*The endothelin receptors: ET<sub>A</sub> and ET<sub>B</sub>*

ET receptors belong to the family of seven transmembrane G-protein-coupled receptors (GPCR). Heterotrimeric guanine nucleotide-binding G proteins, composed of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -subunits on the inner membrane surface of the cells, are key determinants of many signaling processes, including cell proliferation, apoptosis, survival, contraction, migration and/or differentiation. GPCR ligands interact with many downstream effectors, including adenylyl cyclases, phosphodiesterases, phospholipases, tyrosine kinases and ion channels. The duration of the signal is modulated by the activity of the multiple GPCR-mediated signaling pathways, leading to diverse biological responses.<sup>19,20</sup> At physiological concentrations, ET-1 and ET-2, but not ET-3, bind to ET<sub>A</sub> receptors with comparable affinity ( $K_D$  ET-1 = ET-2 ~ 20-60 pM, ET-3 ~ 6500 pM), whereas all three ET ligands bind ET<sub>B</sub> receptors with similar affinity ( $K_D$  ET-1 = ET-2 = ET-3 ~ 15 pM). In humans, ET<sub>A</sub> is located in the vasculature and is mostly expressed by cells of the vascular smooth muscle lineage. In these cells, binding of ET-1 to ET<sub>A</sub> mainly induces vasoconstriction and cell proliferation. ET<sub>B</sub> in the vasculature is mostly expressed by endothelial cells. In these cells, binding of ETs to ET<sub>B</sub> induces vasodilatation, bronchoconstriction and cell proliferation. In the endothelium, ET-1 is mainly released abluminally, rather than into the blood stream, suggesting autocrine/paracrine-localized tissue functions rather than endocrine functions; however, other cell types have been shown to



express and respond to the components of the endothelin axis. For example, mutations in ET<sub>B</sub> in the colon is associated with Hirschprung disease and selective expression of both receptors is seen in cancer cells and cancer-associated stromal cells (see below). In human, the genes encoding for ET<sub>A</sub> and ET<sub>B</sub> are located on chromosomes 4 and 13, respectively.

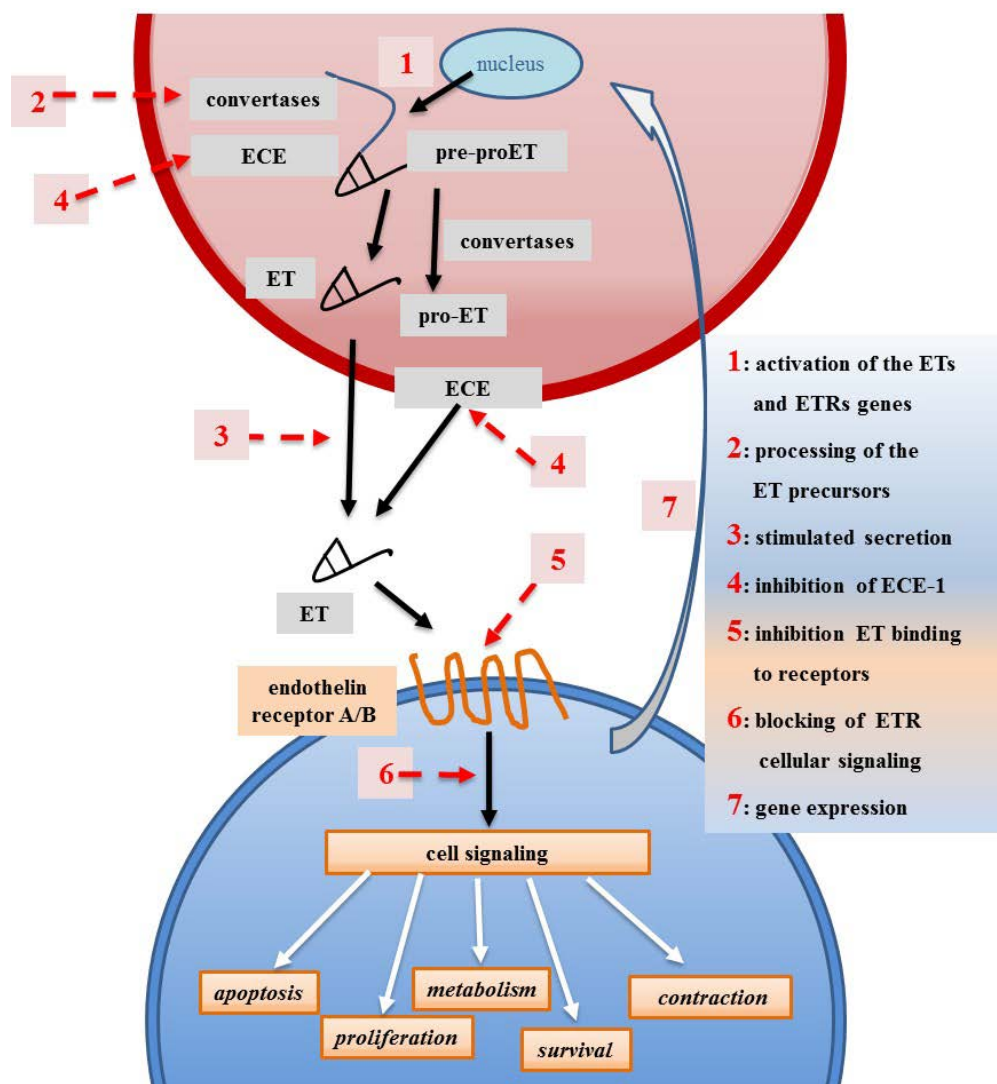
Both ET<sub>A</sub> and ET<sub>B</sub> receptors share common signal-transduction pathways through phosphatidylinositol hydrolysis and an increase in cytosolic calcium. The two receptors can also signal through different G proteins and stimulate a variety of other effector systems such as phospholipases D and A2, PKC or the protein tyrosine kinase-MAP-kinase/ERK pathways.<sup>21,22</sup> The transactivation of the EGF receptor by ET-1 has also been reported.<sup>10</sup> In addition to its potent vasoconstrictor activity, ET-1 is an autocrine/paracrine (co-)mitogen in many cell types, involving several intracellular signaling pathways. Antagonists to endothelin receptors have been developed for the treatment of several cardiovascular diseases<sup>23-27</sup> and have reached clinical use in the case of pulmonary hypertension.<sup>28</sup> Unexpectedly, they have also shown promising effects in the context of cancer, with the potential to control tumor growth and potentiate apoptosis, as well as in fibrotic processes in kidney disorders.

Variants of both endothelin receptors have been described,<sup>29-38</sup> and mutagenesis and chimeric receptor studies have indicated that splice variants of the endothelin receptors may undergo conformational changes. It can be speculated that these changes may allow either the binding of ligands other than the ETs or the binding of cellular proteins able to modify the biological functions of the receptors, resulting in either increased or decreased binding affinities for receptor agonists and antagonists and changes in signaling pathways. Several studies have shown that the loss of ET<sub>B</sub> receptor functions plays an important role in ET-induced proliferation and cell death, as well as the activation of intracellular signal transduction pathways that regulate ET production. These effects of ET<sub>B</sub> receptor functions may be important in tumor development, both in cancer cells and cancer-associated stromal

cells. We have searched for ET<sub>B</sub> variants in human glioblastoma (GBM) cells (Y Berger and L Juillerat-Jeanneret, unpublished data) and in human melanoma cells.<sup>39</sup> Our results suggested cell-selective effects and possibly methylation of the ET<sub>B</sub> promoter. Methylation of the *EDNRB* gene was also observed by others in cancer but was not predictive of survival.<sup>40</sup> However, a quantitative analysis comparing promoter methylation of the *EDNRB* gene in paired human normal and gastric tumors found a correlation between higher methylation rates and the aggressiveness and progression of the cancer.<sup>41</sup>

#### *The therapeutic control of the endothelin axis in human diseases*

The endothelin axis has been widely implicated in the pathophysiology of various human diseases. As ET-1 is a very potent vasoconstrictor peptide, the first therapeutic attempts aimed at treating hypertensive disorders, then it was realized that the components of this axis may also be valuable therapeutic targets in cancer, inflammation, fibrosis and metabolic diseases. For therapeutic purposes, the endothelin axis may be blocked using chemical tools, either at the level of bigET activation (using inhibitors of ECE-1) or by using antagonists to the ET<sub>A</sub> and/or ET<sub>B</sub> endothelin receptors (**Figure 1**). In many academic and industrial groups, programs were initiated for the design, preparation and evaluation of molecules aimed at controlling the functions of the endothelin axis, the bioprocessing of ET-1 by its activating enzymes and its binding to its cognate receptors, ET<sub>A</sub> and/or ET<sub>B</sub>. These two strategies have been evaluated in animal experimental models of human diseases; however, only receptor antagonists have reached clinical trials and clinical use.



**Figure 1.** The metabolic processing of the components of the endothelin axis and the potential sites of control of the functions of the endothelin axis (*insert, 1 to 7*). ET: endothelin; ECE: endothelin converting enzyme-1. Many different types of cells can secrete the ET peptides and express the ET receptors. Following *ET* gene activation, a precursor polypeptide is sequentially hydrolyzed by a signal peptidase, a furin-like convertase, then the ECE-1, either by intracellular ECE isoforms or at the cell surface by cell-membrane inserted ECE, to yield the 21-aa active ET. ET can be either constitutively secreted or can be secreted according to a regulated pathway in response to a stimulus. Then, ET acts on two distinct receptors  $ET_A$  and  $ET_B$ , to induce intracellular signaling. In this drawing, a paracrine effect of ET is shown, however, the effects of the ET axis may be autocrine or endocrine.

## THERAPEUTIC AGENTS

### **Endothelin-receptor antagonists**

As the diseases for which endothelin-receptor antagonists are targeted for therapy are chronic diseases, including hypertensive and fibrogenic disorders, requesting drug intake once or sometimes twice a day for many years, oral bioavailability is required for patient compliance to their treatment. Due to the nanomolar/picomolar  $IC_{50}$  values of ET-1 binding to its cognate receptors, receptor antagonists have to be of low nanomolar affinity. Thus, the molecules developed as therapeutic endothelin-receptor antagonists must have these two properties, oral bioavailability and high affinity. Many research groups have begun work on the discovery and development of endothelin-receptor antagonists for the treatment of such diseases. Many synthetic molecules have been designed, prepared and evaluated, first in *in vitro* cellular models, then in *in vivo* experimental animal models of human diseases, and finally, for some of them, in human clinical trials. Whereas some compounds are being marketed to treat pulmonary arterial hypertension (PAH), several other compounds have been evaluated in late clinical trials for various indications. The compounds were generally active at low nanomolar concentrations and orally bioavailable. They were designed to be either  $ET_A$ -selective antagonists (**Table 1**) with selectivity  $ET_A/ET_B > 100$ , or dual  $ET_A/ET_B$  antagonists (**Table 2**) with selectivity  $ET_A \sim ET_B$ , whereas very few  $ET_B$ -selective antagonists (**Table 3**) with selectivity  $ET_A/ET_B < 100$  have been developed and evaluated in clinical trials. Only two types of endothelin-receptor antagonists have reached clinical use:  $ET_A$ -selective and dual antagonists; however, at high concentrations,  $ET_A$ -selective antagonists may display dual antagonistic properties for  $ET_A/ET_B$ .

#### *Side effects*

The side effects linked to this class of therapeutics, mainly studied for sitaxsentan (**1**),<sup>42</sup> bosentan (**2**)<sup>43,44</sup> and ambrisentan (**3**),<sup>45</sup> are dual, either drug-related side effects, such as drug-drug interaction or liver toxicity, or target-related, such as fluid retention or teratogenicity.

Liver toxicity is the major side effect of endothelin-receptor antagonists, involving two main biological processes. Endothelin-receptor antagonists inhibit the canalicular bile salt export pump which translate in a dose-dependent increase of liver enzymes, leading to dose adjustment or discontinuation of the treatment. For **2**,<sup>43,44</sup> elevation of transaminases is observed in approximately 10% of patients under the standard 125-mg bid regimen. In contrast to **1**, elevation of liver enzymes is usually reversible when **2** is discontinued. The regulatory agencies recommend a monthly control of liver enzymes for patients treated with **2**. Liver toxicity appears to be less frequent with **3**,<sup>45</sup> occurring in 1 to 5% of patients. A more severe and sometimes irreversible liver toxicity is also encountered; its severity and incidence vary, however, with each compound; after three cases of irreversible and fatal liver failure under **1**,<sup>42</sup> published in 2009 and 2010, Pfizer decided to withdraw this drug from the market in 2010. Another common side effect and problematic drawback of endothelin-receptor antagonists is fluid retention. This side effect is mediated by the ET<sub>A</sub> receptor and is due to a direct effect of this class of drugs on the renal collecting duct. It is partially related to the vascular action of the drugs and does not arise from alterations in cardiac function, according to animal studies. This side effect is more frequent with **2**, occurring in 10 to 20% of cases, depending on the daily dose. Older patients are more likely than younger patients to suffer from this side effect. All endothelin-receptor antagonists are teratogenic; therefore, administration to women with child-bearing potential should be accompanied by expert contraception counseling. Drug interactions may also occur. Compound **2** is metabolized by cytochrome P450, CYP2C9 and CYP3A4, and numerous drug interactions have been described. Among the most clinically relevant are a decrease in estrogens and progesterone levels for oral contraception, a decrease in sildenafil levels with concomitant administration, a rise in **2** blood levels with HIV-protease inhibitors, a decrease in the efficacy of anticoagulation with anti-vitamin K agents such as warfarin. Drug interactions are less severe

and less prevalent with **3** and macitentan (**4**).<sup>46,47</sup>

*Molecules developed for therapeutic purposes*

Several previous reviews have described the clinical characteristics of endothelin-receptor antagonists, the “sentan” family of drugs<sup>24-28,48</sup>. Thus, this information will not be discussed in detail in this Perspective. Only brief comments concerning some selected compounds are provided here, in particular concerning the biochemical characteristics of the most recently approved dual ET<sub>A</sub>/ET<sub>B</sub> endothelin-receptor antagonist **4**. The detailed clinical information is provided in the dedicated sections below. Several ET<sub>A</sub>-specific endothelin-receptor antagonists have reached human clinical evaluation for diseases such as PAH, cancer or kidney diseases,<sup>49-52</sup> including **1**, **3**, atrasentan (**5**),<sup>24,53</sup> clazosentan (**6**),<sup>54</sup> zibosentan (**7**),<sup>27,55</sup> which are discussed below.

**Table 1: ET<sub>A</sub>-selective antagonists.**

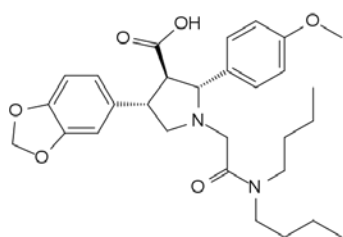
<i>name</i>	<i>structure</i>	<i>comment</i>	<i>company</i>	<i>reference</i>
<b>1</b> <i>sitaxsentan</i> <i>Thelin</i>	The chemical structure of sitaxsentan (1) features a central benzene ring with a 1,3-dioxolane ring fused at the 1 and 2 positions. A propyl chain is attached to the benzene ring at the 4-position, which is further substituted with a thiazole ring. The thiazole ring is linked via a sulfonamide group (-SO <sub>2</sub> NH-) to a pyridine ring substituted with a chlorine atom and a methyl group.	ET <sub>A</sub> /ET <sub>B</sub> : 6000x	Pfizer	42
<b>3</b> <i>ambrisentan</i> <i>Letairis (USA)</i> <i>Volibris (EU)</i> <i>(LU-208075)</i>	The chemical structure of ambrisentan (3) consists of a central carbon atom bonded to two phenyl rings, a methoxy group (-OCH <sub>3</sub> ), and a carboxylic acid group (-COOH). This central carbon is also bonded to a chiral center (indicated by a wedge bond) which is part of a pyridine ring substituted with a methyl group.		Gilead/ GlaxoSmithKlein	45

---

**5** *atrasentan*

*ABT-627*

*Xinlay*



IC<sub>50</sub>: 0.055 nM

Abbott

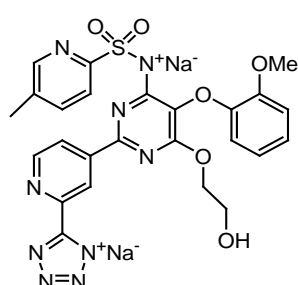
24,53

---

**6** *clazosentan*

*RO-61-1790*

*AXV-034343*



ET<sub>A</sub>/ET<sub>B</sub>: 1000x

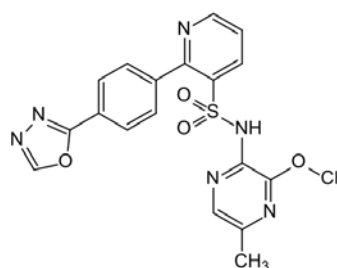
Actelion

54

---

**7** *zibotentan*

*ZD4054*

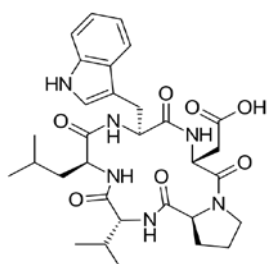


AstraZeneca

27,55

---

**8** *BQ123*

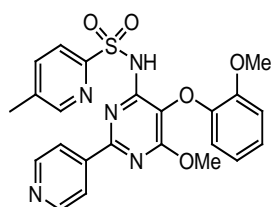


56

---

**9** *avosentan*

*SPP301*



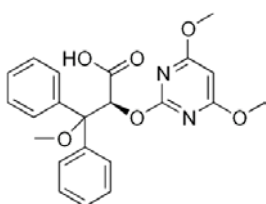
Speedel

57

---

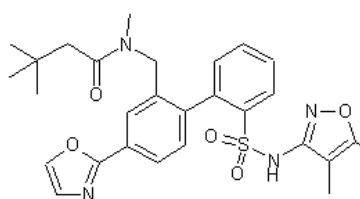
**10** *darusentan*dual/ET<sub>A</sub>-selective    Gilad

58

*LU-135252**HMR-4005***11** *edonentan*

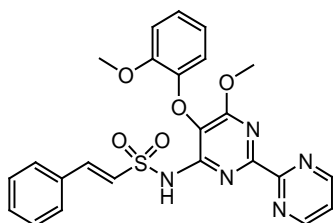
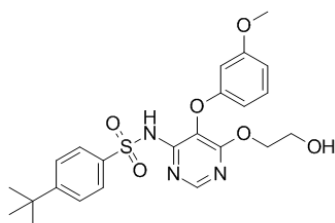
Bristol-Myers-Squibb

59

*BMS 207940***12** *nebentan*

Astellas Pharma

60

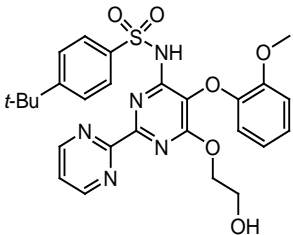
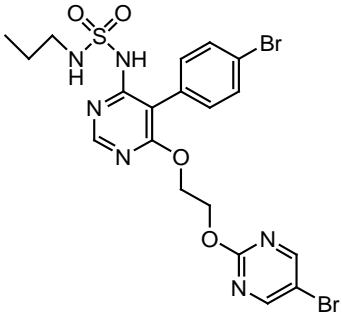
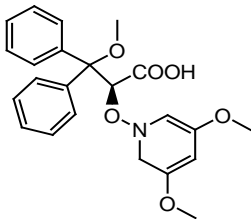
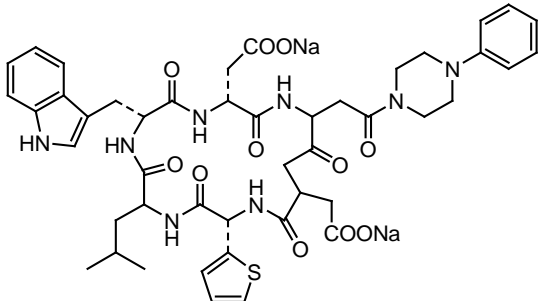
*YM-598***13** *RO-462005*ET<sub>A</sub>/ET<sub>B</sub>: 1000x Hoffmann-La Roche <sup>61,62</sup>

The dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **2**<sup>43,44</sup> was the first molecule approved for clinical use;



however, clinical trials rapidly revealed that **2** tended to induce liver toxicity. Structural modification of **2** produced **4**,<sup>46,47</sup> which has improved an toxicity profile and biological characteristics and is presently approved for clinical treatment of PAH.<sup>47,48,63,64</sup> Minimal accumulation of **4** in the liver suggests that it will be less prone to drug-drug interaction than **2**, an advantage for combination therapies necessary in cancer treatment.<sup>65</sup> The interaction profile of **4** *in vitro* has shown it is also an inhibitor of the drug-resistance molecule P-Glycoprotein (P-Gp), as well as of other proteins involved in cancer chemoresistance, which may enhance its anti-cancer properties, in particular for multidrug-resistant cancers (see below). A comparison of the biochemical characteristics of **2** and **4** is also of interest. Molecular-homology docking of **2** on ET<sub>B</sub> showed the involvement and importance of Arg82, Arg84 and His197 in hydrogen bonding between the antagonist and ET<sub>B</sub>.<sup>66</sup> Compound **4** shows slow apparent ET<sub>A</sub>-receptor association kinetics, consistent with a competitive mode. **2** and structurally related molecules are characterized by fast dissociation kinetics and very short (~1 min) occupancy half-lives at ET<sub>A</sub>, whereas **4** and structurally related molecules display a 20-fold slower dissociation half-life, resulting in insurmountable antagonism. The interaction of **4** with ET<sub>A</sub> depends predominantly on hydrophobic interaction, not on charge-charge interactions, and the tightly packed conformation adopted by **4** fits into a defined pocket of ET<sub>A</sub>, in contrast to the non-compacted structure of **2**. Mutagenesis studies showed that Arg326 and Ile355 are important for **2** but not **4**, binding to ET<sub>A</sub>. The opposite is true for Leu322.<sup>67,68</sup>

**Table 2: dual  $ET_A/ET_B$  antagonists.**

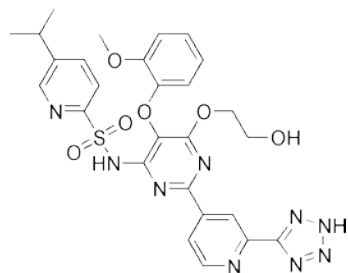
<i>name</i>	<i>structure</i>	$IC_{50}/ET_A/ET_B$	<i>company</i>	<i>reference</i>
<b>2</b> bosentan <i>Tracleer</i> RO470203		$ET_A$ : 45 nM $ET_B$ : 202 nM	Actelion	43,44
<b>4</b> macitentan <i>Opsumit</i>		$ET_A$ : 0.5 nM $ET_B$ : 391 nM	Actelion	46,47
<b>10</b> darusentan LU-135252 HMR-4005		dual/ $ET_A$ -selective	Gilead	58
<b>14</b> TAK-044			Takeda	69

---

**15** *tezosentan*

Actelion

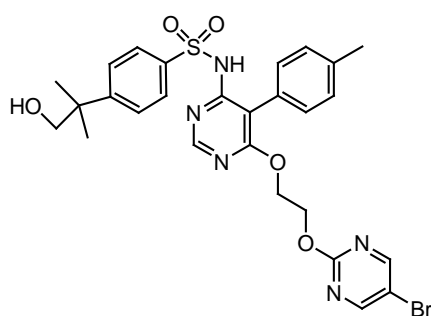
70



---

**16** *T-0201*ET<sub>A</sub>: 0.4 nM Tanabe

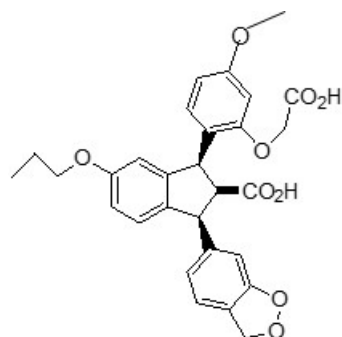
71

ET<sub>B</sub>: 12 nM

---

**17** *SB209670*

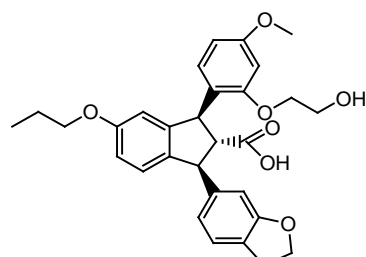
72



---

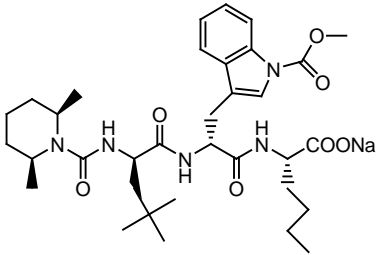
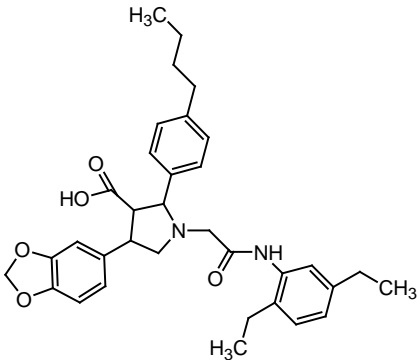
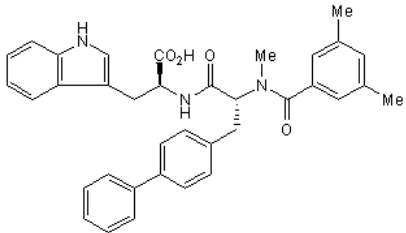
**18** *enrasentan*

73

*SB217242*

ET<sub>B</sub>-selective antagonists, exemplified by BQ788<sup>23</sup> (**19**), have mostly been used in preclinical cellular and animal experimental models.

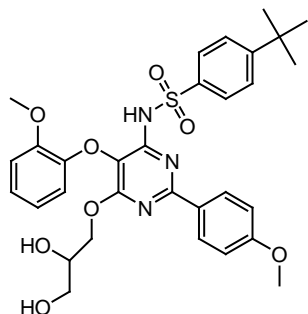
**Table 3: ET<sub>B</sub>-selective antagonists.**

<i>name</i>	<i>structure</i>	<i>IC<sub>50</sub>: ET<sub>A</sub>/ET<sub>B</sub></i>	<i>reference</i>
<b>19</b> BQ788		ET <sub>B</sub> : 1.2 nM ET <sub>A</sub> : 1300 nM	23
<b>20</b> A192621			74
<b>21</b> IRL2500		ET <sub>A</sub> : 94 nM ET <sub>B</sub> : 1.3 nM	75
<b>22</b> RES7011	peptide-based molecule		76

*cyclo(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp*

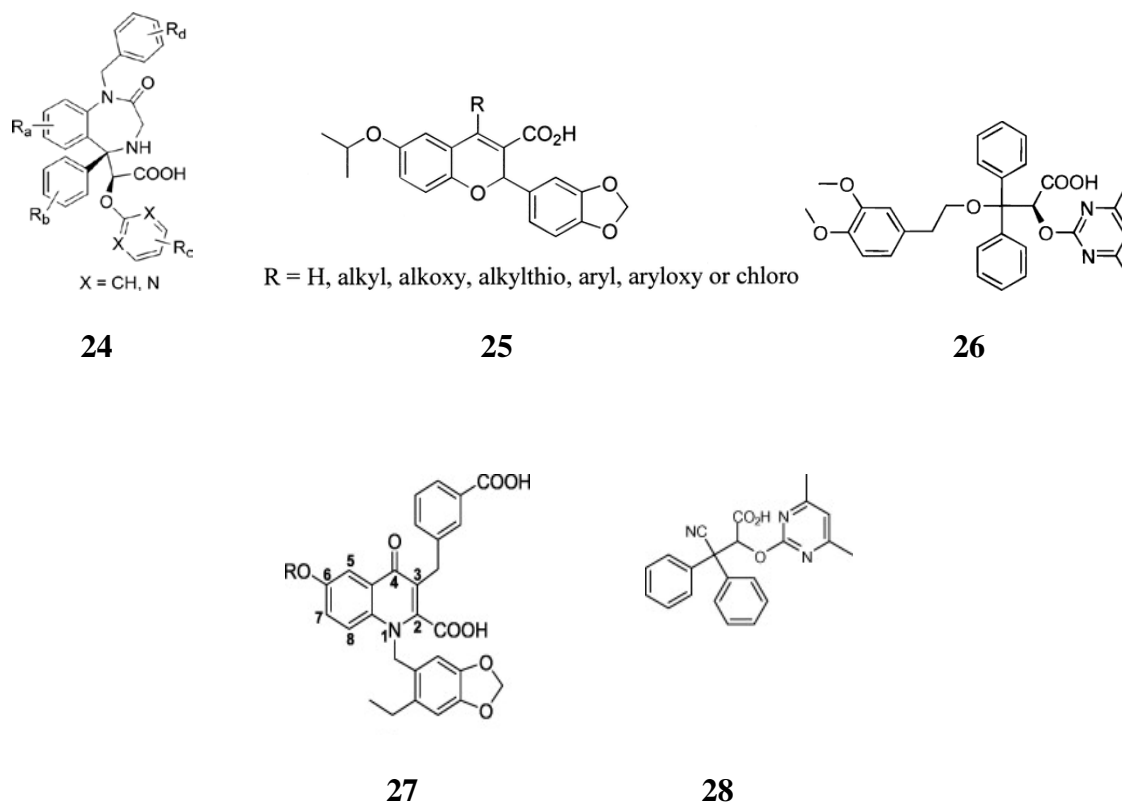
23RO-468443

77



Inspired by the structure of the molecules developed early in experimentation, novel series of endothelin-receptor antagonists have been designed and evaluated. A few examples are shown in **Scheme 1**. Compound **24**<sup>78</sup>: this series is based on a 1,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4] diazepin-2-one scaffold producing derivatives that are potent dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists with affinities in the low-nanomolar range. Compound **25**<sup>79</sup>: this series of ET<sub>A</sub>-selective receptor antagonists has a 2*H*-chromene skeleton and is based on a series of angiotensin-II receptor 1 (AT1) antagonists. The most potent compound binds to the ET<sub>A</sub> with an IC<sub>50</sub> value of 0.19 nM and is 630-fold selective for ET<sub>A</sub> compared to ET<sub>B</sub>. Compound **26**<sup>80</sup>: based on the ET<sub>A</sub>-selective antagonist (*S*)-3-methoxy-2-(4,6-dimethoxypyrimidin-2-yloxy)-3,3-diphenyl propionic acid (LU 135252); structural modifications resulted in the antagonist (*S*)-3-[2-(3,4-dimethoxyphenyl)ethoxy]-2-(4,6-dimethylpyrimidin-2-yloxy)-3,3-diphenyl-propionic acid (LU 302872), with a *K<sub>i</sub>* = 2.15 nM for ET<sub>A</sub> and a *K<sub>i</sub>* = 4.75 nM for ET<sub>B</sub>. Compound **27**<sup>81</sup>: a series of 1,3,6-trisubstituted-4-oxo-1,4-dihydroquinoline-2-carboxylic acid analogs were prepared as ET<sub>A</sub>-selective antagonists, with the most potent displaying an IC<sub>50</sub> = 0.11 nM and a ET<sub>B</sub>/ET<sub>A</sub> selectivity of > 8000x. Compound **28**<sup>82</sup>: a series of novel 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3,3-diphenyl butyric-acid derivatives were synthesized and

evaluated, with the most potent displaying potency comparable to that of **2**.



**Scheme 1.** Examples of endothelin-receptor antagonists based on chemical modifications of previously developed compounds.

Approaches other than modifying previously developed compounds have also been attempted. For example, inhibition of ET-1 precursor activation by ECE-1 (EC 3.4.24.71) may be an alternative to using receptor antagonists. A series of non-peptidic thiol-based ECE-1 inhibitors were able to inhibit DNA synthesis in human GBM cells. This effect was not reversed by exogenous addition of ET-1, suggesting an intracellular effect of ECE-1 inhibitors not involving extracellular production of ET-1.<sup>13</sup> Receptor-specific antibodies may also represent an alternative to synthetic compounds as endothelin-receptor antagonists. In melanoma cell lines, the binding characteristics of a monoclonal antibody (mAb) developed against tumor-specific epitopes of ET<sub>B</sub>, possibly N-terminal truncation at Arg64-Ser65 by

ligand-induced hydrolysis by a metalloprotease, suggest other therapeutic possibilities. This hydrolytic truncation was also shown to abolish ET<sub>B</sub>-mediated EGFR transactivation.<sup>83</sup>

## CLINICAL TRIALS

### **Clinical trials of endothelin-receptor(s) antagonists in human diseases.**

#### *Endothelin-receptor antagonists in cardiovascular diseases other than PAH*

We have previously reviewed in detail clinical trials performed before 2009.<sup>84</sup> In this section, therefore, we will summarize only the main results of these early trials. We will present the more recent trials, in particular clinical trials for PAH and development of the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **4** for PAH, cancer and fibrosis, in more detail. Antagonists of ET<sub>A</sub> or ET<sub>B</sub> and inhibitors of ECE-1 were initially developed for the treatment of cardiovascular diseases. The first antagonists to be tested in healthy human volunteers and patients with chronic heart failure (CHF) were peptidomimetics, the ET<sub>A</sub>-selective BQ123<sup>56</sup> (**8**) and the ET<sub>B</sub>-selective BQ788<sup>23</sup> (**19**). The usual problems of peptide-like molecules, such as poor bioavailability and susceptibility to proteolytic enzymes, arose, but these trials were very useful in defining the physiological functions of ET-1 in health and disease. The information gained<sup>85,86</sup> suggested that ET-1 maintains vascular tone via basal activation of ET<sub>A</sub> on vascular smooth-muscle cells, whereas ET<sub>B</sub> activation reduces basal vascular tone and vasodilatation, which are mediated by nitric oxide (NO) generation in the endothelium. Patients with hypertension responded to infusion of **8** and **19** with more vasodilatation than did healthy subjects. Additionally, ET<sub>A</sub>-selective antagonists, but not dual ET<sub>A</sub>/ET<sub>B</sub> antagonists, improved hypertension. In animal models, dual or ET<sub>A</sub>-selective antagonism ameliorated left-ventricular functions, prevented ventricular remodeling and resulted in prolonged survival, properties not shared by ET<sub>B</sub> antagonists. Altogether, the information obtained from these early molecules suggested an advantage in the treatment of

cardiovascular disease to the development of non-peptidomimetic antagonists to modulate the functions of ET-1 receptors. Therefore, low-molecular-weight synthetic ET<sub>A</sub>-selective or dual ET<sub>A</sub>/ET<sub>B</sub> antagonists, but only very few ET<sub>B</sub>-selective antagonists, were developed and evaluated in clinical trials. From the published results of these early clinical trials, it can be inferred that the endothelin axis, in addition to regulating vascular tone, may also promote vascular-cell migration, proliferation, differentiation and/or growth. In patients with CHF, ET<sub>A</sub> blockade was more favorable than ET<sub>B</sub> blockade; however, in the long-term, a lack of beneficial effects of endothelin-receptor antagonists in heart failure mortality/morbidity was observed.<sup>87</sup> The results of these trials did not meet expectations for CHF patients; however, some encouraging information was obtained for patients with primary and secondary PAH.

Due to its inherent ability to produce potent and prolonged vasoconstriction, ET-1 has been postulated to be a critical mediator of cerebral vasospasm. A phase-IIa trial conducted with the ET<sub>A</sub> antagonist **6** demonstrated a benefit for patients in terms of the severity and incidence of vasospasm. ET<sub>B</sub> antagonism, however, may be detrimental due to inhibition of ET<sub>B</sub>-mediated NO production, as was observed for the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist TAK-044 (**14**).<sup>69</sup>

#### *Endothelin-receptor antagonists for the treatment of PAH*

PAH is a pathological lung condition arising from multiple etiological causes leading to vascular remodeling, an abnormal thickening of the arterial wall and an increase in pulmonary vascular resistance. The hemodynamic consequences of PAH are an increased afterload for the right ventricle and, eventually, right heart failure and ultimately death. When untreated, PAH has a grim prognosis, with a median survival of approximately two to four years from diagnosis. The basic defect in the pathogenesis of PAH is thought to be an endothelial dysfunction with impaired paracrine signaling from endothelial to smooth-muscle cells.<sup>88</sup> A number of biological systems have been implicated, including increased ET-1 expression in vascular endothelial cells from human pulmonary arteries.<sup>88,89</sup> Three therapeutics are



presently registered with the FDA (US Federal Drug Administration) and/or the EMA (European Medicines Agency) for the treatment of PAH. The first endothelin-receptor antagonist, the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **2**<sup>44</sup> was approved in USA in 2001 and Europe in 2002. Then the ET<sub>A</sub>-selective antagonists **1**<sup>42</sup> and **3**<sup>45</sup> were approved, respectively, in 2006 by EMA and in 2007 by FDA, and in 2008 only by EMA, followed by the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **4**,<sup>46,47</sup> approved by both institutions in 2013. Compound **1** has been withdrawn by Pfizer in 2010.

The potential advantage of selective ET<sub>A</sub> antagonism over dual ET<sub>A</sub>/ET<sub>B</sub> blockade remains controversial in the field of PAH.<sup>90</sup> Physiologically, ET<sub>B</sub> mediates vasodilatation through the NO pathway, and it may appear counterproductive to inhibit that pathway; however, in pathological states, cross-talk between ET<sub>A</sub> and ET<sub>B</sub> may occur with heterodimerization of both types of receptors. Under these conditions, ET<sub>B</sub> may also mediate vasoconstriction<sup>91</sup> and proliferation of smooth-muscle cells.<sup>92</sup> As there has been to date no direct comparison of both classes of antagonists in a randomized and blinded trial, there is no formal evidence for clinical use. While ET-1 receptor antagonists have been demonstrated to be clinically useful in PAH, their use as a monotherapy is often insufficient to lastingly relieve symptoms in affected patients, suggesting the value of combination-therapy regimens, which are presently under evaluation.

For the present review, the databases PubMed ([www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed)), the NIH registry for clinical trials ([clinicaltrials.gov](http://clinicaltrials.gov)), the WHO international clinical trials platform (<http://apps.who.int/trialsearch>) and reviews from the last five years<sup>93-95</sup> were consulted. In particular, the systematic review by the Cochrane collaboration published in 2013<sup>96</sup> remains of high interest, as it included and analyzed all but three trials available to date. Fourteen studies were identified according to the search criteria: four for **3**, six for **2**, three for **1** and one for **4**. These studies are summarized in **Table 4**.

**Table 4.** Clinical trials of endothelin-receptor antagonists for patients with PAH.

<i>Drug</i>	<i>dose</i> [mg]	<i>code trial</i>	<i>comparator</i>	<i>duration</i> [weeks]	<i>patients</i>	<i>outcome</i>
<b>1</b>	100/300	STRIDE-1 <sup>97</sup>	placebo	12	untreated	increased peak VO <sub>2</sub>
	50/100	STRIDE-2 <sup>98</sup>	placebo <b>2</b>	18	untreated	improved 6-MDW 6-MWD neutral
				18	untreated	
50/100	STRIDE-4 <sup>99</sup>	placebo	18	untreated	worsened 6-MWD	
<b>2</b>	125/250	ASSET-1 <sup>100</sup>	placebo	16	untreated	improved PVR
	125	Study-351 <sup>101</sup>	placebo	12	untreated	improved 6-MWD
	125/250	BREATHE-1 <sup>44</sup>	placebo	16	untreated	improved 6-MWD
	125 bid	BREATHE-2 <sup>102</sup>	epoprostanol	16	untreated	Neutral PVR
	125 bid	BREATHE-5 <sup>103</sup>	placebo	16	untreated	improved PVR
	125 bid	COMPASS-2 <sup>104</sup>	placebo	>16	sildenafil	neutral TCW
	125 bid	EARLY <sup>105</sup>		24	untreated NYHA II	decreased PVR neutral 6-MWD
	125 bid	SERAPH <sup>106</sup>	sildenafil	16	untreated	no arm difference
<b>3</b>	5/10	ARIES-1 <sup>107</sup>	placebo	12	untreated	improved 6-MWD
	2.5/5	ARIES-2 <sup>107</sup>	placebo	16	untreated	improved 6-MWD
	5	ATHENA-1	none and	24	PDEVi	improved 6-MWD
	10	AMBITION <sup>108</sup>	sildenafil	≥24	untreated	no arm difference in TCW
<b>4</b>	3/10	SERAPHIN <sup>47</sup>	placebo	115	untreated	improved TCW
	10		placebo	115	PDEVi or prost.	improved TCW

---

6-MWD: 6 min walking distance; PVR: pulmonary vascular resistance; Peak VO<sub>2</sub>: maximal oxygen consumption on exercise; TCW: time to clinical worsening; PDEVi: phosphodiesterase V inhibitor; RV: right ventricle; Prost: prostanoids.

In previously untreated patients, all studies but one yielded a positive primary outcome. The earlier trials were of short duration (12-16 weeks) and used mainly the distance achieved during the six-minute walking test (6-MWD) as the primary outcome.<sup>109,110</sup> Only the STRIDE-4 study yielded a negative result in patients treated with 50 mg **1**. The gain was also modest in the SERAPHIN study, but 63% of the patients included in this study had already been treated, mostly with phosphodiesterase-V (PDEV) inhibitors. The short duration of most studies was also criticized by experts and regulatory agencies.<sup>111</sup> For these reasons, more recent trials have been designed with a new composite primary index: time to clinical worsening (TCW). The two most recent studies that applied this new primary outcome (SERAPHIN and AMBITION) were also of longer duration and included significantly more patients than the earlier studies. Most studies have published data on the improvement in symptoms and mortality (Reveal registry).<sup>112</sup> Overall, one half to two thirds of patients did not worsen, while the remaining improved.

The most recently approved antagonist **4** has been studied in SERAPHIN,<sup>47</sup> a multicenter, double-blind, placebo-controlled, parallel-group, event-driven, phase-III outcome study involving 742 patients with symptomatic PAH randomized to three treatment groups (placebo, 3 mg or 10 mg **4** once daily) to assess the long-term effects on morbidity and mortality. Treatment with 10 mg **4** resulted in a sustained 45% relative risk reduction early in treatment. The efficacy of 10 mg **4** on the primary endpoint was consistent across subgroups of age, sex, ethnic origin, geographical region, etiology, monotherapy and combination with

another PAH therapy. The majority of adverse reactions were mild to moderate in intensity. Therefore, **4** was approved in 2013 by the FDA and the EU Commission as an orally available dual ET<sub>A</sub>/ET<sub>B</sub> antagonist for the treatment of PAH.

While earlier studies compared essentially single-drug regimens with a placebo, three more recent trials included patients already treated with a drug of another therapeutic class, such as PDEV inhibitors or prostanoids (**Table 4**). Not surprisingly, positive results are more difficult to demonstrate for this group than for treatment-naïve patients. Only the study with **4** 10 mg which included a large number of patients for an extended period of time was able to show a benefit of adding an endothelin-receptor antagonist to a preexisting PAH therapy.<sup>47</sup> The design of the AMBITION study was different, as **3** and tadalafil were introduced together, not sequentially. This simultaneous double therapy proved superior to single-drug therapy in terms of TCW.<sup>108</sup> Based on this latter study, the most recent guidelines advocate the concurrent use of a dual-drug regimen while also offering the possibility of starting with a single-drug regimen, as not every drug combination has been tested in randomized clinical trials (RCT).<sup>113</sup>

It comes as no surprise that pharmaceutical companies have avoided direct comparison of their product with other available drugs; only very few investigator-driven comparative studies are available. It was not the design of the AMBITION study to compare the two single-drug arms (tadalafil and **3**). Nevertheless, the TCW was similar in these two groups in patients of comparable PAH severity (**Table 4**). Another study has compared two endothelin-receptor antagonists, **1** and **2**.<sup>98</sup> No difference was found in the 6-MWD, the primary outcome of this trial. Overall, no striking difference in efficacy was observed among the four endothelin-receptor antagonists tested in PAH.

None of the fourteen RCT performed with endothelin-receptor antagonists in PAH had death as the primary outcome; however, deaths were duly recorded in every study. These data

showed a trend for less deaths in patients treated with endothelin-receptor antagonists compared to placebo.<sup>112</sup> The Cochrane review published in 2013 concluded that “there is uncertainty as to whether endothelin-receptor antagonists reduce mortality in this population”.<sup>96</sup> The two most recent trials, SERAPHIN and AMBITION, analyzed morbidity and mortality in PAH; however, as non-fatal events define the end of study for the participant, it is logical that mortality was not different between the treated and placebo arms in both studies.<sup>47,108</sup> Finally, as more than eight substances have proven efficacy for PAH it is becoming unethical to perform placebo-controlled studies, especially with mortality as the major endpoint.

In conclusion, the three clinically available oral endothelin-receptor antagonists are considered as first-line therapies for PAH functional class II and III, intravenous epoprostenol being the first choice for patients in class IV.<sup>113</sup> According to the AMBITION study, concurrent therapy with **3** and tadalafil is also recommended as a first-line treatment. In case of unsatisfactory response to a single-drug regimen, sequential use of a double- or triple-drug regimen is proposed.

Several studies with **3** and **4** are presently ongoing (**Table 5**). These trials address mainly specific subtypes of PAH, such as Eisenmenger syndrome or portopulmonary hypertension. In addition, some trials explore the benefit of endothelin-receptor antagonists in other PAH groups such as PAH due to left heart disease or inoperable chronic thromboembolic pulmonary hypertension.<sup>114</sup>

**Table 5.** Ongoing studies of endothelin-receptor antagonists.

<i>study and NCT No</i>	<i>drug</i>	<i>target</i>	<i>primary outcome</i>
ATPAHSS	<b>3</b> , 10 mg	Scleroderma-associated	6-MWD
NCT01042158	+/- sildenafil	PAH	Published 09.2015

NCT02253394	<b>3</b> , 10 mg +/- spironolactone	PAH	6-MWD
MAESTRO NCT01743001	<b>4</b> , 10 mg	Eisenmenger syndrome	6-MWD
MELODY NCT02070991	<b>4</b> , 10 mg	Pulmonary hypertension due to left heart disease	Hemodynamics
MERIT NCT02060721	<b>4</b> , 10 mg	Chronic thromboembolic pulmonary hypertension	6-MWD
PORTICO NCT02382016	<b>4</b> , 10 mg	portopulmonary hypertension	PVR
SCOBA-PH) NCT01330108	Switch <b>2</b> , to <b>3</b> , 10 mg	PAH	Safety, Six-MWD

6-MWD: 6 min walking distance; PVR: pulmonary vascular resistance.

In summary, endothelin-receptor antagonists of *both* ET<sub>A</sub>-selective and dual ET<sub>A</sub>/ET<sub>B</sub> subtypes have shown clinical interest and benefit for patients with PAH; however, even if these therapeutics can delay disease progression, they are not curative.

### ***The functions of the endothelin axis beyond PAH: cancer***

As stated above, ET-1 beside being a potent vasoconstrictor is also an anti-apoptotic and mitogenic peptide whose expression, as well as that of the components of the whole endothelin axis, is largely increased in human cancers.<sup>115-119</sup> Cancer is a proliferative disorder of the cancer cells themselves, as well as of the cancer-associated stromal cells, mainly endothelial cells and fibroblasts. The transformation of fibroblasts into myofibroblasts is a characteristic of not only cancer but also of fibrogenic diseases. In human cancers, depending

on the relative expression of ET<sub>A</sub> and ET<sub>B</sub>, the endothelin axis may act as a growth-promoting or an apoptosis-promoting effector. The main source of ET-1 is the tumor-associated endothelium, whereas ET-1 production by human tumor cell lines has also been demonstrated.<sup>120</sup> Using *ex vivo*, *in vivo* and *in vitro* approaches, the expression of ET-1, ECE-1<sub>a-d</sub>, ET<sub>A</sub> and ET<sub>B</sub> was determined in human prostate, ovarian, breast, cervix, oral, lung and colon carcinoma, GBM, melanoma and Kaposi sarcoma, and the relevant human tumor cells in culture.<sup>13,39,115,116,121-128</sup> In these human cancers, ETs were survival factors, protecting tumor cells against apoptosis.<sup>115,116,128,129</sup> From these data, ET-1, ET<sub>A</sub> and/or ET<sub>B</sub>-receptors have been proposed to be involved in cancer progression. In human cancer samples it was generally observed that the ET-1 precursors, ECE-1 and the ET<sub>A</sub> and/or ET<sub>B</sub> receptors were also expressed by the tumor-associated stroma. Thus, in human cancers, depending on the type of cancer, the main source of ETs may be either the cancer cells, mainly in carcinomas, or the cancer-associated stroma, including the tumor-associated vasculature, in non-carcinoma tumors. Published data indicate that the main endothelin receptor implicated in human carcinomas is the ET<sub>A</sub> receptor, whereas in human GBM and melanoma, it is the ET<sub>B</sub>-receptors. The role and the mechanisms of ET-1-mediated effects in cancer have been mainly studied in preclinical models and have been previously reviewed in detail by others.<sup>123-127,130-132</sup> Thus, we will not repeat the details of these previous reviews. Only some information discussed in previous reviews will be outlined here.

Preclinical cellular and animal experimental models have implicated all components of the endothelin axis in cancer development and progression. ET-1 is a direct cell-proliferation and cell-survival factor for cancer cells and is involved in the transactivation of other tumor-cell receptors, mainly the EGF receptor. ET-1 also promotes cancer-cell epithelial-to-mesenchymal transition (EMT), migration, invasion and metastasis, and resistance to chemotherapy; however, ET-1 is also involved in the interactions between tumor cells and

their environment. ET-1 induces tumor-associated angiogenesis, increasing the release of VEGF and stimulating (myo)fibroblasts and cancer cells to produce pro-angiogenic proteases. ET-1 mediates the recruitment, proliferation and differentiation of fibroblasts into tumor-associated myofibroblasts, and the interaction of cancer cells with inflammatory/immune cells, pro-tumoral macrophages (type-2 macrophages) and anti-tumoral T-lymphocytes. ET-1 also upregulates pro-fibrotic growth factors, contraction- and migration-enhancing factors, thus favoring the development of tumor stroma.<sup>133</sup>

Most *preclinical* studies of endothelin-receptor antagonists have been conducted in experimental models of ovarian and prostate carcinomas.<sup>121,123,124,127,132,134</sup> In epithelial ovarian cancer cells, a link between an autocrine ET-1/ET<sub>A</sub> pathway, EGFR and beta-catenin-beta arrestin-mediated transcriptional activity, EMT, chemoresistance and possibly metastasis was proposed.<sup>135-138</sup> ET-1 via ET<sub>A</sub> transactivates EGF receptors in ovarian cancer cells, and combination of the ET<sub>A</sub> antagonist **7** with an EGFR antagonist (gefitinib) strongly reduced proliferation and invasion of ovarian tumor cells. In an experimental model of multidrug-resistant (taxol or cisplatinum) ovarian tumors, **4** in combination, but not alone, was highly anti-tumoral, reversing drug resistance, suggesting that drug combination should be considered in cancer therapy.<sup>139</sup> In a preclinical study of colon cancer, the specific ET<sub>A</sub> antagonist **7** inhibited cell proliferation, but cell migration was more inhibited by an ET<sub>B</sub> antagonist than by an ET<sub>A</sub> antagonist and cell contraction was more inhibited by combined ET<sub>A</sub> and ET<sub>B</sub> blockage; however, it has to be emphasized that *in vitro*, the concentration of the antagonists necessary to induce or potentiate apoptosis in cancer cells was much higher than the concentrations effective in the context of cardiovascular diseases. These concentration-dependent effects resemble the effects observed when modifications were introduced in the transmembrane domain 3 and cytoplasmic domain 2 of ET-receptors using either mutagenesis or chimeric receptors. Alternatively, the localization of the endothelin



receptor(s) is intracellular in tumor cells, rendering them poorly accessible to the antagonists. Another explanation may be that endothelin-receptor antagonists are substrates for ATP-dependent membrane transporters involved in drug resistance, such as P-Gp.

However, most of these preclinical data, in particular the expression of mainly ET<sub>A</sub> by tumor cells, were established using experimental models of *epithelial* tumors, the so-called “carcinomas”. It was realized only recently that the picture may be different in non-carcinoma cancers, the “mesenchymal” tumors. In human non-carcinoma cancer, the pattern of expression of endothelin receptors is more complex. For example, we observed that in human GBM surgical samples, ET<sub>A</sub> receptors were mostly expressed by blood vessels, while ET<sub>B</sub> receptors were mainly expressed by the tumor cells. The pro-survival functions of ET-1 for GBM and melanoma cells in culture were exerted via the ET<sub>B</sub> receptor.<sup>13,116,140</sup> In GBM cells in culture, exogenous ET-1 enhanced tumor-cell migration and MMP-9 and MMP-13 expression.<sup>141</sup> ET-3 is mainly expressed in the central nervous system and has been involved via ET<sub>B</sub> in the progression of GBM via an autocrine pathway in GBM stem cells able to maintain their stemness-associated properties. Compound **19**, A192621<sup>74</sup> (**20**, **Table 3**) and ET-3 RNA interference blocked growth and induced GBM stem-cell apoptosis. Thus, ET<sub>B</sub> antagonists may be valuable in GBM and oligodendroglioma therapy.<sup>142-144</sup>

Interaction with cancer-associated stroma has also been studied. ET<sub>A</sub> is critical in mediating immune-cell recruitment and T-cell-mediated antitumor activity, while ET<sub>B</sub> is a barrier to this recruitment.<sup>139</sup> Neither **4** nor the combination of **8** and **19** modified immune-cell recruitment to cancer.<sup>139</sup> In an experimental model of breast cancer, macrophages were shown to induce the endothelin-integrin axis in endothelial cells, favoring the adhesion and migration of breast tumor cells across the vascular wall, the early steps of metastasis.<sup>145</sup> ET-1 may stimulate the growth of fibroblasts and it was shown that in co-culture of ovarian carcinoma cells and fibroblasts the growth of both populations was reciprocally stimulated.<sup>146</sup> The high rate of

binding of ET-1 to (myo)fibroblasts, implicates these cells in tumor progression and ET-1 receptors as potential therapeutic targets.<sup>147</sup> Induction and overexpression of ET<sub>B</sub> receptors was observed in colon cancer-associated (myo)fibroblasts and endothelial cells.<sup>115,148</sup> These observations were later confirmed in different human cancers, suggesting that tumor cells can induce ET<sub>B</sub> in their stroma. These stroma-expressed ET<sub>B</sub> can participate in the progression of cancer and be relevant targets for therapeutic purposes. In brain cancers, expression of ET<sub>A</sub> was demonstrated in human gliomas and meningiomas, with high affinity for ET<sub>A</sub> antagonists. In human surgical samples from patients with GBM, ET-1, ECE-1 and ET<sub>A</sub> were mainly expressed by GBM neovessels, while ET<sub>B</sub> is mainly expressed by the tumor cells, suggesting that GBM vessels are likely a source of ET-1 acting on cancer cells via ET<sub>B</sub>. ET<sub>A</sub> was also expressed by tumor-associated vessels. Induction of the ET<sub>B</sub>-type receptor in tumor stroma, mainly in reactive astrocytes and in myofibroblasts ensheathing tumor neovessels in GBM,<sup>116</sup> indicated that the endothelin axis is also important in the interactions between non-epithelial human cancers and their stroma. The dual ET<sub>A</sub>/ET<sub>B</sub>-antagonist **2** induced apoptosis in human GBM cells. ET-1 via ET<sub>B</sub> is a survival factor but not a proliferation factor for GBM cells.<sup>111</sup> Meningiomas have been shown to express mainly ET<sub>A</sub>.<sup>149</sup> The ET-1/ET<sub>B</sub> axis was shown to reduce P-Gp functions at the blood-brain barrier (BBB), an important effect for the transport of chemotherapeutics in brain cancer, and other cerebral disorders.<sup>148</sup> Thus, tumor cells can induce ET<sub>B</sub> in their stroma and stromal ET<sub>B</sub> can participate in the progression of brain cancer and be an additional target of the endothelin axis for therapeutic purposes.

The endothelin axis was also involved in maintaining the properties of cancer stem cells (CSC). Compound **4** alone did not show significant anti-tumor activity, while in combination with chemotherapy, **4** demonstrated specific anti-CSC activity. Lack of stromal ET<sub>B</sub> is associated with diminished infiltration of tumor-associated macrophages.<sup>151</sup> Endothelin receptors also play a permissive role in tumor metastasis, the main cause of mortality of

cancer patients. In an experimental murine model, the ET-1/ET<sub>A</sub> axis was shown to enhance lung metastasis of bladder cancer, preceded and depending on macrophage infiltration of the lung. Thus, the pro-inflammatory functions of the ET-1 axis are important in the context of tumor progression.<sup>130</sup>

Antagonists of either ET<sub>A</sub>, ET<sub>B</sub> or the combination of both have involved the couple ET-1/ET<sub>A</sub> in modulating the functions of cancers in many of these preclinical experiments. However, all together the results suggest that in the context of cancer, dual ET<sub>A</sub>/ET<sub>B</sub> antagonism may be superior to single antagonism. Moreover, many of these beneficial effects of blocking the endothelin receptors were observed when the antagonists were applied very early in the development of these experimental tumors.

Other components of the endothelin axis may also be involved in cancer, such as the expression of variants of ECE-1 (ECE-1a, ECE-1b, ECE-1c and ECE-1d), variants of the receptors, or the ET-2 and/or ET-3. peptides Only limited information exists concerning the inhibition of ECE-1 to control the activation of bigET-1 to ET-1.<sup>13</sup> It was shown in a murine model that germ-line mutations and subsequent promoter hypermethylation of the ET<sub>B</sub> gene and the absence of ET<sub>B</sub> transcripts may be responsible for susceptibility to cancer.<sup>152</sup> Promoter methylation of the *EDNRB* gene in gastric cancer and in oral squamous-cell carcinoma is correlated with the aggressiveness and progression of the cancer.<sup>41,153</sup>

In summary, preclinical experimental cancer models and *ex vivo* studies suggest that malignant cell growth and survival are dependent on ET-1-mediated pathways. Endothelin-receptor antagonists demonstrated promising effects in the context of experimental cancer as molecules with the potential to control survival of tumor cells and regulate vascular functions and angiogenesis induced by tumor cells, an important factor in the mechanisms of cancer progression. In cancer, ET-1 is not a growth factor by itself, but it appears to potentiate tumor-cell growth induced by other growth factors such as bFGF, EGF, IGF-I and IGF-II, through a

PKC-mediated pathway, involving several intracellular signaling pathways. Antagonists of the receptors of the endothelin axis have anti-tumor effects in human cells and in animal cancer models, which have been attributed to their direct anti-survival effects on tumor cells and/or on the tumor-associated vasculature. Based on promising preclinical results, clinical trials in human cancers mostly evaluated the effects of antagonists of ET<sub>A</sub>; however, while ET<sub>A</sub> is important in tumor-cell migration, metastasis and proliferation, ET<sub>B</sub> is critical for angiogenesis and inhibition of anti-tumor immune-cell recruitment, and possibly also for tumor growth and metastasis. Thus, dual ET<sub>A</sub>/ET<sub>B</sub> blockade may be a valid strategy, which was not taken into account in the design of the early clinical trials.

#### *Human clinical trials of endothelin-receptor antagonists in cancer*

The encouraging results of cell and animal experimental models of cancer led to the evaluation of the two ET<sub>A</sub> antagonists **5** and **7**<sup>154,155</sup> in several human clinical trials (up to phase III) for ovarian, prostate, breast, colon, lung, or kidney cancer either alone or in combination with cytotoxic drugs. Owing to the observed interactions between endothelin receptors and EGFR, combinations of ET<sub>A</sub> antagonists and EGFR antagonists were also attempted. Unfortunately, these clinical trials did not show significant effects on their primary end-points, defined as time to progression, onset of metastases and/or overall survival. The main conclusions resulting of the most important human clinical trials of endothelin-receptor antagonists in human cancer are summarized in **Table 10** and discussed below.

We have previously reviewed in detail the clinical trials performed for cancer indications before 2009,<sup>84</sup> and others have reviewed in detail the clinical trials performed more recently.<sup>123,125,127,156,157</sup> Therefore, we will only summarize the main outcomes of these early trials. We will present in more detail the most recent trials, in particular the clinical evaluation of the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **4** in GBM.

**Table 10.** Some examples of clinical trials of ET-1-receptor(s) antagonists in cancer

<b>compound</b>	<b>phase</b>	<b>company</b>	<b>disease</b>	<b>end-points</b>
<i>A) ET<sub>A</sub>-selective antagonists</i>				
	<i>5</i>	<i>Abbott</i>	<i>prostate, renal carcinomas, glioma</i>	
<b>5</b>	II (N=288)		hormone-refractory prostate cancer	time to progression bone metastasis
<b>5</b>	III (N=942)		non-metastatic hormone-refractory prostate cancer	time to progression
<b>5</b>	III		metastatic hormone-refractory prostate cancer	time to progression
	<i>7</i>	<i>AstraZeneca</i>	<i>prostate carcinoma</i>	
<b>7</b>	II (N=312)		hormone-resistant bone metastatic prostate cancer	time to progression and survival
<b>7</b>	III (N=312) ENTHUSEMI		hormone-resistant bone metastatic prostate cancer	safety and efficacy and overall survival
<i>B) Dual ET<sub>A</sub>/ET<sub>B</sub> antagonists</i>				
	<i>2 and 4</i>	<i>Actelion</i>	<i>melanoma and GBM</i>	
<b>2</b>	II (N=35) II (N=40)		melanoma	safety/tolerability and disease progression
<b>4</b>			GBM	launched December 2011 completion October 2017. safety and tolerability, markers of brain tumors.

Early clinical trials evaluating the efficacy of endothelin-receptor(s) antagonists in human

cancers mostly concentrated on prostate carcinomas and ET<sub>A</sub>-selective antagonists. ET-1 is produced in the normal prostate, and ET<sub>B</sub> is mainly expressed by prostate luminal epithelial cells, whereas ET<sub>A</sub> is found predominantly in the stromal component of the prostate. ET<sub>A</sub> expression is increased in prostate cancer and is associated with progression and osteoblastic bone metastasis,<sup>122</sup> whereas ET<sub>B</sub> may be silenced by methylation of its promoter. The effect of ET-1 receptor antagonism in prostate cancer has been evaluated, mainly using **5** (a very potent ET<sub>A</sub> antagonist; K<sub>i</sub>=0.034 nM) and **7** (a very selective ET<sub>A</sub> antagonist). Results of these early clinical trials generally demonstrated safety and tolerability but no significant improvement in survival or time to disease progression. For the treatment of castration-resistant prostate cancer, combination therapies based on docetaxel are the standard. Following attempts to add endothelin-receptor antagonists, the results for ET<sub>A</sub> antagonists were initially encouraging in pre-clinical assays and early human clinical trials. Drug combinations have been attempted in a phase-I/II trial of **5** in combination with paclitaxel, docetaxel, carboplatin or liposomal doxorubicin,<sup>156-159</sup> demonstrating no differences in efficacy and patient survival over standard therapeutic regimens but no added toxicity compared to chemotherapeutics. A phase I/II clinical trial of **5** combined with docetaxel in patients with hormone-refractory prostate cancer showed that **5** pharmacokinetics was not influenced by docetaxel, though docetaxel clearance was increased by concomitant administration of **5**, suggesting a pharmacokinetic drug-drug interaction. Compound **5** combined with doxorubicin was evaluated in a phase-I/II trial in patients with platinum-resistant recurrent ovarian cancer. No obvious toxicity was observed, with some indication of prolonged survival in a limited number of patients.<sup>160</sup> Phase-III trials have nevertheless been launched, combining an ET<sub>A</sub> antagonist with standard chemotherapeutics, and the results are now available. Docetaxel and **5** versus docetaxel and placebo was evaluated in men with advanced castration-resistant prostate cancer in a randomized phase-III trial (SWOG 50421).

In combination with docetaxel, **5** did not improve overall survival and disease-free survival compared to placebo. A phase-III, randomized, placebo-controlled study of docetaxel in combination with **7** in patients with metastatic castration-resistant prostate cancer (ENTHUSE trial) did not result in any benefit compared to docetaxel alone.<sup>161</sup> A large phase-III placebo-controlled trial evaluating the use of **7** for bone metastasis in patients with castration-resistant prostate cancer did not show a statistically significant advantage; however, it has an acceptable toxicity profile.<sup>155</sup>

In non-carcinoma cancer, **5** has been evaluated in phase I in patients with recurrent GBM. No dose-limiting toxicities were observed, but its efficacy was not better than those of other cytotoxic regimens.<sup>162</sup> Based on data suggesting that endothelin may play a role in the pathophysiology of melanoma<sup>39,140</sup> and that the dual endothelin-receptor antagonist **2** may have anti-tumor activity in human melanoma cells,<sup>39</sup> a multicenter, open-label, single-arm, prospective, proof-of-concept phase-II study assessed the effects of **2** monotherapy on tumor response in patients with stage-IV metastatic melanoma. Among the 35 patients included in this study, disease stabilization was observed in six of the 32 patients analyzed. These results suggest that **2** might have benefits in disease stabilization in certain patients with metastatic melanoma.<sup>163</sup> No adverse events were observed. Thus a larger, open-label, single-arm phase-II trial combining first-line dacarbazine and either **2** or placebo (N=40 patients per group) was launched in patients naïve to chemotherapy or immunotherapy for stage-IV metastatic cutaneous melanoma. Median time to tumor progression (primary endpoint) was not significantly different between the two groups.<sup>164</sup> However, some hope of a clinical benefit of blocking the endothelin receptors in human cancer was raised by evaluating the newly developed dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **4**. Compound **4** in combination with dose-dense temozolomide, a standard chemotherapeutic for GBM therapy, is being evaluated in two phase-I/Ib open-label studies in patients with newly diagnosed and recurring GBM (clinical

trial NCT01499251. Sponsor: Actelion, start date: December 2011, estimated completion date: October 2017; an open-label, single-arm phase-I study to assess safety and tolerability, and expression of markers of brain-tumor tissues.). Based on the promising preliminary results of these trials, Actelion will approach Health Authorities for further discussions about the future development of **4** in new and recurring GBM in a phase-IIb/III study (information posted on the Actelion website).

The deceptive results of most human clinical trials with endothelin-receptor antagonists<sup>156,165</sup> merit evaluation and discussion. For progression, cancer cells require key pathways that influence cell growth and death, cell migration, and the development of a vascular network and a permissive environment mainly composed of stromal cells, vascular cells, (myo)fibroblasts, and immune/inflammatory cells. These pathways include the endothelin axis but also other pathways. Preclinical models have demonstrated that the blockade of the endothelin axis may reduce tumor growth, driving the development of antagonists of endothelin receptors and their evaluation in human clinical trials for various cancers.<sup>127</sup> However, in these preclinical models, mainly the ET<sub>A</sub>-dependent pathways and the anti-proliferative effects of antagonists on the carcinoma tumor cells were evaluated. Human tumors develop according to a time-frame that is much longer than and very likely very different from the time-frame and development of animal experimental tumors. Moreover, novel compound are often tested in patients with cancer after one and sometimes two or more established therapies have failed, meaning that many compounds, including ET receptor antagonists are tested at the later stages of cancer. Thus, the expression of *both* ET<sub>A</sub> and ET<sub>B</sub> endothelin receptors might be very different and much more complex in human cancers than in experimental models. The type of human tumors (epithelial versus non-epithelial origin) is also relevant. It has also been shown that stromal cells, including tumor-associated (myo)fibroblasts and macrophages expressing both ET<sub>A</sub> and ET<sub>B</sub>, are mandatory for tumor



progression in humans. This fact has not been considered in early clinical trials. Therefore, we believe, but this reflects only our opinion, that in cancer dual antagonists are more appropriate and promising than single antagonists. We also believe that combination therapy with ET antagonists are also mandatory. But it has to be kept in mind that patients that failed antitumor therapies many times have also developed drug resistance, and would likely failed any therapy. Thus in our opinion, ET antagonist must be added early in therapy, in addition to standard treatments.

### ***Endothelin receptor antagonists beyond PAH: fibrotic diseases and inflammation***

In response to tissue injury including wounding, surgery, cancer, or drug-induced injury, the repair processes may result in two distinct phenomena: a normal regenerative process, limited in time, in which injured cells are replaced by cells of the same type, and a chronic, uncontrolled fibrotic process, in which connective tissue replaces normal tissue, associated with abnormal deposition of extracellular matrix (ECM). Fibrosis is defined by the overgrowth, hardening and scarring of tissues, mainly by activated (myo)fibroblasts, and finally in the replacement of normal tissue with permanent scar tissue.<sup>166</sup> Myofibroblasts may be generated from a variety of cellular sources: resident mesenchymal cells, circulating bone-marrow-derived fibrocytes, or epithelial and endothelial cells by cell-transition mechanisms. Myofibroblasts are activated by a variety of stimuli, including ET-1.<sup>167</sup> ET-1 plays a fundamental role in the pathogenesis of fibrosis and may be a key mediator of profibrotic effects of other agents, such as TGF $\beta$ 1 which induces ET-1 expression, forming an autocrine amplifying loop. ET-1 is chemotactic for cells of the fibroblast lineage and causes fibroblast proliferation and ECM accumulation and contraction, mediated by the two ET-1 receptors, ET<sub>A</sub> and ET<sub>B</sub>, expressed on these cells.<sup>115,168</sup> Thus, antagonists of both ET-1 receptors may have therapeutic potential to prevent the development of fibrosis. Correspondingly, the dual

ET-1 receptor antagonist **2** was shown to decrease collagen-I and -III synthesis by fibroblasts.

In the lung, fibrosis encompasses a variety of idiopathic disorders characterized by a progressive replacement of normal alveolar wall by dense connective tissue that eventually prevents normal gas exchange. The endothelin axis may be implicated in the pathogenesis of lung fibrosis according to experiments in animal models and immunohistochemistry performed on human lung tissue from affected individuals. ET-1 was demonstrated to induce  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) expression and the myofibroblast phenotype in human lung fibroblasts through the ET<sub>A</sub>-JNK-AP-1 pathway.<sup>169</sup> Despite its enormous impact on human health, there are currently no approved treatments to cure this disease. Two compounds have nevertheless been recently approved for the treatment of idiopathic lung fibrosis, as they demonstrated involvement in slowing down the disease: pirfenidone and nintedanib. Based on preliminary data, a one-year clinical double-blind and randomized study has been performed using **2** versus placebo in 158 patients (Build-1).<sup>170,171</sup> The primary endpoint was the exercise capacity. This study was negative concerning this primary endpoint. A post hoc analysis revealed, however, a positive trend in less severely ill patients for the criteria “TCW or death”. Similar findings were published for the Build-2 study that involved patients with lung fibrosis secondary to systemic sclerosis. According to these findings, a new randomized clinical study has been launched (Build-3)<sup>170,171</sup> involving 390 patients with established pulmonary fibrosis. A similar three-year phase-III study was launched in early 2009 with the ET<sub>A</sub>-selective antagonist **3** (Artemis study). A phase-II study (MUSIC) evaluated **4** for the treatment of idiopathic pulmonary fibrosis.<sup>172</sup> These studies evaluated TCW as the primary endpoint. Unfortunately, all studies produced negative results, the Artemis study being prematurely stopped due to an increased progression of the disease in the treated arm.

Within the kidney, injury to glomerular or tubular cells is the initiating cause of many acute and chronic diseases, leading to progressive dysfunction and end-stage renal disease. The

glomerulus is the main filtration barrier that determines global kidney function. Inflammatory and non-inflammatory stresses affect the glomerulus and lead to alterations in its structure and thus, in its permeability and functions, resulting in chronic kidney disease (CKD). In hypertension, diabetes and other kidney diseases, the standard treatment is angiotensin II antagonism; however, this treatment only modestly slows disease progression. ET-1 via ET<sub>A</sub> activation has also been implicated, in addition to the renin-angiotensin system, in the pathophysiology of CKD and particularly in diabetic nephropathy. Animal models have shown beneficial effects of the blockade of the endothelin axis on proteinuria and kidney functions. ET-1 is the major isoform of the endothelin peptides in the kidney, regulating sodium and water excretion, and the vast majority of endothelin receptors of renal cells are of the ET<sub>B</sub> subtype, in contrast to most peripheral organs. Smooth muscle cells of the renal vasculature mainly express ET<sub>A</sub>, while the endothelium express ET<sub>B</sub>, stimulating the release of vasodilators. Most of the dilatory effects of ET-1, as well as vasoconstriction, mesangial cell proliferation and inflammation mainly occur via ET<sub>A</sub>; thus, selective ET<sub>A</sub> blockade may be more beneficial in renal disease, and dual antagonists may block the beneficial vasodilatory effects of ET<sub>B</sub>, causing sodium retention.<sup>173,174</sup> Thus, in the diseased kidney, ET-1-promoting effects on renal cell injury, proteinuria, glomerulosclerosis, inflammation, fibrosis and hypertrophy associated with CKD are mainly mediated by ET<sub>A</sub>.

Preclinical models of CKD and of type-I and type-II diabetic nephropathy have suggested that endothelin-receptor antagonists may be of therapeutic interest in renal diseases. In particular, ET<sub>A</sub> antagonists have been shown to improve renal injury and fibrosis, and this possibility has been evaluated in clinical trials.<sup>173,174</sup> A phase-II study with the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **14** has revealed favorable systemic hemodynamic effects with a trend toward increased renal plasma flow in seven patients. A phase-III study with the ET<sub>A</sub> selective antagonist **9**,<sup>57</sup> performed in 286 patients with diabetic nephropathy and already taking

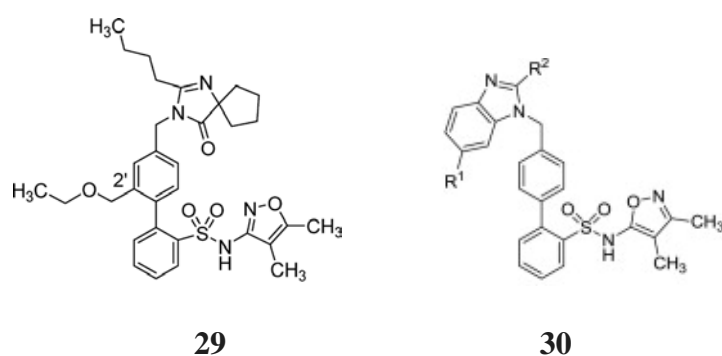
angiotensin blockers showed that albuminuria decreased significantly in the **9**-treated group compared to placebo. The ASCEND trial testing **9** versus placebo in patients with type-II diabetes and nephropathy has been prematurely terminated due to an increase in congestive heart failure events and mortality in the **9** arm. The RADAR phase-II study evaluating **5** for albuminuria reduction in patients with type-II diabetes and nephropathy showed some positive outcomes without obvious edema.<sup>51</sup> Completed and ongoing clinical trials for endothelin-receptor antagonists are summarized in **Table 7** (adapted from <sup>174</sup>).

**Table 7.** Completed and ongoing clinical trials for endothelin-receptor antagonists for kidney diseases. (adapted from <sup>174</sup>)

<i>drug</i>	<i>study phase/ N patients</i>	<i>disease</i>	<i>end-points and outcome</i>
<b>8</b>	N=22	non-diabetic CKD	reduced proteinuria
<b>14</b>	N=7	non-diabetic CKD	tendency to increased renal blood flow
<b>9</b>	phase II N=286	diabetic nephropathy	modified albumin/creatinine ratio dose-dependent
	phase III (ASCEND) N=1392	diabetic nephropathy	reduced albumin/creatinine ratio in the long-term
<b>1</b>	phase II N=27	non-diabetic CKD	reduced proteinuria better than nifedipine
<b>5</b>	phase IIa N=89	diabetic nephropathy	reduced albumin/creatinine ratio
	phase IIb (RADAR) N=211	diabetic nephropathy	reduced albumin/creatinine ratio
	Phase III (SONAR) N=4150 (planned)	diabetic nephropathy	primary endpoint: time to serum creatinine doubling

In summary, the outcomes of preclinical and early clinical phase-II trials suggest that ET<sub>A</sub>

antagonists may be of therapeutic interest and benefit in patients with kidney diseases, the main side effects being fluid retention, which may be minimized by careful drug dosing and the use of diuretics.<sup>174</sup> This information has yet to be confirmed in large multicenter phase-III trials. As several studies suggested an interest in the antagonists of the endothelin axis in these disorders in addition to the renin-angiotensin system, dual angiotensin receptor type 1 (AT<sub>1</sub>)/ET<sub>A</sub> antagonist were also designed and evaluated in preclinical setting only. Combining key structural elements present in an AT<sub>1</sub> receptor antagonist (irbesartan, structure not disclosed) with structural elements in a biphenylsulfonamide ET<sub>A</sub> receptor antagonist provided compound **29**<sup>175</sup> which potently blocked both AT<sub>1</sub> and ET<sub>A</sub> receptors, with improved pharmacokinetic properties and efficacy compared to AT<sub>1</sub> or ET<sub>A</sub> receptor antagonists alone.<sup>175</sup> Another dual angiotensin receptor type 1 (AT<sub>1</sub>, IC<sub>50</sub>=8.5 nM)/ET<sub>A</sub> (IC<sub>50</sub>=8.9 nM) antagonist, compound **30**,<sup>176</sup> was designed and evaluated in a rodent model, showing increased potency compared to losartan (a standard AT<sub>1</sub> antagonist in clinical use) without detrimental effects on heart rate.<sup>176</sup> However, to the best of our knowledge, no clinical trial has been launched with these dual antagonists. (**Scheme 2**)



**Scheme 2:** Dual angiotensin type-1 (AT<sub>1</sub>R) and ET<sub>A</sub> receptors antagonists.

### Other potential clinical indications for endothelin-receptor antagonists

Compound **8** or the combination of **8** and **19** are presently being evaluated in a clinical trial (NCT02062346, start date: April 2014, completion: March 2017) in patients with vasculitis.

The CONSCIOUS-3 phase-III clinical trial assessed the efficacy of **5** in patients with aneurysmal subarachnoid hemorrhage, improving vasospasm-related morbidity and mortality.<sup>177</sup> In an experimental model of arthritis, **2** was shown to have a beneficial effect. The results suggested that the detrimental effects of the endothelin axis in arthritis were mediated by TNF- $\alpha$ .<sup>178</sup> ETs have immunomodulatory properties as pro-inflammatory cytokines and inducers of leukocyte adhesion molecules and have been involved in sepsis. In an experimental model of LPS-induced lung injury, an ET<sub>A</sub> antagonist reduced neutrophil recruitment and lung injury. Endothelin receptor antagonists may be useful as an adjunctive therapy for preserving the organs during infectious diseases.<sup>179</sup> In Crohn' disease, ECE-1, ET<sub>A</sub> and ET<sub>B</sub> are key players of the inflammatory and fibrogenic process, in particular involving the submucosal smooth muscle proliferation.<sup>180</sup> In inflammation and sepsis, blockage of both endothelin receptors attenuated the inflammatory responses.<sup>181,182</sup> However, very few clinical trials have evaluated these possibilities, which will not be discussed further in the present Perspective.

## CONCLUSIONS AND PERSPECTIVES

The selective expression of the components of the endothelin axis in human diseases has been demonstrated, and some information has emerged suggesting that targeting specific components of this axis may be of therapeutic interest in human diseases. Within five years of the discovery of the endothelin axis, orally available endothelin-receptor antagonists became available, and their effects were first evaluated in human clinical trials in the context of cardiovascular diseases, heart failure, PAH, resistant arterial hypertension, stroke, subarachnoid hemorrhage, then for kidney diseases and various cancers. They were of greatest interest for the treatment of PAH and are now in clinical use for this indication. The results of most clinical trials for other indications were either neutral or negative, leading to

the discontinuation of endothelin-receptor antagonist programs in many but not all pharmaceutical companies. Several clinical reviews have analyzed the causes of the failures of clinical trials,<sup>25,121,123,131,156,183-185</sup> some of which may raise further questions about the therapeutic value of endothelin-receptor antagonists.

Since the very initial stages of development of antagonists of ET-1 receptors, there has been a debate about whether blocking ET<sub>A</sub> selectively, or whether additional blocking of ET<sub>B</sub> with dual ET<sub>A</sub>/ET<sub>B</sub> antagonists would be an optimal therapeutic option. ET<sub>B</sub>-selective antagonists were rapidly eliminated from development programs due to side effects. Thus, the debate no longer concerns the usefulness of antagonists of endothelin receptors in human pathologies. Instead, it is concerned with whether ET<sub>A</sub>-selective or dual ET<sub>A</sub>/ET<sub>B</sub> blockers should be used. It is therefore of importance to consider not only receptor selectivity (ET<sub>A</sub>-, ET<sub>B</sub>- or dual selectivity) but also the particular disease involved when developing new antagonists.

The selectivity profile of the ideal endothelin-receptor antagonist depends on the clinical indications, the organs and the type of cells involved in the disease, the stage of progression of the disease and the therapeutic context. Of utmost importance, the heterogeneity of human chronic diseases which develop over long-term must be considered, on the contrary of acute experimental animal models. In PAH, presently, ET<sub>A</sub>-selective and dual ET<sub>A</sub>/ET<sub>B</sub> antagonists have been approved, and it was not possible to identify a clinically relevant advantage for one class of drug or the other in this context. However, it was observed in clinical trials for PAH that the ET<sub>A</sub>-selective drug **3** induced more fluid retention than the dual ET<sub>A</sub>/ET<sub>B</sub> compounds **2** or **4**. In fibrosis-related disorders, the evaluation of the clinical interest of endothelin receptor(s) antagonists has been only very recently initiated, and more feed-back is necessary to reach conclusive information; however, human clinical trials have clearly noted a deleterious effect, in particular fluid retention, of blocking endothelin receptors. For example, phase III clinical trials of **9** for diabetic nephropathy or **5** in the context of oncology of ET<sub>A</sub>-

selective compounds led to early study termination due to water retention or increased mortality. However, it has been observed in most studies, that the side effects are dose-related and that for most trials the dosage applied were likely too high. In cancer, the situation is more complex. ET<sub>A</sub> is important in tumor cell migration, metastasis and proliferation, while ET<sub>B</sub> is critical to angiogenesis and inhibition of anti-tumor immune cell recruitment. However, the type and grade of cancer, its tissue origin, its stage of progression, the exact cells involved and the level of heterogeneity of the tumor must be considered. In human cancer, but possibly not in experimental animal tumors, long-term cancer progression required, also impacted on the expression of the endothelin axis in cancer stroma, and blocking both receptors may be more appropriate for cancer indications.

A second conclusion reached by clinical trials, and it is not surprising in human therapy, was that to achieve optimal therapeutic efficacy drug combination must be considered, adding endothelin-receptor antagonists as an adjuvant to other regimens, either concurrent or sequential double or even triple therapy. It remains to be demonstrated however, which receptor is the better target and which combined-schedule therapy should be proposed to patients according to their level of risk. In unpublished experiments using human GBM cells in culture and drug combination of the dual ET<sub>A</sub>/ET<sub>B</sub> antagonist **2** and alkylating agents (carmustine, temozolomide or alkeran), we have shown that cell growth (defined as inhibition of DNA synthesis) was additively, rather than synergistically, decreased by combination of **2** with either carmustine or alkeran.

In summary, several excellent, orally available, ET<sub>A</sub>-selective or dual ET<sub>A</sub>/ET<sub>B</sub> endothelin-receptor antagonists, as well as agonists and inhibitors, with acceptable side effects<sup>186</sup> have been designed, developed and evaluated in preclinical and human clinical settings, showing beneficial therapeutic effects in several life-threatening diseases, By themselves, they are not curative for these diseases, but they are beneficial in several ways; however, improvement is



still needed, in particular, to develop a means to control the side effects associated with endothelin-receptor antagonism and achieve more tissue-selective delivery of the therapeutics. Strategies to consider may include the design of cell-, tissue- or organ-specific targeting therapies, the development of drugs with disease-specific markers, or the use of functionalized targeted nanoparticles.

**Conflicts of interests.** JDA has received travel support from Actelion, and has been invited in advisory boards for Glaxo Smith Kline and Actelion.

### **Authors' biographies**

**John-David Aubert** graduated from the Faculty of Biology and Medicine of the University of Lausanne (UNIL), Switzerland, and specialized in internal and respiratory medicine, first in Lausanne University Hospital, then in UBC Research Laboratory in Vancouver (Prof JC Hogg) as a post-doctoral assistant. In Lausanne in 1993, he was involved in the creation of the new lung-transplantation program and is now its medical director and associate professor. He also leads the pulmonary hypertension clinic and has been president of the Swiss Society for Pulmonary Hypertension. He has been appointed president of the Research Committee of the Swiss Lung League for 2016-2018. His main research interests focus on chronic allograft dysfunction after lung transplantation and clinical aspects of pulmonary hypertension.

**Lucienne Juillerat-Jeanneret** obtained her PhD from the University of Geneva, Switzerland. After post-doctoral experiences at the University of Geneva and the University Hospital of Lausanne (CHUV-UNIL), she joined the University Institute of Pathology of Lausanne as a tenured senior lecturer and a teacher at the University of Lausanne (UNIL) and the Swiss Federal Institute of Technology of Lausanne (EPFL). Her main research interests are focused

on the interface between biomedicine, chemistry and biomaterials, the design and development of innovative devices or modified drugs to deliver therapeutics. The strategies investigated include nanotherapeutics and targeted chemotherapeutics for the treatment of cancer and degenerative diseases. She is also involved in the development of novel approaches for diagnosis and tissue engineering.

### **Abbreviations**

AT: angiotensin receptor; CHF: chronic heart failure; CKD: chronic kidney disease; EMA: European medicines agency; EMT: epithelial to mesenchymal transition; ET: endothelin; ET<sub>A</sub>: endothelin receptor A; ET<sub>B</sub>: endothelin receptor B; ECE: endothelin converting enzyme; ECM: extracellular matrix; EGF: epidermal growth factor; ECM: extracellular matrix; FDA: US federal drug administration; GBM: glioblastoma; GPCR: G-protein coupled receptor; 6MWD: 6 min walking distance; MMP: matrix metalloproteinase; NEP: neutral endopeptidase; NYHA: New York heart association; PAH: pulmonary arterial hypertension  
P-Gp: P-glycoprotein; RCT: randomized clinical trial; TCW: time to clinical worsening.

\*Corresponding authors:

Dr John-David Aubert, MD, Associate professor

Pneumology Division and Transplantation Center, Centre Hospitalier Universitaire Vaudois (CHUV), Bugnon 46, CH-1011 Lausanne, Switzerland

Phone: +41 21 314 1376; e-mail : [john-david.aubert@chuv.ch](mailto:john-david.aubert@chuv.ch)

Dr Lucienne Juillerat-Jeanneret, PhD, Privat Docent

Transplantation Center and Institute of Pathology, CHUV-UNIL, Chemin des Boveresses 155, CH1011, Lausanne, Switzerland

Phone: +41 21 314 7214/ 7117; e-mail: [lucienne.juillerat@chuv.ch](mailto:lucienne.juillerat@chuv.ch)

## References

1. Xu, D.; Emoto, N.; Giaid, A.; Slaughter, C.; Kaw, S.; de Wit, D.; Yanagisawa, M. ECE-1: A Membrane-bound Metalloprotease that Catalyzes the Proteolytic Activation of Big Endothelin-1. *Cell*. **1994**, *78*, 473-485.
2. Russell, F. D.; Skepper, J. N.; Davenport, A. P. Human Endothelial Cell Storage Granules: A Novel Intracellular Site for Isoforms of the Endothelin-converting Enzyme. *Circ. Res.* **1998**, *83*, 314-321.
3. Davenport, A. P. International Union of Pharmacology. XXIX. Update on Endothelin Receptor Nomenclature. *Pharmacol. Rev.* **2002**, *54*, 219-226.
4. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. A Novel Potent Vasoconstrictor Peptide Produced by Vascular Endothelial Cells. *Nature*. **1988**, *332*, 411-415.
5. Ling, L.; Maguire, J. J.; Davenport, A. P. Endothelin-2, the Forgotten Isoform: Emerging Role of Endothelin-2 in the Cardiovascular System, Ovarian Development, Immunology and Cancer. *Br. J. Pharmacol.* **2013**, *168*, 283-295.
6. Wang, R.; Löhr, C. V.; Fischer, K.; Dashwood, W. M.; Greenwood, J. A.; Ho, E.; Williams, D. E.; Ashktorab, H.; Dashwood, M. R.; Dashwood, R. H. Epigenetic Inactivation of Endothelin-2 and Endothelin-3 in Colon Cancer. *Int. J. Cancer.* **2013**, *132*, 1004-1012.
7. Denault, J. B.; Claing, A.; D'Orléans-Juste, P.; Sawamura, T.; Kido, T.; Masaki, T.; Leduc, R. Processing of Proendothelin-1 by Human Furin Convertase. *FEBS Lett.* **1995**, *362*, 276-280.
8. Coppola, J. M.; Hamilton, C. A.; Bhojani, M. S.; Larsen, M. J.; Ross, B. D.; Rehemtulla, A. Identification of Inhibitors Using a Cell-based Assay for Monitoring Golgi-resident Protease Activity. *Anal. Biochem.* **2007**, *364*, 19-29.

9. Muller, L.; Barret, A.; Etienne, E.; Meidan, R.; Valdenaire, O.; Corvol, P.; Tougard, C. Heterodimerization of Endothelin-converting-enzyme-1 Isoforms Regulates the Subcellular Distribution of this Metalloprotease. *J. Biol. Chem.* **2003**, *278*, 545-555.
10. Azarani, A.; Boileau, G.; Crine, P. Recombinant Human Endothelin-converting Enzyme ECE-1b Is Located in an Intracellular Compartment When Expressed in Polarized Madin-Darby Canine Kidney Cells. *Biochem. J.* **1998**, *333*, 439-448.
11. Turner, A. J.; Barnes, K.; Schweizer, A.; Valdenaire, O. Isoforms of Endothelin-converting Enzyme: Why and Where? *Trends Pharmacol. Sci.* **1998**, *19*, 483-486.
12. Valdenaire, O.; Lepailleur-Enouf, D.; Egidy, G. Thouhard, A.; Barret, A.; Vranckx, R.; Tougard, C.; Michel, J. B. A Fourth Isoform of Endothelin-converting Enzyme (ECE-1) is Generated from an Additional Promoter: Molecular Cloning and Characterization. *Eur. J. Biochem.* **1999**, *264*, 341-349.
13. Berger, Y.; Dehmlow, H.; Blum-Kaelin, D.; Kitas, E. A.; Löffler, B. M.; Aebi, J. D.; Juillerat-Jeanneret, L. Endothelin-converting Enzyme-1 Inhibition and Growth of Human Glioblastoma Cells. *J. Med. Chem.* **2005**, *48*, 483-498.
14. Juillerat-Jeanneret, L. Inhibiting the Enzymes of the Endothelin and Renin-angiotensin Systems in Cancer. *Curr. Enzyme Inhibit.* **2006**, *2*, 353-362.
15. Russell, F. D.; Coppell, A. L.; Davenport, A. P. In Vitro Enzymatic Processing of Radiolabel Led Big ET-1 in Human Kidney. *Biochem. Pharmacol.* **1998**, *55*, 697-701.
16. Hayasaki-Kajiwara, Y.; Naya, N.; Shimamura, T.; Iwasaki, T.; Nakajima, M. Endothelin Generating Pathway through Endothelin1-31 in Human Cultured Bronchial Smooth Muscle Cells. *Br. J. Pharmacol.* **1999**, *127*, 1415-1421.
17. Tabrizchi, R. SLV-306 Solvay. *Curr. Opin. Investig. Drugs* **2003**, *4*, 329-332.

18. Parvanova, A.; van der Meer, I. M.; Iliev, I.; Perna, A.; Gaspari, F.; Trevisan, R.; Bossi, A.; Remuzzi, G.; Benigni, A.; Ruggenti, P. Effect on Blood Pressure of Combined Inhibition of Endothelin-converting Enzyme and Neutral Endopeptidase with Dabigatran in Patients with Type 2 Diabetes Who Have Albuminuria: a Randomised, Crossover, Double-blind, Placebo-controlled Trial. *Lancet Diabetes Endocrinol.* **2013**, *1*, 19-27.
19. Cabrera-Vera, T. M.; Vanhauwe, J.; Thomas, T. O.; Medkova, M.; Preinerger, A.; Mazzoni, M. R.; Hamm, H. E. Insights into G Protein Structure, Function, and Regulation. *Endocr. Rev.* **2003**, *24*, 765–781.
20. Lappano, R.; Maggiolini, M. G Protein-coupled Receptors: Novel Targets for Drug Discovery in Cancer. *Nat. Rev. Drug Disc.* **2011**, *10*, 47-60.
21. Sasaki, Y.; Hori, S.; Oda, K.; Okada, T.; Takimoto, M. Both ET(A) and ET(B) Receptors Are Involved in Mitogen-activated Protein Kinase Activation and DNA Synthesis of Astrocytes: Study Using ET(B) Receptor-deficient Rats (aganglionosis Rats). *Eur. J. Neurosci.* **1998**, *10*, 2984-2993.
22. Bremnes, T.; Paasche, J. D.; Mehlum, A.; Sandberg, C.; Bremnes, B.; Attramadal, H. Regulation and Intracellular Trafficking Pathways of the Endothelin Receptors. *J. Biol. Chem.* **2000**, *275*, 17596-17604.
23. Ishikawa, K.; Ihara, M.; Noguchi, K.; Mase, T.; Mino, N.; Saeki, T.; Fukuroda, T.; Fukami, T.; Ozaki, S.; Nagase, T. Biochemical and Pharmacological Profile of a Potent and Selective Endothelin B-receptor Antagonist, BQ788. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4892-4896.
24. Jimeno, A.; Carducci, M. Atrasentan: Targeting the Endothelin Axis in Prostate Cancer. *Exp. Opin. Invest. Drugs.* **2004**, *13*, 1631-1640.
25. Battistini, B.; Berthiaume, N.; Kelland, N. F.; Webb, D. J.; Kohan, D. E. Profile of

Past and Current Clinical Trials Involving Endothelin Receptor Antagonists: The Novel “sentan” Class of Drug. *Exp. Biol. Med.* **2006**, *231*, 653-695.

26. Liu, G. Recent Advances in Endothelin Antagonism. *Ann. Rev. Med. Chem.* **2000**, *8*, 73-82.

27. Morris, C. D.; Rose, A.; Curwen, J.; Hughes, A. M.; Wilson, D. J.; Webb, D. J. Specific Inhibition of the Endothelin A Receptor with ZD4054: Clinical and Pre-clinical Evidence. *Br. J. Cancer.* **2005**, *92*, 2148-2152.

28. Price, L. C.; Howard, L. S. Endothelin Receptor Antagonists for Pulmonary Arterial Hypertension: Rationale and Place in Therapy. *Am. J. Cardiovasc. Drugs.* **2008**, *8*, 171-185.

29. Bourgeois, C.; Robert, B.; Rebourcet, R.; Mondon, F.; Mignot, T. M.; Duc-Goiran, P.; Ferré, F. Endothelin-1 and ETA Receptor Expression in Vascular Smooth Muscle Cells from Human Placenta: A New ETA Receptor Messenger Ribonucleic Acid Is Generated by Alternative Splicing of Exon 3. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3116-3123.

30. Elshourbagy, N. A.; Adamou, J. E.; Gagnon, A. W.; Wu, H. L.; Pullen, M.; Nambi, P. Molecular Characterization of a Novel Human Endothelin Receptor Splice Variant. *J. Biol. Chem.* **1996**, *271*, 25300-25307.

31. Imamura, F.; Arimoto, I.; Fujiyoshi, Y.; Doi, T. W276 Mutation in the Endothelin Receptor Subtype B Impairs G<sub>q</sub> Coupling but Not G<sub>i</sub> or G<sub>o</sub> Coupling. *Biochemistry.* **2000**, *39*, 686-692.

32. Lo, K. W.; Tsang, Y. S.; Kwong, J.; To, K. F.; Teo, P. M.; Huang, D. P. Promoter Hypermethylation of the EDNRB Gene in Nasopharyngeal Carcinoma. *Int. J. Cancer.* **2002**, *98*, 651-655.

33. Miyamoto, Y.; Yoshimasa, T.; Arai, H.; Takaya, K.; Ogawa, Y.; Itoh, H.; Nakao, K. Alternative RNA Splicing of the Human Endothelin-A Receptor Generates Multiple

Transcripts. *Biochem. J.* **1996**, *313*, 795-801.

34. Nambi, P.; Wu, H. L.; Ye, D.; Gagnon, A.; Elshourbagy, N. Characterization of a Novel Porcine Endothelin-B Receptor Splice Variant. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 247-253.

35. Pao, M. M.; Tsutsumi, M.; Liang, G.; Uzvolgyi, E.; Gonzales, F. A.; Jones, P. A. The Endothelin Receptor B (EDNRB) Promoter Displays Heterogeneous, Site Specific Methylation Patterns in Normal and Tumor Cells. *Hum. Mol. Genet.* **2001**, *10*, 903-910.

36. Shyamala, V.; Moulthrop, T. H.; Stratton-Thomas, J.; Tekamp-Olson, P. Two Distinct Human Endothelin B Receptors Generated by Alternative Splicing Form a Single Gene. *Cell. Mol. Biol. Res.* **1994**, *40*, 285-296.

37. Tsutsumi, M.; Liang, G.; Jones, P. A. Novel Endothelin B Receptor Transcripts with the Potential of Generating a New Receptor. *Gene.* **1999**, *228*, 43-49.

38. Zhang, Y. F.; Jeffery, S.; Burchill, S. A.; Berry, P. A.; Kaski, J. C.; Carter, N. D. Truncated Human Endothelin Receptor A Produced by Alternative Splicing and Its Expression in Melanoma. *Br. J. Cancer.* **1998**, *78*, 1141-1146.

39. Berger, Y.; Bernasconi, C. C.; Juillerat-Jeanneret, L. Targeting the Endothelin Axis in Human Melanoma: Combination of Endothelin Receptor Antagonism and Alkylating Agents. *Exp. Biol. Med.* **2006**, *231*, 1111-1119.

40. Roh, J. L.; Wang, X. V.; Manola, J.; Sidransky, D.; Forastiere, A. A.; Koch, W. M. Clinical Correlates of Promoter Hypermethylation of Four Target Genes in Head and Neck Cancer: A Cooperative Group Correlative Study. *Clin. Cancer Res.* **2013**, *19*, 2528-2540.

41. Tao, K.; Wu, C.; Wu, K.; Li, W.; Han, G.; Shuai, X.; Wang, G. Quantitative Analysis of Promoter Methylation of the EDNRB Gene in Gastric Cancer. *Med. Oncol.* **2012**, *29*, 107-112.



42. Barst, R. J.; Langleben, D.; Frost, A.; Horn, E. M.; Oudiz, R.; Shapiro, S.; McLaughlin, V.; Hill, N.; Tapson, V. F.; Robbins, I. M.; Zwicke, D.; Duncan, B.; Dixon, R. A.; Frumkin, L. R.; STRIDE-1 Study Group. Sitaxsentan Therapy for Pulmonary Arterial Hypertension. *Am. J. Respir. Crit. Care Med.* **2004**, *169*, 441-447.
43. Clozel, M.; Breu, V.; Gray, G. A.; Kalina, B.; Löffler, B. M.; Burri, K.; Cassal, J. M.; Hirth, G.; Müller, M.; Neidhart, W.; Ramuz, H. Pharmacological Characteristics of Bosentan, a New Potent Orally Active Nonpeptide Endothelin Receptor Antagonist. *J. Pharm. Exp. Ther.* **1994**, *270*, 228-235.
44. Rubin, L. J.; Badesch, D. B.; Barst, R. J.; Galie, N.; Black, C. M.; Keogh, A.; Pulido, T.; Frost, A.; Roux, S.; Leconte, I.; Landzberg, M.; Simonneau, G. Bosentan Therapy for Pulmonary Arterial Hypertension. *N. Engl. J. Med.* **2002**, *346*, 896-903.
45. Galié, N.; Badesch, D.; Oudiz, R.; Simonneau, G.; McGoon, M. D.; Keogh, A. M.; Frost, A. E.; Zwicke, D.; Naeije, R.; Shapiro, S.; Olschewski, H.; Rubin, L. J. Ambrisentan Therapy for Pulmonary Arterial Hypertension. *J. Am. Coll. Cardiol.* **2005**, *46*, 529-535.
46. Bolli, M. H.; Boss, C.; Binkert, C.; Buchmann, S.; Bur, D.; Hess, P.; Iglarz, M.; Meyer, S.; Rein, J.; Rey, M.; Treiber, A.; Clozel, M.; Fischli, W.; Weller, T. The Discovery of *N*-5-(4-bromophenyl)-6-2-(5-Bromo-2-Pyrimidinyl)oxyethoxy-4-pyrimidinyl-*N'*-propyl-sulfamide (Macitentan), an Orally Active, Potent Dual Endothelin Receptor Antagonist. *J. Med. Chem.* **2012**, *55*, 7849-7861.
47. Pulido, T.; Adzerikho, I.; Channick, R. N.; Delcroix, M.; Galiè, N.; Ghofrani, H. A.; Jansa, P.; Jing, Z. C.; Le Brun, F. O.; Mehta, S.; Mittelholzer, C. M.; Perchenet, L.; Sastry, B. K.; Sitbon, O.; Souza, R.; Torbicki, A.; Zeng, X.; Rubin, L. J.; Simonneau, G.; SERAPHIN Investigators Macitentan and Morbidity and Mortality in Pulmonary Arterial Hypertension. *N. Engl. J. Med.* **2013**, *369*, 809-818.

48. Houde, M.; Labonté, J.; D'Orléans-Juste, P. Peptide and Non-peptide Antagonists Targeting Endothelin Receptors in Physiology and Pathology. *Curr. Pharm. Des.* **2011**, *17*, 2613-2625.

49. Ishikawa, K.; Fukami, T.; Nagase, T.; Fujita, K.; Hayama, T.; Niiyama, K.; Mase, T.; Ihara, M.; Yano, M. Cyclic Pentapeptide Endothelin Antagonists with High ETA Selectivity. Potency- and Solubility-enhancing Modifications. *J. Med. Chem.* **1992**, *35*, 1239–1242.

50. Chiappori, A. A.; Haura, E.; Rodriguez, F. A.; Boulware, D.; Kapoor, R.; Neuger, A. M.; Lush, R.; Padilla, B.; Burton, M.; Williams, C.; Simon, G.; Antonia, S.; Sullivan, D. M.; Bepler, G. Phase I/II Study of Atrasentan, an Endothelin A Receptor Antagonist, in Combination with Paclitaxel and Carboplatin as First-line Therapy in Advanced Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2008**, *14*, 1464–1469.

51. de Zeeuw, D.; Coll, B.; Andress, D.; Brennan, J. J.; Tang, H.; Houser, M.; Correa-Rotter, R.; Kohan, D.; Lambers Heerspink, H. J.; Makino, H.; Perkovic, V.; Pritchett, Y.; Remuzzi, G.; Tobe, S. W.; Toto, R.; Viberti, G.; Parving, H. H. The Endothelin Antagonist Atrasentan Lowers Residual Albuminuria in Patients with type 2 Diabetic Nephropathy. *J. Am. Soc. Nephrol.* **2014**, *25*, 1083–1093.

52. Quinn, D. I.; Tangen, C. M.; Hussain, M.; Lara, P. N.; Goldkorn, A.; Moinpour, C. M.; Garzotto, M. G.; Mack, P. C.; Carducci, M. A.; Monk, J. P.; Twardowski, P. W.; Van Veldhuizen, P. J.; Agarwal, N.; Higano, C. S.; Vogelzang, N. J.; Thompson, I. M. Docetaxel and Atrasentan Versus Docetaxel and Placebo for Men with Advanced Castration-resistant Prostate Cancer (SWOG S0421): A Randomized Phase 3 Trial. *Lancet Oncol.* **2013**, *14*, 893-900.

53. Buchholz, M.; Reissig, H. U. Enantioselective Synthesis of the Pyrrolidine Core of Endothelin Antagonist ABT-627 (Atrasentan) via 1,2-Oxazines. *Eur. J. Org. Chem.* **2003**,

2003, 3524-3533.

54. Paul LM van Giersbergen, P. L. M.; Dingemans, J. Tolerability, Pharmacokinetics, and Pharmacodynamics of Clazosentan, a Parenteral Endothelin Receptor Antagonist. *Eur. J. Clin. Pharm.* **2007**, *63*, 151-158.

55. Tomkinson, H.; Kemp, J.; Oliver, S.; Swaisland, H.; Taboada, M.; Morris, T. Pharmacokinetics and Tolerability of Zibotentan (ZD4054) in Subjects with Hepatic or Renal Impairment: Two Open-label Comparative Studies. *BMC Clin. Pharmacol.* **2011**, *11*, 3.

56. Ishikawa, K.; Fukami, T.; Nagase, T.; Fujita, K.; Hayama, T.; Niiyama, K.; Mase, T.; Ihara, M.; Yano, M. Cyclic Pentapeptide Endothelin Antagonists with High ETA Selectivity. Potency- and Solubility-enhancing Modifications. *J. Med. Chem.* **1992**, *35*, 1239–1242.

57. Dutzler, R.; Ernst, B.; Hediger, M. A.; Keppler, D.; Mohr, P.; Neidhart, W.; Märki H.P. Channels and Transporters. *Chimia* **2010**, *64*, 662-666.

58. Epstein, B. J. Efficacy and Safety of Darusentan: a Novel Endothelin Receptor Antagonist. *Ann. Pharmacother.* **2008**, *42*, 1060-1069.

59. Murugesan, N.; Gu, Z.; Spergel, S.; Young, M.; Chen, P.; Mathur, A.; Leith, L.; Hermsmeier, M.; Liu, E. C.; Zhang, R.; Bird, E.; Waldron, T.; Marino, A.; Koplowitz, B.; Humphreys, W. G.; Chong, S.; Morrison, R. A.; Webb, M. L.; Moreland, S.; Trippodo, N.; Barrish, J. C. Biphenylsulfonamide Endothelin Receptor Antagonists. 4. Discovery of N-[[2'-[[[(4,5-Dimethyl-3-isoxazolyl)amino]sulfonyl]-4-(2-oxazolyl)[1,1'-biphenyl]-2-yl]methyl]-N,3,3-trimethyl butanamide (BMS-207940), a Highly Potent and Orally Active ET(A) Selective Antagonist. *J. Med. Chem.* **2003**, *46*, 125-137.

60. Sudoh, K.; Yuyama, H.; Noguchi, Y.; Fujimori, A.; Ukai, M.; Ohtake, A.; Sato, S.; Sasamata, M.; Miyata, K. Pharmacological Characterization of YM598, a Selective Endothelin-A Receptor Antagonist. *J. Cardiovasc. Pharmacol.* **2004**, *44* (S1), S390-S393.

61. Clozel, M.; Breu, V.; Gray, G. A.; Löffler, B. M. In Vivo Pharmacology of Ro 46-2005, the First Synthetic Nonpeptide Endothelin Receptor Antagonist: Implications for Endothelin Physiology. *J. Cardiovasc. Pharmacol.* **1993**, *22* (S8), S377-S379.
62. Breu, V.; Löffler, B. M.; Clozel, M. In Vitro Characterization of Ro 46-2005, a Novel Synthetic Non-Peptide Endothelin Antagonist of ET<sub>A</sub> and ET<sub>B</sub> Receptors. *FEBS Lett.* **1993**, *334*, 210-214.
63. Sidharta, P. N.; van Giersbergen, M.; Halabi, A.; Dingemans, J. Macitentan: Entry-into-humans Study with a New Endothelin Receptor Antagonist. *Eur. J. Clin. Pharmacol.* **2011**, *67*, 977-984.
64. Patel, T.; McKeage, K. Macitentan: First Global Approval. *Drugs.* **2014**, *74*, 127-133.
65. Weiss, J.; Theile, D.; Rüppell, M. A.; Speck, T.; Spalwisch, A.; Haefeli, W. E. Interaction Profile of Macitentan, a new Non-selective Endothelin-1 Receptors Antagonist, In Vitro. *Eur. J. Pharmacol.* **2013**, *701*, 168-175.
66. Rayalu, D. J.; Selvaraj, C.; Singh, S. K.; Ganeshan, R.; Kumar, N. U.; Seshapani, P. Homology Modeling, Active Site Prediction, and Targeting the Anti-hypertension Activity through Molecular Docking on Endothelin-B Receptor Domain. *Bioinformation.* **2012**, *8*, 81-86.
67. Gatfield, J.; Mueller Grandjean, C.; Sasse, T.; Clozel, M.; Nayler, O. Slow Receptor Dissociation Kinetics Differentiate Macitentan from Other Endothelin Receptor Antagonists in Pulmonary Arterial Smooth Muscle Cells. *PLOS ONE.* **2012**, *7*, e47662.
68. Gatfield, J.; Mueller Grandjean, C.; Bur, D.; Bolli, M. H.; Nayler, O. Distinct ET<sub>A</sub> Receptor Binding Mode of macitentan as Determined by Site Directed Mutagenesis. *PLOS ONE.* **2014**, *9*, e107809.
69. Shaw, M. D.; Vermeulen, M.; Murray, G. D.; Pickard, J. D.; Bell, B. A.; Teasdale, G.

M. Efficacy and Safety of the Endothelin Receptor Antagonist TAK-044 in Treating Subarachnoid Hemorrhage. *J. Neurosurg.* **2000**, *93*, 992-997.

70. Clozel, M.; Ramuz, H.; Clozel, J. P.; Breu, V.; Hess, P.; Löffler, B. M.; Coassolo, P.; Roux, S. Pharmacology of Tezosentan, New Endothelin Receptor Antagonist Designed for Parenteral Use. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 840-846.

71. Hoshino, T.; Yamauchi, R.; Kikkawa, K.; Yabana, H.; Murata, S. Pharmacological Profile of T-0201, a Highly Potent and Orally Active Endothelin Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 643-649.

72. Ohlstein, E. H.; Nambi, P.; Douglas, S. A.; Edwards, R. M.; Gellai, M.; Lago, A.; Leber, J. D.; Cousins, R. D.; Gao, A.; Frazee, J. S.; Peishoff, C. E.; Bean, J. W.; Eggleston, D. S.; Elshourbagy, N. A.; Kumar, C.; Lee, J. A.; Yue, T. L.; Louden, C.; Brooks, D. P.; Weinstock, J.; Feuerstein, G.; Poste, G.; Ruffolo, R. R.; Gleason, J. G.; Elliott, J. D. SB 209670, a Rationally Designed Potent Nonpeptide Endothelin Receptor Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8052-8056.

73. Ohlstein, E. H.; Nambi, P.; Lago, A.; Hay, D. W.; Beck, G.; Fong, K. L.; Eddy, E. P.; Smith, P.; Ellens, H.; Elliott, J. D. Nonpeptide Endothelin Receptor Antagonists. VI: Pharmacological Characterization of SB 217242, a Potent and Highly Bioavailable Endothelin Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 609-615.

74. von Geldern, T. W.; Tasker, A. S.; Sorensen, B. K.; Winn, M.; Szczepankiewicz, B. G.; Dixon, D. B.; Chiou, W. J.; Wang, L.; Wessale, J. L.; Adler, A.; Marsh, K. C.; Nguyen, B.; Opgenorth, T. J. Pyrrolidine-3-Carboxylic Acids as Endothelin Antagonists. 4. Side Chain Conformational Restriction Leads to ET<sub>B</sub> selectivity. *J. Med. Chem.* **1999**, *42*, 3668-3678.

75. Balwierzak, J. L.; Bruseo, C. W.; DelGrande, D.; Jeng, A. Y.; Savage, P.; Shetty, S.

S. Characterization of a Potent and Selective Endothelin-B Receptor Antagonist, IRL 2500. *J. Cardiovasc. Pharmacol.* 1995, 26 (Suppl. 3), S393-S396.

76. Ikemura, T.; Ohmori, K.; Tanaka, T.; Matsuda, Y.; Kitamura, S. Effects of Endothelin B Antagonist RES-701-1 on Endothelin-induced Contractile Responses In-vivo and In-vitro in Guinea-pigs. *J. Pharm. Pharmacol.* **1996**, 48, 100-105.

77. Breu, V.; Clozel, M.; Burri, K.; Hirth, G.; Neidhart, W.; Ramuz, H. In-vitro Characterisation of Ro 46-8443, the First Non-peptide Antagonist Selective for the Endothelin ET<sub>B</sub> Receptor. *FEBS Lett.* **1996**, 383, 37-41.

78. Bolli, M. H.; Marfurt, J.; Grisostomi, C.; Boss, C.; Binkert, C.; Hess, P.; Treiber, A.; Thorin, E.; Morrison, K.; Buchmann, S.; Bur, D.; Ramuz, H.; Clozel, M.; Fischli, W.; Weller, T. Novel Benzo[1,4]diazepin-2-one Derivatives as Endothelin Receptor Antagonists. *J. Med. Chem.* **2004**, 47, 2776-2795.

79. Ishizuka, N.; Matsumura, K.; Sakai, K.; Fujimoto, M.; Mihara, S.; Yamamori, T. Structure-Activity Relationships of a Novel Class of Endothelin-A Receptor Antagonists and Discovery of Potent and Selective Receptor Antagonist, 2-(Benzo[1,3]Dioxol-5-yl)-6-isopropoxy-4-(4-Methoxyphenyl)-2*H*-chromene-3-carboxylic Acid (S-1255). 1. Study on Structure-activity Relationships and Basic Structure Crucial for ET<sub>A</sub> Antagonism. *J. Med. Chem.* **2002**, 45, 2041-2055.

80. Amberg, W.; Hergenröder, S.; Hillen, H.; Jansen, R.; Kettschau, G.; Kling, A.; Klinge, D.; Raschack, M.; Riechers, H.; Unger, L. Discovery and Synthesis of (*S*)-3-[2-(3,4-Dimethoxyphenyl)Ethoxy]-2-(4,6-dimethylpyrimidin-2-yloxy)-3,3-diphenylpropionic Acid (LU 302872), a Novel Orally Active Mixed ET<sub>A</sub>/ET<sub>B</sub> Receptor Antagonist. *J. Med. Chem.* **1999**, 42, 3026-3032.

81. Patel, H. J.; Olgun, N.; Lengyel, I.; Reznik, S.; Stephani, R. A. Synthesis and

Pharmacological Activity of 1,3,6-trisubstituted-4-oxo-1,4-dihydroquinoline-2-carboxylic Acids as Selective ET<sub>A</sub> Antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6840-6844.

82. Xia, J.; Song, J.; Zhen, L.; Zhang, X.; Lei, X.; Zheng, L.; Wang, Q.; Sun, H. Synthesis and In-vitro Evaluation of Ambrisentan Analogues as Potential Endothelin Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3894-3897.

83. Allard, B.; Wijkhuisen, A.; Borrull, A.; Deshayes, F.; Priam, F.; Lamourette, P.; Ducancel, F.; Boquet, D.; Couraud, J. Y. Generation and Characterization of Rendomab-B1, a Monoclonal Antibody Displaying Potent and Specific Antagonism of the Human Endothelin B Receptor. *MAbs.* **2013**, *5*, 56-69.

84. Aubert, J. D.; Juillerat-Jeanneret, L. Therapeutic Potential of Endothelin Receptor Modulators: Lessons from Human Clinical Trials. *Expert Opin. Ther. Targets.* **2009**, *13*, 1069-1084.

85. Kiowski, W.; Lüscher, T. F.; Linder, L.; Bühler, F. R. Endothelin-1-induced Vasoconstriction in Humans. Reversal by Calcium Channel Blockade but Not by Nitrovasodilators or Endothelium-derived Relaxing Factor. *Circulation.* **1991**, *83*, 469-475.

86. Haynes, W. G.; Webb, D. J. Contribution of Endogenous Generation of Endothelin-1 to Basal Vascular Tone. *Lancet* **1994**, *344*, 852-854.

87. Ertl, G.; Bauersachs, J. Endothelin Receptor Antagonists in Heart Failure: Current Status and Future Directions. *Drugs.* **2004**, *64*, 1029-1040.

88. Morrell, N. W.; Adnot, S.; Archer, S. L.; Dupuis, J.; Jones, P. L.; MacLean, M. R.; McMurtry, I. F.; Stenmark, K. R.; Thistlethwaite, P. A.; Weissmann, N.; Yuan, J. X.; Weir, E. K. Cellular and Molecular Basis of Pulmonary Arterial Hypertension. *J. Am. Coll. Cardiol.* **2009**, *54*, S20-S31.

89. Tuder, R. M.; Archer, S. L.; Dorfmueller, P.; Erzurum, S. C.; Guignabert, C.;

Michelakis, E.; Rabinovitch, M.; Schermuly, R.; Stenmark, K. R.; Morrell, N. W. Relevant Issues in the Pathology and Pathobiology of Pulmonary Hypertension. *J. Am. Coll. Cardiol.* **2013**, *62*, D4-D12.

90. Macchia, A.; Marchioli, R.; Tognoni, G.; Scarano, M.; Marfisi, R.; Tavazzi, L.; Rich, S. Systematic Review of Trials Using Vasodilators in Pulmonary Arterial Hypertension: Why a New Approach is Needed. *Am. Heart J.* **2010**, *159*, 245-257.

91. Sauvageau, S.; Thorin, E.; Caron, A.; Dupuis, J. Endothelin-1-induced Pulmonary Vasoreactivity is Regulated by ET(A) and ET(B) Receptor Interactions. *J. Vasc. Res.* **2007**, *44*, 375-381.

92. Porter, K. E.; Riches, K. Vascular Smooth Muscle as a Target for Novel Therapeutics. *Curr. Diab. Rep.* **2015**, *15*, 72.

93. Tonelli, A. R.; Zein, J.; Ioannidis, J. P. Geometry of the Randomized Evidence for Treatments of Pulmonary Hypertension. *Cardiovasc. Therap.* **2013**, *31*, e138-e146.

94. Montani, D.; Chaumais, M. C.; Guignabert, C.; Günther, S.; Girerd, B.; Jaïs, X.; Algalarrondo, V.; Price, L. C.; Savale, L.; Sitbon, O.; Simonneau, G.; Humbert, M. Targeted Therapies in Pulmonary Arterial Hypertension. *Pharmacol. Ther.* **2014**, *141*, 172-191.

95. Humbert, M.; Ghofrani, H. A. The Molecular Targets of Approved Treatments for Pulmonary Arterial Hypertension. *Thorax.* **2015**, *71*, 73–83.

96. Liu, C.; Chen, J.; Gao, Y.; Deng, B.; Liu, K. Endothelin Receptor Antagonists for Pulmonary Arterial Hypertension. *Cochrane Database Syst. Rev.* **2013**, *2*, 1-68.

97. Langleben, D.; Brock, T.; Dixon, R.; Barst, R.; STRIDE-1 Study Group. Stride 1: Effects of the Selective ET(A) Receptor Antagonist, Sitaxsentan Sodium, in a Patient Population with Pulmonary Arterial Hypertension That Meets Traditional Inclusion Criteria of Previous Pulmonary Arterial Hypertension Trials. *J. Cardiovasc. Pharmacol.* **2004**, *44*



(Suppl. 1), S80-S84.

98. Barst, R. J.; Langleben, D.; Badesch, D.; Frost, A.; Lawrence, E. C.; Shapiro, S.; Naeije, R.; Galie, N. Treatment of Pulmonary Arterial Hypertension with the Selective Endothelin-A Receptor Antagonist Sitaxsentan. *J. Am. Coll. Cardiol.* **2006**, *47*, 2049-2056.

99. Sandoval, J.; Torbicki, A.; Souza, R.; Ramirez, A.; Kurzyna, M.; Jardim, C.; Jerjes-Sanchez Diaz, C.; Teal, S. A.; Hwang, L. J.; Pulido, T. Safety and Efficacy of Sitaxsentan 50 and 100 mg in Patients with Pulmonary Arterial Hypertension. *Pulm. Pharmacol. Therap.* **2012**, *25*, 33-39.

100. Barst, R. J.; Mubarak, K. K.; Machado, R. F.; Ataga, K. I.; Benza, R. L.; Castro, O.; Naeije, R.; Sood, N.; Swerdlow, P. S.; Hildesheim, M.; Gladwin, M. T. Exercise Capacity and Haemodynamics in Patients with Sickle Cell Disease with Pulmonary Hypertension Treated with Bosentan: Results of the ASSET Studies. *Br. J. Haematol.* **2010**, *149*, 426-435.

101. Channick, R. N.; Simonneau, G.; Sitbon, O.; Robbins, I. M.; Frost, A.; Tapson, V. F.; Badesch, D. B.; Roux, S.; Rainisio, M.; Bodin, F.; Rubin, L. J. Effects of the Dual Endothelin-receptor Antagonist Bosentan in Patients with Pulmonary Hypertension: A Randomized Placebo-controlled Study. *Lancet.* **2001**, *358*, 1119-1123.

102. Humbert, M.; Barst, R. J.; Robbins, I. M.; Channick, R. N.; Galiè, N.; Boonstra, A.; Rubin, L. J.; Horn, E. M.; Manes, A.; Simonneau, G. Combination of Bosentan with Epoprostenol in Pulmonary Arterial Hypertension: BREATHE-2. *Eur. Respir. J.* **2004**, *24*, 353-359.

103. Galiè, N.; Beghetti, M.; Gatzoulis, M. A.; Granton, J.; Berger, R. M.; Lauer, A.; Chiossi, E.; Landzberg, M.; Bosentan Randomized Trial of Endothelin Antagonist Therapy-5 (BREATHE-5) Investigators. Bosentan Therapy in Patients with Eisenmenger Syndrome: A Multicenter, Double-blind, Randomized, Placebo-controlled Study. *Circulation.* **2006**, *114*,

48-54.

104. McLaughlin, V.; Channick, R. N.; Ghofrani, H. A.; Lemarié, J. C.; Naeije, R.; Packer, M.; Souza, R.; Tapson, V. F.; Tolson, J.; Al Hiti, H.; Meyer, G.; Hoeper, M. M. Bosentan Added to Sildenafil Therapy in Patients with Pulmonary Arterial Hypertension. *Eur. Respir. J.* **2015**, *46*, 405-413.

105. Simonneau, G.; Galiè, N.; Jansa, P.; Meyer, G. M.; Al-Hiti, H.; Kusic-Pajic, A.; Lemarié, J. C.; Hoeper, M. M.; Rubin, L. J. Long-term Results from the EARLY Study of Bosentan in WHO Functional Class II Pulmonary Arterial Hypertension Patients. *Int. J. Cardiol.* **2014**, *172*, 332-339.

106. Wilkins, M. R.; Paul, G. A.; Strange, J. W.; Tunariu, N.; Gin-Sing, W.; Banya, W. A.; Westwood, M. A.; Stefanidis, A.; Ng, L. L.; Pennell, D. J.; Mohiaddin, R. H.; Nihoyannopoulos, P.; Gibbs, J. S. Sildenafil Versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) Study. *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 1292-1297.

107. Galiè, N.; Olschewski, H.; Oudiz, R. J.; Torres, F.; Frost, A.; Ghofrani, H. A.; Badesch, D. B.; McGoon, M. D.; McLaughlin, V. V.; Roecker, E. B.; Gerber, M. J.; Dufton, C.; Wiens, B. L.; Rubin, L. J.; Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-blind, Placebo-controlled, Multicenter, Efficacy Studies (ARIES) Group. Ambrisentan for the Treatment of Pulmonary Arterial Hypertension: Results of the Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-blind, Placebo-controlled, Multicenter, Efficacy (ARIES) Study 1 and 2. *Circulation.* **2008**, *117*, 3010–3019.

108. Galiè, N.; Barberà, J. A.; Frost, A. E.; Ghofrani, H. A.; Hoeper, M. M.; McLaughlin, V. V.; Peacock, A. J.; Simonneau, G.; Vachiery, J. L.; Grünig, E.; Oudiz, R. J.; Vonk-Noordegraaf, A.; White, R. J.; Blair, C.; Gillies, H.; Miller, K. L.; Harris, J. H.; Langley, J.;

Rubin, L. J.; AMBITION Investigators. Initial Use of Ambrisentan plus Tadalafil in Pulmonary Arterial Hypertension. *N. Engl. J. Med.* **2015**, *373*, 834-844.

109. Miyamoto, S.; Nagaya, N.; Satoh, T.; Kyotani, S.; Sakamaki, F.; Fujita, M.; Nakanishi, N.; Miyatake, K. Clinical Correlates and Prognostic Significance of Six-minute Walk Test in Patients with Primary Pulmonary Hypertension. Comparison with Cardiopulmonary Exercise Testing. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, 487-492.

110. Hoepfer, M. M.; Schwarze, M.; Ehlerding, S.; Adler-Schuermeyer, A.; Spiekerkoetter, E.; Niedermeyer, J.; Hamm, M.; Fabel, H. Long-term Treatment of Primary Pulmonary Hypertension with Aerosolized Iloprost, a Prostacyclin Analogue. *N. Engl. J. Med.* **2000**, *342*, 1866-1870.

111. Gomberg-Maitland, M.; Bull, T. M.; Sagar, R.; Barst, R. J.; Elgazayerly, A.; Fleming, T. R.; Grimminger, F.; Rainisio, M.; Stewart, D. J.; Stockbridge, N.; Ventura, C.; Ghofrani, A. H.; Rubin, L. J. New Trial Designs and Potential Therapies for Pulmonary Artery Hypertension. *J. Am. Coll. Cardiol.* **2013**, *62*, D82-D91.

112. Benza, R. L.; Gomberg-Maitland, M.; Miller, D. P.; Frost, A.; Frantz, R. P.; Foreman, A. J.; Badesch, D. B.; McGoon, M. D. The REVEAL Registry Risk Score Calculator in Patients Newly Diagnosed with Pulmonary Arterial Hypertension. *Chest.* **2012**, *141*, 354-362.

113. Galliè, N.; Humbert, M.; Vachiery, J. L.; Gibbs, S.; Lang, I.; Torbicki, A.; Simonneau, G.; Peacock, A.; Vonk Noordegraaf, A.; Beghetti, M.; Ghofrani, A.; Gomez Sanchez, M. A.; Hansmann, G.; Klepetko, W.; Lancellotti, P.; Matucci, M.; McDonagh, T.; Pierard, L. A.; Trindade, P. T.; Zompatori, M.; Hoepfer, M. ESC/ERS Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and

the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). International Society for Heart and Lung Transplantation (ISHLT). *Eur. Heart J.* **2016**, *37*, 67-119.

114. Ghofrani, H. A.; D'Armini, A. M.; Grimminger, F.; Hoeper, M. M.; Jansa, P.; Kim, N. H.; Mayer, E.; Simonneau, G.; Wilkins, M. R.; Fritsch, A.; Neuser, D.; Weimann, G.; Wang, C. Riociguat for the Treatment of Chronic Thromboembolic Pulmonary Hypertension. *New Eng. J. Med.* **2013**, *369*, 319-329.

115. Egidy, G.; Juillerat-Jeanneret, L.; Jeannin, J.-F.; Korth, P.; Bosman, F. T.; Pinet, F. Modulation of Human Colon Tumor-stromal Interactions by the Endothelin System. *Am. J. Pathol.* **2000**, *157*, 1863-1874.

116. Egidy, G.; Eberl, L. P.; Valdenaire, O.; Irmeler, M.; Majdi, R.; Diserens, A. C.; Fontana, A.; Janzer, R. C.; Pinet, F.; Juillerat-Jeanneret, L. The Endothelin System in Human Glioblastoma. *Lab. Invest.* **2000**, *80*, 1681-1689.

117. Douglas, M. L.; Richardson, M. M.; Nicol, D. L. Endothelin Axis Expression Is Markedly Different in the Two Main Subtypes of Renal Cell Carcinoma. *Cancer.* **2004**, *100*, 2118-2124.

118. Grant, K.; Loizidou, M.; Taylor, I. Endothelin-1: A Multifunctional Molecule in Cancer. *Br. J. Cancer.* **2003**, *88*, 163-166.

119. Grimshaw, M. J. Endothelins in Breast Tumour Cell Invasion. *Cancer Lett.* **2005**, *222*, 129-138.

120. Kusuhara, M.; Yamaguchi, K.; Nagasaki, K.; Hayashi, C.; Suzaki, A.; Hori, S.; Handa, S.; Nakamura, Y.; Abe, K. Production of Endothelin in Human Cancer Cell Lines. *Cancer Res.* **1990**, *50*, 3257-3261.

121. Nelson, J.; Bagnato, A.; Battistini, B.; Nisen, P. The Endothelin Axis: Emerging Role

in Cancer. *Nat. Rev. Cancer*. **2003**, *3*, 110-116.

122. Nelson, J. B.; Love, W.; Chin, J. L.; Saad, F.; Schulman, C. C.; Sleep, D. J.; Qian, J.; Steinberg, J.; Carducci, M.; Atrasentan Phase 3 Study Group. Phase 3, Randomized, Controlled Trial of Atrasentan in Patients with Nonmetastatic Hormone-refractory Prostate Cancer. *Cancer*. **2008**, *113*, 2478-2487.

123. Rosanò, L.; Spinella, F.; Bagnato, A. Endothelin 1 in Cancer: Biological Implications and Therapeutic Opportunities. *Nat. Rev. Cancer*. **2013**, *13*, 637-651.

124. Bagnato, A.; Rosanò, L. Understanding and Overcoming Chemoresistance in Ovarian Cancer: Emerging Role of the Endothelin Axis. *Curr. Oncol*. **2012**, *19*, 36-38.

125. Bagnato, A.; Loizidou, M.; Pflug, B. R.; Curwen, J.; Growcott, J. Role of the Endothelin Axis and Its Antagonists in the Treatment of Cancer. *Br. J. Pharmacol*. **2011**, *163*, 220-233.

126. Bagnato, A.; Natali, P. G. Endothelin Receptors as Novel Targets in Tumor Therapy. *J. Transl. Med*. **2004**, *2*, 16.

127. Bagnato, A. The Endothelin Axis as Therapeutic Target in Human Malignancies: Present and Future. *Curr. Pharm. Des*. **2012**, *18*, 2720-2733.

128. Peduto Eberl, L.; Bovey, R.; Juillerat-Jeanneret, L. Endothelin-receptor Antagonists Are Pro-apoptotic and Anti-proliferative in Human Colon Cancer Cells. *Br. J. Cancer*. **2003**, *88*, 788-795.

129. Peduto Eberl, L.; Egidy, G.; Pinet, F.; Juillerat-Jeanneret, L. Endothelin Receptor Blockade Potentiates FasL-induced Apoptosis in Colon Carcinoma Cells via the Protein Kinase C-pathway. *J. Cardiovasc. Pharm*. **2000**, *35*, S354-S356.

130. Said, N.; Theodorescu, D. Permissive Role of Endothelin Receptors in Tumor

Metastasis. *Life Sci.* **2012**, *91*, 522-527.

131. Irani, S.; Salajegheh, A.; Smith, R. A.; Lam, A. K. A Review of the Profile of Endothelin Axis in Cancer and Its Management. *Crit. Review Oncol. Hematol.* **2014**, *89*, 314-321.

132. Knowles, J.; Loizidou, M.; Taylor, I. Endothelin-1 and Angiogenesis in Cancer. *Curr. Vasc. Pharm.* **2005**, *3*, 309-314.

133. Knowles, J. P.; Shi-Wen, X.; Haque, S. U.; Bhalla, A.; Dashwood, M. R.; Yang, S.; Taylor, I.; Winslet, M. C.; Abraham, D. J.; Loizidou, M. Endothelin-1 Stimulates Colon Cancer Adjacent Fibroblasts. *Int. J. Cancer.* **2012**, *130*, 1264-1272.

134. Nelson, J. B. Endothelin Receptor Antagonists. *World J. Urol.* **2005**, *23*, 19-27.

135. Rosanò, L.; Spinella, F.; Di Castro, V.; Nicotra, M. R.; Dedhar, S.; de Herreros, A. G.; Natali, P. G.; Bagnato, A. Endothelin-1 Promotes Epithelial-to-mesenchymal Transition in Human Ovarian Cancer Cells. *Cancer Res.* **2005**, *65*, 11649-11657.

136. Rosanò, L.; Cianfrocca, R.; Spinella, F.; Di Castro, V.; Nicotra, M. R.; Lucidi, A.; Ferrandina, G.; Natali, P. G.; Bagnato, A. Acquisition of Chemoresistance and EMT Phenotype is Linked with Activation of the Endothelin A Receptor Pathway in Ovarian Carcinoma Cells. *Clin. Cancer Res.* **2011**, *17*, 2350-2360.

137. Rosanò, L.; Cianfrocca, R.; Tocci, P.; Spinella, F.; Di Castro, V.; Spadaro, F.; Salvati, E.; Biroccio, A. M.; Natali, P. G.; Bagnato, A. Beta-arrestin-1 is a Nuclear Transcriptional Regulator of Endothelin-1-induced Beta-catenin Signaling. *Oncogene.* **2013**, *32*, 5066-5077.

138. Cianfrocca, R.; Tocci, P.; Spinella, F.; Di Castro, V.; Bagnato, A.; Rosanò, L. The Endothelin A Receptor and Epidermal Growth Factor Receptor Signaling Converge on Beta-catenin to Promote Ovarian Cancer Metastasis. *Life Sci.* **2012**, *91*, 550-556.

139. Coffman, L.; Mooney, C.; Lim, J.; Bai, S.; Silva, I.; Gong, Y.; Yang, K.; Buckanovich, R. J. Endothelin Receptor-A is Required for the Recruitment of Antitumor T Cells and Modulates Chemotherapy Induction of Cancer Stem Cells. *Cancer Biol. Ther.* **2013**, *14*, 184-192.
140. Lahav, R.; Heffner, G.; Patterson, P. H. An Endothelin Receptor B Antagonist Inhibits Growth and Induces Cell Death in Human Melanoma Cells In Vitro and In Vivo. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11496-11500.
141. Hsieh, W. T.; Yeh, W. L.; Cheng, R. Y.; Lin, C.; Tsai, C. F.; Huang, B. R.; Wu, C. Y.; Lin, H. Y.; Huang, S. S.; Lu, D. Y. Exogenous Endothelin-1 Induces Cell Migration and Matrix Metalloproteinase Expression in U251 Human Glioblastoma Multiforme. *J. Neurooncol.* **2014**, *118*, 257-269.
142. Liu, Y.; Ye, F.; Yamada, K.; Tso, J. L.; Zhang, Y.; Nguyen, D. H.; Dong, Q.; Soto, H.; Choe, J.; Dembo, A.; Wheeler, H.; Eskin, A.; Schmid, I.; Yong, W. H.; Mischel, P. S.; Cloughesy, T. F.; Kornblum, H. I.; Nelson, S. F.; Liao, L. M.; Tso, C. L. Autocrine endothelin-3/Endothelin Receptor B Signaling Maintains Cellular and Molecular Properties of Glioblastoma Stem Cells. *Mol. Cancer Res.* **2011**, *9*, 1668-1685.
143. Paolillo, M.; Russo, M. A.; Curti, D.; Lanni, C.; Schinelli, S. Endothelin B Receptor Antagonists Block Proliferation and Induce Apoptosis in Glioma Cells. *Pharmacol. Res.* **2010**, *61*, 306-315.
144. Anguelova, E.; Beuvon, F.; Leonard, N.; Chaverot, N.; Varlet, P.; Couraud, P. O.; Dumas-Duport, C.; Cazaubon, S. Functional Endothelin ET B Receptors Are Selectively Expressed in Human Oligodendrogliomas. *Brain Res. Mol. Brain Res.* **2005**, *137*, 77-88.
145. Chen, C. C.; Chen, L. L.; Hsu, Y. T.; Liu, K. J.; Fan, C. S.; Huang, T. S. The Endothelin-integrin Axis is Involved in Macrophage-induced Breast Cancer Cell

Chemotactic Interactions with Endothelial Cells. *J. Biol. Chem.* **2014**, *289*, 10029-10044.

146. Moraitis, S.; Miller, W. R.; Smyth, J. F.; Langdon, S. P. Paracrine Regulation of Ovarian Cancer by Endothelin. *Eur. J. Cancer.* **1999**, *35*, 1381-1387.

147. Haque, S. U.; Dashwood, M. R.; Heetun, M.; Shiwen, X.; Farooqui, N.; Ramesh, B.; Welch, H.; Savage, F. J.; Ogunbiyi, O.; Abraham, D. J.; Loizidou, M. Efficacy of the Specific Endothelin A Receptor Antagonist Zibotentan (ZD4054) in Colorectal Cancer: A Preclinical Study. *Mol. Cancer Ther.* **2013**, *12*, 1556-1567.

148. Hoosein, M. M.; Dashwood, M. R.; Dawas, K.; Ali, H. M.; Grant, K.; Savage, F.; Taylor, I.; Loizidou, M. Altered Endothelin Receptor Subtypes in Colorectal Cancer. *Eur. J. Gastroenterol. Hepatol.* **2007**, *19*, 775-782.

149. Harland, S. P.; Kuc, R. E.; Pickard, J. D.; Davenport, A. P. Expression of Endothelin A Receptors in Human Gliomas and Meningiomas, with High Affinity for the Selective Antagonist PD156707. *Neurosurgery.* **1998**, *43*, 890-898.

150. Hartz, A. M.; Bauer, B.; Fricker, G.; Miller, D. S. Rapid Regulation of P-glycoprotein at the Blood-brain Barrier by Endothelin-1. *Mol. Pharmacol.* **2004**, *66*, 387-394.

151. Binder, C.; Hagemann, T.; Sperling, S.; Schulz, M.; Pukrop, T.; Grimshaw, M. J.; Ehrenreich, H. Stromal Endothelin B Receptor-deficiency Inhibits Breast Cancer Growth and Metastasis. *Mol. Cancer Ther.* **2009**, *8*, 2452-2460.

152. Watanabe, J.; Kaneko, Y.; Kurosumi, M.; Kobayashi, Y.; Sakamoto, M.; Yoshida, M. A.; Akiyama, M.; Matsushima, Y. High-incidence Spontaneous Tumors in JF1/Ms Mice: Relevance of Hypomorphic Germline Mutation and Subsequent Promoter Methylation of *Ednrb*. *J. Cancer Res. Clin. Oncol.* **2014**, *140*, 99-107.

153. De Freitas Cordeiro-Silva, M.; Stur, E.; Agostini, L. P.; de Podestá, J. R.; de Oliveira, J. C.; Soares, M. S.; Mendonça, E. F.; Gouvea, S. A.; Von Zeidler, S. V.; Louro,



D. Promoter Hypermethylation in Primary Squamous Cell Carcinoma of the Oral Cavity and Oropharynx: A Study of a Brazilian Cohort, I. *Mol. Biol. Rep.* **2012**, *39*, 10111-10119.

154. Jimeno, A.; Carducci, M. Atrasentan: a Novel and Rationally Designed Therapeutic Alternative in the Management of Cancer. *Exp. Rev. Anticancer Ther.* **2005**, *5*, 419-427.

155. Nelson, J. B.; Fizazi, K.; Miller, K.; Higano, C.; Moul, J. W.; Akaza, H.; Morris, T.; McIntosh, S.; Pemberton, K.; Gleave, M. Phase 3, Randomized, Placebo-controlled Study of zibotentan (ZD4054) in Patients with Castration-resistant Prostate Cancer Metastatic to Bone. *Cancer.* **2012**, *118*, 5709-5718.

156. Kohan, D. E.; Cleland, J. G.; Rubin, L. J.; Theodorescu, D.; Barton, M. Clinical Trials with Endothelin Receptor Antagonists: What Went Wrong and Where Can We Improve? *Life Sci.* **2012**, *91*, 528-539.

157. Lalich, M.; McNeel, D. G.; Wilding, G.; Liu, G. Endothelin Receptor Antagonists in Cancer Therapy. *Cancer Invest.* **2007**, *25*, 785-794.

158. Armstrong, A. J.; Creel, P.; Turnbull, J.; Moore, C.; Jaffe, T. A.; Haley, S.; Petros, W.; Yenser, S.; Gockerman, J. P.; Sleep, D.; Hurwitz, H.; George, D. J. A Phase I-II Study of Docetaxel and Atrasentan in Men with Castration-resistant Metastatic Prostate Cancer. *Clin. Cancer Res.* **2008**, *14*, 6270-6276.

159. Pinto, A.; Merino, M.; Zamora, P.; Redondo, A.; Castelo, B.; Espinosa, E. Targeting the Endothelin Axis in Prostate Carcinoma. *Tumour Biol.* **2012**, *33*, 421-426.

160. Witteveen, P. O.; van der Mijn, K. J.; Los, M.; Kronemeijer, R. H.; Groenewegen, G.; Voest, E. E. Phase 1/2 Study of Atrasentan Combined with Peggylated Liposomal Doxorubicin in Platinum-resistant Recurrent Ovarian Cancer. *Neoplasia.* **2010**, *12*, 941-945.

161. Fizazi, K.; Higano, J. B.; Nelson, J. B.; Gleave, M.; Miller, K.; Morris, T.; Nathan, F. E.; McIntosh, S.; Pemberton, K.; Moul, J. W.; Moul, J. W. Phase III, Randomized,

Placebo-controlled Study of Docetaxel in Combination with Zibotentan in Patients with Metastatic Castration-resistant Prostate Cancer. *J. Clin. Oncol.* **2013**, *31*, 1740–1747.

162. Phuphanich, S.; Carson, K. A.; Grossman, S. A.; Lesser, G.; Olson, J.; Mikkelsen, T.; Desideri, S.; Fisher, J. D.; New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium Phase I Safety Study of Escalating Doses of Atrasentan in Adults with Recurrent Malignant Glioma. *Neuro-Oncology.* **2008**, *10*, 617-623.

163. Kefford, R.; Beith, J. M.; Van Hazel, G. A.; Millward, M.; Trotter, J. M.; Wyld, D. K.; Kusic, R.; Shreeniwas, R.; Morganti, A.; Ballmer, A.; Segal, E.; Nayler, O.; Clozel, M. A phase II Study of Bosentan, a Dual Endothelin Receptor Antagonist, as Monotherapy in Patients with Stage IV Metastatic Melanoma. *Invest. New Drugs.* **2007**, *25*, 247-252.

164. Kefford, R. F.; Clingan, P. R.; Brady, B.; Ballmer, A.; Morganti, A.; Hersey, P. A Randomized, Double-blind, Placebo-controlled Study of High-dose Bosentan in Patients with Stage IV Metastatic Melanoma Receiving First-line Dacarbazine Chemotherapy. *Mol. Cancer.* **2010**, *9*, 69.

165. Shao, N.; Wang, Y.; Jiang, W. Y.; Qiao, D.; Zhang, S. G.; Wu, Y.; Zhang, X. X.; Wang, J. L.; Ding, Y.; Feng, N. H. Immunotherapy and Endothelin Receptor Antagonists for Treatment of Castration-resistant Prostate Cancer. *Int. J. Cancer.* **2013**, *133*, 1743-1750.

166. Wynn, T. A. Cellular and Molecular Mechanisms of Fibrosis. *J. Pathol.* **2008**, *214*, 199-210.

167. Clozel, M.; Salloukh, H. Role of Endothelin in Fibrosis and Anti-fibrotic Potential of Bosentan. *Ann. Med.* **2005**, *37*, 2-12.

168. Poncet, S.; Meyer, S.; Richard, C.; Aubert, J.; Juillerat-Jeanneret, L. The Expression and Function of the Endothelin System in Contractile Properties of Vaginal Myofibroblasts of Women with Uterovaginal Prolapse. *Am. J. Obst. Gynecol.* **2005**, *192*, 426-432.

169. Weng, C. M.; Yu, C. C.; Kuo, M. L.; Chen, B. C.; Lin, C. H. Endothelin-1 Induces Connective Tissue Growth Factor Expression in Human Lung Fibroblasts by ETAR-dependent JNK/AP-1 Pathway. *Biochem. Pharmacol.* **2014**, *88*, 402-411.
170. Antoniu, S. A. Targeting the Endothelin Pathway in the Idiopathic Pulmonary Fibrosis: The Role of Bosentan. *Expert Opin. Ther. Targets.* **2008**, *12*, 1077-1084.
171. King, T. E. Bosentan for Idiopathic Pulmonary Fibrosis. *Curr. Opin. Investig. Drugs.* **2008**, *9*, 1171-1179.
172. Raghu, G.; Million-Rousseau, R.; Morganti, A.; Perchenet, L.; Behr, J.; MUSIC Study Group. Macitentan for the Treatment of Idiopathic Pulmonary Fibrosis: The Randomized Controlled MUSIC Trial. *Eur. Respir. J.* **2013**, *42*, 1622-1632.
173. Yuan, W.; Li, Y.; Wang, J.; Li, J.; Gou, S.; Fu, P. Endothelin-receptor Antagonists for Diabetic Nephropathy: A Meta-Analysis. *Nephrology.* **2015**, *20*, 459-466.
174. Kohan, D. E.; Barton, M. Endothelin and Endothelin Antagonists in Chronic Kidney Disease. *Kidney Int.* **2014**, *86*, 896-904.
175. Murugesan, N. Gu, Z.; Fadnis, L.; Tellew, J. E.; Baska, R. A. F.; Yang, Y.; Beyer, S. M.; Monshizadegan, H.; Dickinson, K. E.; Valentine, M. T.; Humphreys, W. G.; Lan, S. J.; Ewing, W. R.; Carlson, K. E.; Kowala, M. C.; Zahler, R.; John E. Macor, J. E. Dual Angiotensin II and Endothelin A Receptor Antagonists: Synthesis of 2'-Substituted N-3-Isoxazolyl Biphenylsulfonamides with Improved Potency and Pharmacokinetics. *J. Med. Chem.* **2005**, *48*, 171-179.
176. Bai, R.; Wei, Z.; Liu, J.; Xie, W.; Yao, H.; Wu, X.; Jiang, J.; Wang, Q.; Xu, J. Synthesis and Biological Evaluation of 4'-(Benzimidazole-1-yl)methylbiphenyl-2-sulfonamide Derivatives as Dual Angiotensin II/Endothelin a Receptor Antagonists. *Bioorg. Med. Chem.* **2012**, *20*, 4661-4667.

177. Macdonald, R. L.; Higashida, R. T.; Keller, E.; Mayer, S. A.; Molyneux, A.; Raabe, A.; Vajkoczy, P.; Wanke, I.; Bach, D.; Frey, A.; Nowbakht, P.; Roux, S.; Kassell, N. Randomized Trial of Clazosentan in Patients with Aneurysmal Subarachnoid Hemorrhage Undergoing Endovascular Coiling. *Stroke*. **2012**, *43*, 1463–1469.
178. Donate, P. B.; Cunha, T. M.; Verri, W. A.; Junta, C. M.; Lima, F. O.; Vieira, S. M.; Peres, R. S.; Bombonato-Prado, K. F.; Louzada, P.; Ferreira, S. H.; Donadi, E. A.; Passos, G. A.; Cunha, F. Q. Bosentan, an Endothelin Receptor Antagonist, Ameliorates Collagen-induced Arthritis: The Role of TNF- $\alpha$  in the Induction of Endothelin System Genes. *Inflamm. Res.* **2012**, *61*, 337-348.
179. Freeman, B. D.; Machado, F. S.; Tanowitz, H. B.; Desruisseaux, M. S. Endothelin-1 and Its Role in the Pathogenesis of Infectious Diseases. *Life Sci.* **2014**, *118*, 110-119.
180. Suekane, T.; Ikura, Y.; Arimoto, J.; Nakagawa, M.; Kitabayashi, C.; Naruko, T.; Watanabe, T.; Fujiwara, Y.; Oshitani, N.; Maeda, K.; Tanzawa, K.; Hirakawa, K.; Arakawa, T.; Ueda, M. Enhanced Expression of Endothelin-converting Enzyme and Endothelin Receptors in Human Colonic Tissues of Crohn's Disease. *J. Clin. Biochem. Nutr.* **2008**, *42*, 126-132.
181. Kowalczyk, A.; Kleniewska, P.; Kolodziejczyk, M.; Skibska, B.; Goraca, A. The Role of Endothelin-1 and Endothelin Receptor Antagonists in Inflammatory Response and Sepsis. *Arch. Immunol. Ther. Exp.* **2015**, *63*, 41-52.
182. Guo, J.; Li, Y.; He, Z.; Zhang, B.; Li, Y.; Hu, J.; Han, M.; Xu, Y.; Li, Y.; Gu, J.; Dai, B.; Chen, Z. Targeting Endothelin Receptors A and B Attenuates the Inflammatory Response and Improves Locomotor Function Following Spinal Cord Injury in Mice. *Int. J. Mol. Med.* **2014**, *34*, 74-82.
183. Reichetzeder, C.; Tsuprykov, O.; Hocher, B. Endothelin Receptor Antagonists in

Clinical Research-lessons Learned from Preclinical and Clinical Kidney Studies. *Life Sci.* **2014**, *118*, 141-148.

184. Remuzzi, G.; Perico, N.; Benigni, A. New Therapeutics that Antagonize Endothelin: Promises and Frustrations. *Nat. Rev. Drug Disc.* **2002**, *1*, 986-1001.

185. Gagliardini, E.; Zoja, C.; Benigni, A. ET and Diabetic Nephropathy: Preclinical and Clinical Studies. *Semin. Nephrol.* **2015**, *35*, 188-196.

186. Maguire, J. J.; Davenport, A. P. Endothelin@25 - New Agonists, Antagonists, Inhibitors and Emerging Research Frontiers: IUPHAR Review 12. *Br. J. Pharmacol.* **2014**, *171*, 5555-5572.

## TOC graphic

