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Review

Clinical trials with endothelin receptor antagonists: What went wrong and where can we improve?☆

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ABSTRACT

In the early 1990s, within three years of cloning of endothelin receptors, orally active endothelin receptor antagonists (ERAs) were tested in humans and the first clinical trial of ERA therapy in humans was published in 1995. ERAs were subsequently tested in clinical trials involving heart failure, pulmonary arterial hypertension, resistant arterial hypertension, stroke/subarachnoid hemorrhage and various forms of cancer. The results of most of these trials – except those for pulmonary arterial hypertension and scleroderma-related digital ulcers – were either negative or neutral. Problems with study design, patient selection, drug toxicity, and drug dosing have been used to explain or excuse failures. Currently, a number of pharmaceutical companies who had developed ERAs as drug candidates have discontinued clinical trials or further drug development. Given the problems with using ERAs in clinical medicine, at the *Twelfth International Conference on Endothelin* in Cambridge, UK, a panel discussion was held by clinicians actively involved in clinical development of ERA therapy in renal disease, systemic and pulmonary arterial hypertension, heart failure, and cancer. This article provides summaries from the panel discussion as well as personal perspectives of the panelists on how to proceed with further clinical testing of ERAs and guidance for researchers and decision makers in clinical drug development on where future research efforts might best be focused.

Contents

☆ This article represents a summary of the panel discussion "Clinical trials with endothelin receptor antagonists: What went wrong and where can we improve" moderated by Donald E. Kohan and held at the *Twelfth International Conference on Endothelin*, Clare College, The University of Cambridge, United Kingdom, on September 13, 2011. Panelists were Donald E. Kohan (renal disease and arterial hypertension), John G. Cleland (heart failure), Lewis J. Rubin (pulmonary arterial hypertension) and Dan Theodorescu (cancer). This article was submitted directly to and handled by Dr. Frank Porecca, Editor-in-Chief, *Life Sciences*, for assignment of peer reviewers and final decision.

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Introduction

Twenty years ago – only a few years after cloning of the two mammalian endothelin (ET) receptors (Arai et al., 1990; Sakurai et al., 1990) – orally active ET receptor antagonists (ERAs) were discovered (Atkinson and Pelton, 1992; Bazil et al., 1992; Breu et al., 1993; Clozel et al., 1993, 1994; Fukuroda et al., 1992; Ihara et al., 1991, 1992; Spinella et al., 1991), opening new therapeutic opportunities for treating human disease (Battistini et al., 2006). Many pharmaceutical companies identified and synthesized orally active ET_A receptor-selective or non-selective, ET_A/ET_B ERAs as drug candidates, which rapidly were put into clinical testing (Barton and Kohan, 2011; Battistini et al., 2006). In 1995, only 5 years after cloning of ET receptors (Arai et al., 1990; Sakurai et al., 1990), the first clinical study using ERA therapy in patients with severe heart failure was published (Kiowski et al., 1995). At the time when this and other studies were conducted, the biology of ET and its receptors in health and disease was only beginning to be understood (Barton and Yanagisawa, 2008). Subsequently, a large number of Phase II and III trials were conducted for a variety of disorders, including heart failure, cancer, pulmonary arterial hypertension, arterial hypertension, proteinuric renal disease, and autoimmune diseases (Battistini et al., 2006). Despite such intensive efforts, ERAs have been approved by the U.S. Food and Drug Administration for only two drugs and only two indications: bosentan and ambrisentan in pulmonary arterial hypertension (Rubin et al., 2002; Galie et al., 2008a,b), and bosentan in scleroderma-related digital ulcers (Dhillon, 2009) (Fig. 1). Currently, clinical testing is ongoing for subarachnoid hemorrhage, proteinuric renal disease, and coronary artery disease (Barton and Yanagisawa, 2008).

We have recently discussed some of the causes that led to failure of clinical trials using ERAs (including study design, patient selection, and drug dosing) (Barton and Kohan, 2011), and have re-emphasized the need for access to all data obtained in previous clinical ERA trials as initially proposed by Kelland and Webb (2007). In view of the therapeutic opportunities, combined with the difficulties that the field has experienced, a panel discussion by clinicians actively involved in clinical testing of ERAs was held at the *Twelfth International Conference on Endothelin*. This article represents a summary of the panel discussion; the following four sections were written by the relevant panelist on renal disease, heart failure, pulmonary arterial hypertension, or cancer. Each section describes data and personal views on the current state of ERA drug therapy in human disease; of particular importance, guidance is provided on how to best move forward to realize the potential of this class of drugs.

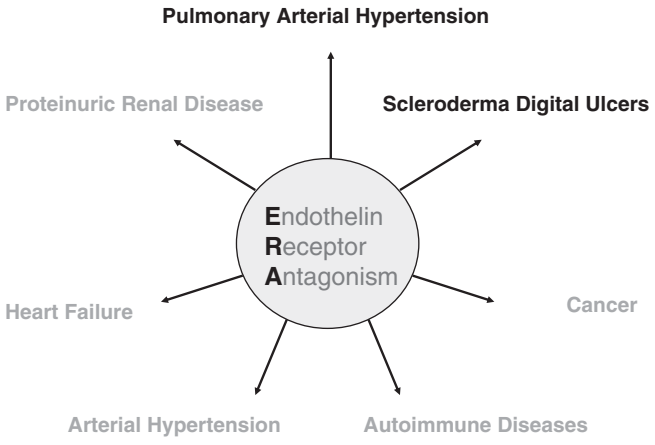

Fig. 1. Human diseases for which ERA therapy has been evaluated in clinical trials. Black print indicates disease indication for which the use of ERAs as therapeutics has been approved by the U.S. Food and Drug Administration, gray print indicates disease indications for which clinical trials have been completed or are currently ongoing but have not yet received approval for clinical use by the FDA or other regulatory agencies. See text for details.

Endothelin receptor antagonism in patients with chronic kidney disease and arterial hypertension

Endothelins are important regulators of kidney function and arterial pressure (Dhaun et al., 2006; Kohan et al., 2011a,b,c). Endogenous ET controls renal cell growth and proliferation, fluid and electrolyte excretion, renal vascular tone, immune function and other parameters (Dhaun et al., 2006; Kohan et al., 2011a,b,c; Schneider et al., 2007). Renal ET-1 production is increased in numerous forms of renal disease (Barton, 2010; Kohan, 2010); as will be described, the ET system plays an important role in renal diseases and blockade of this system has substantial potential benefit in helping to prevent kidney disease progression.

Chronic kidney disease and arterial hypertension are good targets for endothelin receptor antagonists

ET has been strongly implicated in the pathogenesis and progression of experimental chronic kidney disease (CKD), including diabetic nephropathy, glomerulonephritis, hypertensive nephrosclerosis, reduced renal mass and others (Barton, 2010; Benigni et al., 1998, 2004; Dhaun et al., 2006; Kohan, 2010; Orisio et al., 1993). ERAs, and particularly ET_A receptor blockers, confer substantial nephroprotective effects in various models of CKD (Barton, 2008; Benigni et al., 1998, 2004; Dhaun et al., 2006; Kohan, 2010; Neuhofer and Pittrow, 2009). In an exciting study, combined ERA and angiotensin receptor blocker treatment induced regression of renal injury in experimental diabetes (Gagliardini et al., 2009). Clinical trials (Phase II or III) with various ERAs (including atrasentan, avosentan, darusentan, and sitaxsentan) showed reduced proteinuria in patients with CKD (Dhaun et al., 2011; Honing et al., 2000; Kohan et al., 2011a,b,c; Mann et al., 2010; Weber et al., 2009; Wenzel et al., 2009). ET also has been strongly linked with hypertension (Bakris et al., 2010; Battistini et al., 2006; Kohan et

al., 2011a,b,c; Krum et al., 1998; Lazich and Bakris, 2011; Nakov et al., 2002). ERAs, whether targeting ET_A or both ET_A and ET_B receptors, significantly reduced arterial pressure in patients with essential hypertension, treatment-resistant hypertension and/or CKD (Bakris et al., 2010; Goddard et al., 2004; Lazich and Bakris, 2011; Nakov et al., 2002).

Despite these successes with ERAs in CKD and hypertension, no ERAs have been approved by any regulatory agency and clinical trials have markedly decreased. Currently, only atrasentan is being actively studied for use in patients with CKD (Andress et al., 2012; Kohan et al., 2011a,b,c) while there are no on-going clinical trials with ERAs in arterial hypertension.

The reasons for this death of ERA trials in CKD, as well as the current lack of trials with ERAs in hypertension, are varied, and as will be discussed, illustrate how problems with study design, selection of primary endpoints, and adverse effects have greatly limited the clinical application of ERAs in diseases that have shown every indication that they will be highly responsive to ET receptor antagonism.

Adverse effects of eras in human trials in CKD: could they have been avoided?

ERAs are associated with adverse effects, including fluid retention, hepatotoxicity, testicular toxicity and teratogenesis. Like inhibitors of the angiotensin system such as ACEI and ARBs, ERAs are absolutely contraindicated in pregnancy. ERA-induced testicular toxicity in experimental animals is described in drug company product literature (for both ET_A receptor selective and non-selective antagonists) or in dissertations without presenting the actual data (Grass, 2006). In the product literature for bosentan, a non-selective sulphonamide ERA, it is stated that many ERAs induce marked atrophy of the seminiferous tubules of the testes, reduce sperm counts, and decrease male fertility in rats when administered for longer than 10 weeks. It is also stated that these effects of bosentan in humans appear to be irreversible. Despite such findings, amazingly no peer-reviewed study involving either experimental animals or patients devoted to ERA-induced testicular toxicity has been published. An unpublished open-label study in patients with pulmonary hypertension determined that bosentan can reduce sperm count in men. Given the potential seriousness of this side effect, one cannot help but wonder why the scientific community has not investigated this in more detail, and particularly under the scrutiny of peer review. Whether ERA treatment may differently affect testicular toxicity in humans under disease conditions such as diabetes (which so far has been studied only in experimental animals (Cai et al., 2000)) is currently unknown.

Hepatotoxicity is a concern with ERAs, and particularly those that contain a sulfonamide moiety (Hoeper, 2009; Hoeper et al., 2009; McGoon et al., 2009). Sitaxentan, a sulfonamide-based ERA, was being studied in CKD and had demonstrated an antiproteinuric effect (Dhaun et al., 2011). However, in 2010, four cases of fatal liver failure, possibly idiosyncratic, were reported in approximately 2000 sitaxsentan-treated patients. This contrasts with no cases of liver failure in over 90,000 patients on either bosentan or ambrisentan (Galie et al., 2011). Consequently, sitaxsentan was withdrawn from the market.

Perhaps the most glaring example of how an ERA adverse effect has affected clinical trials in CKD relates to ERA-induced fluid retention. All ERAs used in clinical trials, regardless of receptor isoform specificity, cause fluid retention (Battistini et al., 2006), including bosentan, darusentan, tezosentan, ambrisentan, sitaxsentan, avosentan, zibotentan and atrasentan. The degree of fluid retention is dose-dependent and is related to the individual's propensity, being exacerbated by congestive heart failure and renal insufficiency. The importance of fluid retention is best illustrated by a recently failed trial using avosentan (ASCEND trial) in patients with diabetic nephropathy with glomerular filtration rates between 15 and 60 ml/min (i.e., moderate to advanced CKD (Mann et al., 2010)). This Phase III trial was conducted without any preceding publications on avosentan-induced fluid retention in health or in kidney disease. While avosentan significantly reduced proteinuria, it caused marked fluid retention (about 50% of patients in the avosentan groups had fluid retention from mild to severe, as compared to about 33% in the placebo group). Consequently, the trial was prematurely terminated. In retrospect, the doses employed in this trial were too high since a Phase II study, published a year before the results of the ASCEND trial were published, showed that avosentan exerted antiproteinuric effects at substantially lower doses than those used in the ASCEND trial and caused only modest fluid retention (Wenzel et al., 2009). Clearly, the question must be raised as to the choice of doses employed in the ASCEND trial, and how this might have been better informed if studies, particularly peer-reviewed, were conducted in advance of undertaking a large Phase III trial.

Problems with study design have impacted clinical trials of eras in arterial hypertension

The substantial preclinical literature and some clinical studies suggested that ERAs could be effective antihypertensive agents, particularly in the setting of resistant hypertension (Barton et al., 2006; Barton and Yanagisawa, 2008). In a Phase III trial in resistant hypertension (DORADO), darusentan, a relatively ET_A receptor-selective antagonist, reduced proteinuria and lowered blood pressure in patients with CKD (Weber et al., 2009). In a second Phase III trial (DORADO-AC), darusentan reduced ambulatory blood pressure to a greater degree than the active control, guanfacine (Bakris et al., 2010). However, the primary endpoint of office blood pressure reduction was not met, hence the company decided to discontinue further development of darusentan for the clinical indication of resistant hypertension. Thus, due to the unfortunate choice of the primary endpoint, as well as other issues such as side effects (particularly dose-related fluid-retention) and economical issues caused by costs required to perform additional Phase I and II trials in arterial hypertension (Barton and Kohan, 2011; Lazich and Bakris, 2011; Webb, 2010), development of ERAs for the treatment of arterial hypertension has been essentially abandoned.

Using lessons from the past to inform future era trials in kidney disease and arterial hypertension

Moving forward, it is obvious that, as has been pointed out previously (Kelland and Webb, 2007), there must be increased efforts to fully disclose and publish peer-reviewed studies, both experimental and clinical, about ERA actions, pharmacology and adverse effects. It is realized that the clock on ERAs is ticking; many of these drugs are nearing the end of their patent life and companies must be highly selective about clinical conditions for which they seek indications. Nonetheless, CKD in particular, and possibly resistant hypertension, remain highly attractive ERA targets.

How do we optimize future trials studying ERAs in kidney disease and hypertension? First, patients must be carefully selected, excluding the elderly (perhaps >80 years old) and patients with congestive heart failure or advanced CKD (i.e. stage 4 or greater). The issue of testicular safety in young men remains to be fully elucidated, so treatment of these individuals must be approached cautiously. Second, the dose of ERA must be carefully chosen and adjusted; a recent Phase IIA trial with atrasentan in diabetic nephropathy demonstrated that careful ERA dosing can largely avoid significant fluid retention, yet still have a substantial antiproteinuric effect (Kohan et al., 2011a,b,c). In addition, the judicious use of diuretics, particularly early in the course of ERA treatment, may substantially mitigate fluid retention. Third, great care must be paid to study design, including identification of the optimal endpoints and disease markers. Finally, while not extensively discussed in this review, the bulk of literature supports the notion that selective ET_A receptor antagonists are likely to have a greater beneficial effect on kidney disease and blood pressure as compared to non-selective ERAs. With careful attention to these aforementioned issues, there is

every reason to believe that ERAs will indeed prove to be safe and efficacious in the treatment of kidney disease and possibly hypertension, giving us the first new agents for treatment of these disorders in many years.

Endothelin receptor antagonism in patients with heart failure

Heart failure is common and may afflict people at any age but most patients in most countries are aged >60 years (Cleland et al., 2011, 2003). Up to one in three people will develop heart failure at some time in their life (Bleumink et al., 2004; Lloyd-Jones et al., 2002) but this might be a serious under-estimate due to inadequate case ascertainment and frequent failure to identify or highlight heart failure as a complication of other cardiac problems (Cleland et al., 2007, 2009a,b). Most people who die of cardiac disease will first develop heart failure (Torabi et al., 2008, 2009).

Prevalence and mortality of heart failure

The life-time risk of developing heart failure may be high, but the prevalence at any moment is modest and probably at most 3% of adults or about 2% of the entire population will have heart failure (Cleland et al., 2001). This may be >100 million people worldwide at any one time although differences in age and pathophysiology are likely to be heterogeneous amongst regions. The disparity between incidence and prevalence reflects the high mortality (Torabi et al., 2008). Once patients develop heart failure, annual mortality is high, ranging from about 5% per year in stable, well-treated patients with mild disease to more than 30% in patients who have new-onset heart failure or who have experienced a recent hospitalization for worsening symptoms (Cleland et al., 2011, 2009a,b; Harjola et al., 2011). Heart failure is often a terminal process with prognosis measured in days, weeks or months rather than years. However, expert care can restore many patients to a good quality of life for prolonged periods.

Etiology and current therapies of heart failure

Effective management of hypertension and coronary artery and valve disease will delay the onset of heart failure and reduce its incidence in younger people. However, as life expectancy increases and the proportion of the population aged >70 years rises, the prevalence of heart failure will increase (Cleland et al., 2001). Patients who previously would have died of stroke or myocardial infarction will now live longer after the onset of cardiovascular disease, which will fuel a further increase in the prevalence and reported incidence of heart failure, even if age-adjusted rates fall. Moreover, contemporary pharmacological therapy may have tripled life expectancy and therefore, provided the patient can be stabilized on therapy, this will also increase the prevalence of heart failure (Cleland and Clark, 2003).

Heart failure is a complex, multi-dimensional clinical problem with diverse pathophysiology both in terms of etiology and consequences. It is a systemic disease caused by cardiac dysfunction. Ischemic heart disease, hypertension and idiopathic dilated cardiomyopathy are key etiologies, while pulmonary hypertension (Damy et al., 2010), atrial fibrillation (Shelton et al., 2010), renal dysfunction (de Silva et al., 2006a,b), and anemia (de Silva et al., 2006a,b) may be causes and/or consequences of heart failure. Some treatments, such as diuretics, may be applied generically to all forms of heart failure, but most are directed at specific subgroups such as valve disease, electrical disturbances or left ventricular systolic dysfunction. Good patient management requires in-depth knowledge of the disease and its treatment as well as a more holistic assessment of the patients' needs.

Endothelin in heart failure

Endothelin has many cardiovascular effects that may drive the progression of cardiovascular disease and heart failure. It is a powerful constrictor of both systemic and pulmonary arterioles and veins (Serneri et al., 1995). These effects may be mitigated by increased prostaglandin synthesis that develops with heart failure. Prostaglandin synthesis is enhanced by ACE inhibitors and blocked by the administration of aspirin (Cleland, 2006) and may increase the vasoconstrictor effects of ET (Haynes and Webb, 1993). Endothelin may also cause myocyte hypertrophy, both vascular and myocardial, and fibrosis. Its effects on renal sodium handling are less certain (Modesti et al., 1998). Renal cortical vasoconstriction may cause sodium retention but effects on the proximal renal tubule and other nephron segments, possibly mediated by the ET_A receptor, may causes natriuresis (Burnier and Forni, 2012; Smolander et al., 2009, Kohan et al., 2011a,b,c). Endothelin also has positive inotropic and chronotropic effects on the myocardium (Barton and Yanagisawa, 2008; Concas et al., 1989; Moravec et al., 1989; Watanabe et al., 1989). Thus, as with most biological systems, the effects of interference are difficult to predict.

Plasma concentrations of ET are increased in heart failure regardless of phenotype or disease etiology but in proportion to the severity of symptoms (Rodeheffer et al., 1992). It is likely that much of the actions of endothelins are mediated by local concentrations (paracrine) but infusing endothelin-1 to achieve the plasma concentrations observed in disease causes vasoconstriction (Cowburn et al., 1998, 1999), indicating that ET can also be considered an endocrine system. Infusing endothelin-3, which is more selective for the ET_B receptor, has similar hemodynamic effects to endothelin-1 (Cowburn et al., 1999). In patients with heart failure, the ET_A receptor appears mainly responsible for the hemodynamic consequences of ET excess while the ET_B receptor appears mainly involved in ET clearance (Cowburn et al., 2005). Blockade of ET_B receptors has adverse hemodynamic consequence (Cowburn et al., 2005). The paradoxical effects of ET_B stimulation and blockade indicate the complexity of the system, likely different populations of ET_B receptors and the consequences of blocking ET clearance.

Plasma concentrations of ET or its precursors are strongly related to prognosis in heart failure (Hulsmann et al., 1998; Omland et al., 1994; Pacher et al., 1996; Rodeheffer et al., 1992). There is increasing interest in the role of pulmonary hypertension and right heart dysfunction in patients with heart failure, which seem to be better guides to prognosis than left ventricular dysfunction (Damy et al., 2010). Plasma ET is more closely related to pulmonary vascular resistance than other hemodynamic features of heart failure and this relationship may be causal (Cody et al., 1992; Givertz et al., 2000; Good et al., 1994; Ooi et al., 2002). Thus, ET could be an important driver of this pathway of progression of heart failure. Experimental prevention studies of myocardial infarction in mice and rats suggest that ERA therapy initiated prior to or *immediately after* infarction can also prevent myocardial remodeling (Mulder et al., 2000, 1998, 1997; Sakai et al., 1996) but only one experimental study investigated ERA therapy in animals with *established* heart failure and found no benefit on survival (Vetter et al., 2006). A meta-analysis of prevention studies initiating ERA immediately after experimental myocardial infarction also found no benefit on survival (Lee et al., 2003).

Clinical studies of ERAs in heart failure

Administration of bosentan, a non-selective ERA, to patients with severe heart failure resulted in hemodynamic benefits, with striking reductions in systemic and pulmonary vascular resistances and atrial pressures and a rise in cardiac output (Kiowski et al., 1995; Schalcher et al., 2001; Sutsch and Barton, 1999; Sutsch et al., 1998). However, blockade was associated with a reflex increase in circulating ET and further activation of the renin–angiotensin system. The encouraging hemodynamic results led to the first of a series of randomized

controlled trials that had rather disappointing results (Table 1). Various excuses were made for the neutral or negative results, including dose, receptor selectivity or patient population but, so far, no change in strategy has resulted in a convincingly positive result and many studies even showed trends to harm. In studies of chronic heart failure the main problem appears to be fluid retention, as evidence by weight gain, more peripheral and pulmonary edema, and plasma volume expansion, as evidenced by a fall in hemoglobin (Coletta et al., 2002; Packer et al., 2005). There is no evidence of a beneficial effect on cardiac remodeling despite improved hemodynamics (Anand et al., 2004; Prasad et al., 2006). In studies of acute heart failure, hypotension, renal dysfunction and reductions in arterial oxygen tension, the latter suggesting worsening pulmonary ventilation/perfusion matching, appear to be important problems (Coletta et al., 2002; Kaluski et al., 2003; McMurray et al., 2007).

Heart failure has also been reported as a side effect of ERAs used for other indications. Over a median follow-up of 4 months, avosentan (ET_A selective) reduced blood pressure and micro-albuminuria in patients with type-2 diabetes mellitus but caused weight gain and a fall in hemoglobin, suggesting fluid retention and plasma volume expansion (Mann et al., 2010). This was accompanied by strong trends for worsening renal function and an increased risk of developing heart failure (2.2% on placebo versus >6.0% with avosentan; $p=0.05$) (Mann et al., 2010). Mortality was 2.6% on placebo compared to >6.0% with avosentan (ns). In a large study of prostate cancer, atrasentan (ET_A selective) increased the risk of developing heart failure from 3.0% to 6.7% ($p=0.009$) (Nelson et al., 2008) without any effect on survival, and fluid retention/edema development has also been recently reported to occur in patients with prostate cancer treated with the ET_A receptor-specific antagonist zibotentan (Nelson et al., 2012), a drug devoid of any activity on the ET_B receptor (Rosano et al., 2007). Fluid retention and edema during ERA therapy have also been reported in studies of resistant hypertension (Weber et al., 2009), idiopathic pulmonary arterial hypertension (Galie et al., 2008a,b), thrombo-embolic pulmonary hypertension (Jais et al., 2008), coronary artery disease (Raichlin et al., 2008; Reriani et al., 2010), and even in mountain sickness (Modesti et al., 1998).

Fundamentally, ERAs appear to have delivered their expected hemodynamic effects but this has been offset by fluid retention and indiscriminate vasodilatation, an effect that may depend on the dose used (Kelland and Webb, 2006). A healthy vasomotor system constricts and dilates selectively to distribute blood flow in an efficient manner to vital organs according to their metabolic demands (Cleland and Oakley, 1991). Vasodilatation that is 'unintelligent' may direct blood away from where it is most needed. Moreover, a fall in perfusion pressure may have adverse consequences in heart failure, including activation of the renin–angiotensin and sympathetic nervous systems, leading to sodium retention and further derangement in blood flow distribution. A low blood pressure is a bad prognostic sign in heart failure (Raphael et al., 2009). ERAs have worsened arterial oxygen saturation

Table 1
Clinical trials investigating the effects of ERA therapy on symptoms, ventricular remodeling, or clinical outcome in patients with heart failure. Trial references: *a*, (Packer et al., 2005); *b*, (Coletta et al., 2002); c, (Louis et al., 2001); *d*, (Luscher et al., 2002); *e*, (Anand et al., 2004); *f*, (Prasad et al., 2006); g, (Coletta and Cleland, 2001); *h*, (Louis et al., 2001); *i*, (O'Connor et al., 2003); *j*, (Kaluski et al., 2003); *k*, (Cotter et al., 2004); *l*, (McMurray et al., 2007); ERA = endothelin receptor antagonist. # = death or hospitalization for worsening heart failure during or within 48 h of completion of infusion. ACS = acute coronary syndrome. & = readmission but not otherwise specified. NA = not available.

Trial	Agent	Receptor	N	Duration of follow-up	Death		Worsening HF		Comments on ERA
Chronic heart failure					Placebo	ERA	Placebo	ERA	
REACH-1[a]	Bosentan 500 mg bid	$ET_{A/B}$	369	~6 m	8/125 (6.4%)	17/244 (7.0%)	27/125 (21.6%)	47/244 (19.3%)	Early excess of worsening heart failure events
ENABLE[b]	Bosentan 125 mg bd	$ET_{A/B}$	1611	18 m	173/808 (21.4%)	160/805 (19.9%)	321/808 # (39.7%)#	312/805# (38.8%)	Fluid retention
ENCOR[c]	Enrasentan Dose-ranging	$ET_{A/B}$	419	9 m	NA (3.5%)	NA (5.9%)	Adverse trend with enrasentan		More adverse events
HEAT[d]	Darusentan Dose-ranging	ET_A	157	1 m	0/33 (0.0%)	4/124 (3.2%)	4/33 (12.1%)	29/124 (23.4%)	Headaches
EARTH[e]	Darusentan Dose-ranging	ET_A	642	6 m	4/110 (3.6%)	26/532 (4.9%)	9/110 (8.2%)	53/532 (10.0%)	No benefit on LV remodeling
Chronic heart failure					Enalapril	Enrasentan	Enalapril	Enrasentan	
Pennell[f]	Enrasentan	$ET_{A/B}$	72	6 m	1/36 (2.8%)	1/36 (2.8%)	10/36 (27.8%)	8/36 (22.2%)	More favorable LV remodeling with enalapril ($p=0.001$)

	Target	Duration and dose	N	Duration of follow-up	Death	Worsening HF	Comments on tezosentan	Target	Duration and dose
Acute heart failure all conducted with intravenous tezosentan ($ET_{A/B}$)									
RITZ-1[g]	Symptoms	24–72 h 50 mg/h	669	1 m	17/339 (5.0%)	24/336 (7.1%)	39# (11.5%)	51# (15.4%)	Symptoms not improved
RITZ-2[h]	Hemodynamics	24 h 50–100 mg/h	285	1 m	5/94 (5.3%)	16/191 (8.4%)	22/94 (23.4%)	26/191 (13.6%)	Concern about renal function
RITZ-4[i]	ACS	24–48 h 25–50 mg/h	192	72 h	3/95 (3.2%)	3/97 (3.1%)	12/95 (12.6%)	20/97 (20.6%)	More symptomatic hypotension. No adverse effect on ACS
RITZ-5[j]	Pulmonary edema	24 h 50–100 mg/h	84	1 m	2/42 (4.8%)	5/42 (11.9%)	16/42& (38.1%)	16/42& (38.1%)	Fall in arterial oxygen saturation
Cotter et al.[k]	Hemodynamics	24 h 0.2–25 mg/h	129	1 m	0/26 (0.0%)	5/103 (4.9%)	5/26 (19.2%)	17/103 (16.5%)	1 mg/h dose may suffice
VERITAS[l]	Symptoms/ outcome	24–72 h 1 mg/h	1435	1 m	34/708 (4.8%)	28/727 (3.9%)	235/708# (33.2%)	232/727# (31.9%)	Symptoms not improved. Fall in arterial oxygen saturation

when given during acute heart failure episodes (Kaluski et al., 2003). Failure to identify, manage, or understand these intricate aspects of ET pathophysiology has undoubtedly contributed to the failure of clinical research in this arena. In particular, the effects of ERAs on salt and water homeostasis need to be understood. There is preliminary evidence to suggest that an ET_A receptor-mediated mechanism in the collecting duct is responsible for fluid retention following ET_A receptor blockade (Kohan et al., 2011a,b,c).

Is there still a future for ERAs in heart failure?

Surely there must be, but it would be foolish to conduct further research without either better targeting of these agents or a better understanding of their effects on organ perfusion at the tissue level and on renal salt and water handling. Pulmonary hypertension primarily due to increased pulmonary vasomotor tone and subsequent right heart failure appear to be an obvious target. However, in heart failure, pulmonary hypertension is commonly due to left atrial hypertension and secondary pulmonary vasoconstriction; typically, the pulmonary artery systolic pressure at rest is only 40–50 mm Hg (Damy et al., 2010). However, when the right ventricle starts to fail, pulmonary vascular resistance may continue to climb without an increase in pulmonary artery pressure but a downward spiral in blood flow leading to death (Damy et al., 2012). Finding a molecule (or a dose) that does not cause sodium retention or an effective antidote to this effect (Kohan et al., 2011a,b,c) might also transform the fortunes of ERAs. Clearly, as with other neuro-endocrine antagonists, it may depend not only on what you do but also on how you do it!

Endothelin receptor antagonists for therapy of pulmonary arterial hypertension unrelated to heart failure

Early studies in experimental models of PAH as well as clinical studies in PAH patients (Giaid et al., 1993) have demonstrated remarkable up-regulation of ET-1 in the pulmonary arterial vascular bed in diseased but not normal pulmonary arteries suggesting ET-1 might be a novel therapeutic target. This was also suggested by increased circulating ET-1 levels in pulmonary hypertension (Cody et al., 1992; Stewart et al., 1991; Yoshibayashi et al., 1991). Indeed, while ET-1, a vasoconstrictor and a smooth muscle mitogen (Komuro et al., 1988) has been implicated in the pathogenesis of a variety of diseases (Barton and Yanagisawa, 2008), ET receptor blockade has met with success as an efficacious therapy for one particular condition — pulmonary arterial hypertension (PAH).

Bosentan therapy in pulmonary arterial hypertension

The initial effort to evaluate bosentan, a non-selective ET_A and ET_B receptor antagonist, in PAH, consisted of a small, double-blind, placebo-controlled, multicenter study of 32 functional class III subjects who were randomized to receive bosentan or placebo (Channick et al., 2001). After 12 weeks, the mean six-minute walking distance improved by 70 m in the bosentan arm, while no improvement was seen with placebo. Bosentan improved hemodynamic parameters measured at cardiac catheterization as well, including an increase in cardiac index and reduced mean pulmonary artery pressure and pulmonary vascular resistance. Functional class also improved in subjects treated with bosentan. A second double-blind, placebo-controlled study evaluated bosentan in 213 patients with PAH (either idiopathic or associated with connective tissue disease) who were randomized to placebo or bosentan 125 or 250 mg bid for a minimum of 16 weeks (62.5 mg bid for 4 weeks then target dose) (Rubin et al., 2002). The primary endpoint was the change in exercise capacity (assessed by six-minute walk), and secondary endpoints included changes in Borg dyspnea index, functional class, and time from randomization to clinical worsening. After 16 weeks, the difference between treatment groups in the mean change in six-minute walk was 44 m in favor of bosentan. No dose response for efficacy could be ascertained. The risk of clinical worsening was reduced by bosentan compared to placebo.

Abnormal hepatic function (as indicated by elevated levels of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST)), syncope, and flushing occurred more frequently in the bosentan group. Abnormal hepatic function was dose-dependent, being more frequently reported as an adverse event in the high dosage bosentan group (250 mg bid) than in the low dosage group (125 mg bid). McLaughlin et al. reported that open label, first-line therapy with bosentan, with the addition or transition to other therapies as needed, resulted in Kaplan–Meier survival estimates of 96% at 12 months and 89% at 24 months (McLaughlin et al., 2005). At the end of 12 and 24 months, 85% and 70% of patients, respectively, remained alive and on bosentan monotherapy. Sitbon et al. compared open label survival in functional class III IPAH treated with bosentan with historical data from similar patients treated with epoprostenol, a parenterally-administered prostacyclin that was the first and only approved therapy for PAH prior to bosentan (Sitbon et al., 2005). Baseline characteristics for the 139 patients treated with bosentan and the 346 patients treated with epoprostenol suggested that the epoprostenol cohort had more severe disease. Kaplan–Meier survival estimates after 1 and 2 years were 97% and 91%, respectively, in the bosentan-treated cohort and 91% and 84% in the epoprostenol cohort. Bosentan therapy has also been evaluated by Galie et al. in a multicenter, double-blind, randomized, and placebo-controlled study in patients with functional class III Eisenmenger syndrome (Galie et al., 2006). Fifty-four patients were randomized 2:1 to bosentan vs. placebo for 16 weeks. Bosentan did not worsen oxygen saturation, and compared with placebo, bosentan reduced pulmonary vascular resistance index, decreased mean pulmonary arterial pressure, and increased exercise capacity. Open label data with bosentan suggests clinical improvements in HIV patients with PAH (Sitbon et al., 2004), and preliminary data suggests benefits in those with inoperable chronic thromboembolic pulmonary hypertension (Jais et al., 2008), as well as PAH patients with early stage disease (Galie et al., 2008a,b).

Ambrisentan therapy in pulmonary arterial hypertension

Ambrisentan is a relatively selective antagonist of the ET_A receptor. A Phase-II dose-ranging study supported the efficacy and safety of ambrisentan in patients with PAH, and subsequently two pivotal Phase-III clinical trials of ambrisentan in PAH confirmed these findings (Galie et al., 2005). Ambrisentan belongs to the group of carboxylic ERAs which – unlike sulfonamide-based ERAs – are devoid of hepatotoxicity. In fact, patients on ERAs with elevated liver function tests on sulfonamide-based ERAs such as bosentan or sitaxentan have been successfully switched to ambrisentan (Eriksson et al., 2011; McGoon et al., 2009). Consequently, as of 2011 liver function tests are no longer required for patients receiving ambrisentan (MedPageToday.com).

Macitentan therapy in pulmonary arterial hypertension

Macitentan, a non-selective $ET_{A/B}$ receptor antagonist with beneficial effects in experimental pulmonary arterial hypertension (Iglarz et al., 2008; Bolli et al., 2012) and diabetes-associated end-organ injury (Sen et al., 2012), has been recently tested in a Phase III clinical SERAPHIN trial in patients with pulmonary arterial hypertension. It is the first study using a morbidity/mortality composite endpoint (Reuters.com). According to data announced on April 30, 2012 (Reuters.com), treatment with macitentan was associated with a 45% risk reduction with the 10 mg dose and an approx. 30% risk reduction with the 3 mg dose, suggesting a dose-dependent effect of the drug.

The full results of the SERAPHIN trial are to be presented at research conferences in Fall of 2012 (Reuters.com).

Previous experience with sitaxentan in pulmonary arterial hypertension

Sitaxentan, an ERA with even greater ET_A selectivity than ambrisentan, successfully evaluated for the therapy of pulmonary arterial hypertension in two randomized, double-blind, placebo-controlled trials (Barst et al., 2006, 2004) and improving exercise capacity and functional class after 12 weeks of treatment, had received regulatory approval for PAH in Europe in 2007. However, sitaxentan was withdrawn from the market after several fatal cases of hepatic failure in 2010 (Galie et al., 2011).

Factors determining the therapeutic efficacy of eras in patients with pulmonary arterial hypertension

Why has ERA therapy uniquely, but consistently, been effective in pulmonary vascular disease? The answer is unclear. Possibilities include 1) the pathogenic role of ET may be most prominent in the highly unique milieu of pulmonary vascular endothelial and smooth muscle cells, which behave quite differently in a variety of circumstances and in response to many stimuli from their systemic counterparts; 2) the pulmonary vasculature is responsive to relatively low doses of ERAs, while higher, and more toxic doses, may be necessary for systemic vascular diseases. Also, the current clinical data suggests that selective and non-selective ERAs are similarly efficacious in improving clinical outcome in PAH patients. More information in this regard is expected from the results of ongoing Phase III clinical trials in PAH (Raja, 2010). In addition, recently identified factors such as race- and sex differences in response to ERA therapy (Gabler et al., 2012) as well drug–drug interactions observed during ERA therapy (Venitz et al., 2012; Pulido et al., 2009; Srinivas, 2009; Walker et al., 2009; Harrison et al., 2010; Spangler and Saxena, 2010) have to be taken into consideration when treating PAH patients. Regardless of the explanation why ERAs are an effective remedy in PAH, ERAs were the first oral therapy for PAH, and remain a critical component of the therapeutic algorithm for this life-threatening disease. Further research is necessary to determine long-term effects on disease modification.

Endothelin receptor antagonism in patients with cancer

Endothelin is synthesized by cancer cells of different origin and stimulates cancer cell growth (Bagnato and Rosano, 2008; Nelson et al., 2003). More recently, the amount of ET-1 expression in tumor tissue has been found to be a highly sensitive prognostic marker of survival in patients with bladder cancer (Fig. 2) and circulating levels of big ET-1 might predict early diagnosis in patients with invasive breast cancer (Kalles et al., 2012). Moreover, ET-1 – through its ET_A receptor – has been shown to crucially contribute to cancer cell metastasis (Said et al., 2011) and lymphatic angiogenesis (Spinella et al., 2009). ET_B-receptor-mediated signaling has been identified as an inhibitory factor of T cell homing to tumors, which could be enhanced by ET_B antagonists to enable tumor response to otherwise ineffective immunotherapy (Buckanovich et al., 2008; Kandalaft et al., 2009). Endothelin – acting on the unblocked ET_B receptor during chronic ET_A receptor blockade – possibly might therefore interfere with targeted immunotherapies in certain forms of cancer, actions that may be unrelated to the anti-inflammatory and immunomodulatory effects of ERAs (Lattmann et al., 2005; Nett et al., 2006; Sasser et al., 2007). In ovarian cancer, endothelin has been identified to promote epithelial-to-mesenchymal transition (Rosano et al., 2005); moreover, cell invasiveness and metastasis have been linked to ET_A-receptor dependent, beta arrestin-mediated mechanisms that result in activation of beta catenin signaling (Rosano et al., 2009). There is also evidence that the ET-1/ET_A axis plays a propagating role for pain transmission in bone metastasis in patients with therapy refractory prostate carcinoma (Cella et al., 2006; Jimeno and Carducci, 2006), indicating at least three therapeutic avenues from which cancer patients might benefit and experience improvements in quality of life or disease progression from ERA therapy.

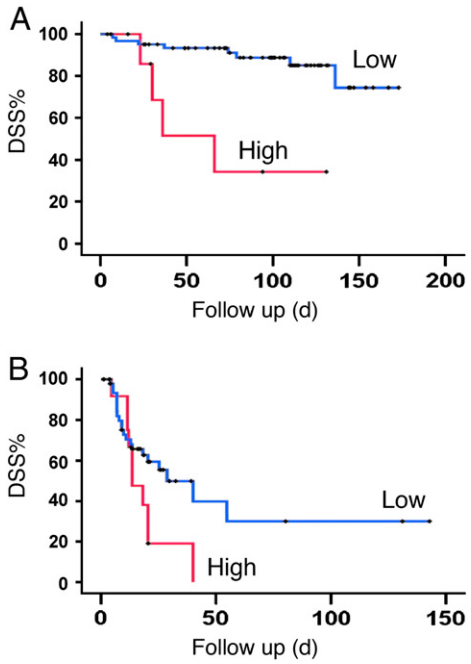

Fig. 2. Kaplan–Meier curves of the association between tumor of ET-1 protein expression (assessed by immunohistochemistry) and disease-specific survival (DSS): A, in 92 patients with bladder cancer with non-muscle invasive disease; B, in 102 patients with muscle invasive stage disease.
Figure reproduced from Said et al. (2011) with permission of *The Journal of Clinical Investigation*.

Evidence for a role of endothelin in metastatic colonization

In 2008, a review entitled "Metastasis: a therapeutic target for cancer" (Steeg and Theodorescu, 2008) argued rather convincingly that targeting the last step in the metastatic process, namely the outgrowth at a distant site, termed "metastatic colonization", holds great therapeutic promise. Such targeting can be of the tumor cell itself or of the cancer cell–microenvironmental interactions that promote tumor growth. The latter has been shown in elegant work by the Pollard and Karin labs to involve the host innate immune system and specifically macrophages (Grivennikov et al., 2010; Qian and Pollard, 2010). In 2005, we reported that ET-1 secreted by tumors with low levels of the RhoGDI2 metastasis suppressor was sensitive to inhibition of metastatic colonization with the use of ET_A receptor antagonists (Titus et al., 2005). This was also seen in head and neck and other cancers (Growcott, 2009). This result led to further experiments that led to the striking discovery that indeed, while ET_A antagonists could reduce metastatic colonization, they would not have any effect on established clinical tumors at metastatic sites.

Further work over the next few years identified tumor secreted ET-1 as a necessary mediator facilitating metastatic colonization via chemoattraction of host macrophages to the metastatic site. More importantly, recently published work (Said et al., 2011) explained why ET_A antagonists were progressively less effective as the tumor grew in the lung indicating that the therapeutic window would best be in the adjuvant setting. Given this data, it appears reasonable to test the hypothesis in a clinical trial, that blockade of ET_A receptors via orally bioavailable small molecule antagonists will delay or reduce the incidence of metastatic colonization in patients with high-risk bladder cancer.

Outcomes of previous clinical trials in cancer patients using ERAs

In the last 10 years, two large pharmaceutical companies, Abbott and Astra Zeneca, embarked on a systematic development and evaluation program on their own ET_A antagonists, atrasentan (Xinlay™) and zibotentan (Nelson et al., 2012), respectively, in cancer. Since a role of ET-1 in cancer was first shown in prostate tumors and subsequent work implicated this molecule in prostate cancer bone metastasis, the clinical work was focused on this cancer type (Lalich et al., 2007; Russo et al., 2010). Following a promising Phase II program in advanced metastatic disease, both companies undertook similar Phase III trials which encompassed early metastatic disease, advanced metastatic disease and combination therapy with ET_A antagonists and a taxane (the most effective chemotherapeutic in routine practice today). Unfortunately, all 6 trials have proven to be negative, which in retrospect, had we known of the critical yet time-sensitive role of ET-1 in metastatic colonization, these trials, in patients with established disease, would likely not have been done. Of course, the "retrospectoscope" is 20:20 and we are fortunate that two companies stepped up the plate and based on basic science data at the time undertook the risky and costly challenge in doing these trials. Recent clinical studies in oncology however suggest that ERAs may also be useful as adjuvants to enhance anti-tumor effects of interferons in patients with renal cell carcinoma (Groenewegen et al., 2012), or to inhibit tumor growth in ovarian cancer patients by enhancing paclitaxel efficacy (Kim et al., 2011). Also, preclinical studies suggest the usefulness of ET_B receptor agonists to enhance reduction in tumor volume induced by radiation therapy (Gulati et al., 2011), an approach which is now being tested in clinical trials (Tolcher et al., 2011).

Proposing to assess the efficacy of ERAs in cancer metastasis

The research strategy is self-evident. We propose the "repurposing" of atrasentan (Xinlay™ Abbott), zibotentan (Nelson et al., 2012) or another ET_A specific or ET_A-selective inhibitor from pulmonary applications to a Phase II trial setting to test the hypothesis formulated above in a clinical trial: will blockade of ET_A via orally bioavailable small molecule antagonists delay or reduce the incidence of metastatic colonization and lymphatic angiogenesis in patients with high-risk bladder cancer? In this proposed randomized Phase II trial, 108 or so high-risk patients after cystectomy would be provided with ET_A antagonists orally and kept on them for 2 years which is the time frame where most recurrences would occur in this patient population. Powered to detect a 15% difference in recurrence compared to historical controls, this trial would provide proof of principle of the concept that has been discovered in experimental studies of metastasis. Given the novel scientific foundation this trial is based upon, candidate biomarkers of response in the patients' primary tumors could also be evaluated and hence this trial would make use of biospecimens collected in the course of routine practice (i.e. the cystectomy specimen). The trial design, patient population, selected agents (toxicity etc.) and biospecimen collection (Lee et al., 2007; Said et al., 2011) make this a very feasible research proposition. This trial would not only be of great utility in bladder cancer but in other malignant diseases as well. For example, data from other laboratories such as that of Anna Bagnato who studies ovarian cancer have shown the importance of ET-1 in early dissemination in this disease.

Why re-evaluate ERAs as therapeutics in oncology?

The trial design proposed above would embody several unique aspects compared to other clinical investigations: 1) Rationally directed therapeutic approach at the innate immune system to block development of metastasis (i.e. metastatic colonization); 2) Repurposing (Collins, 2011; Huang et al., 2011; Lee et al., 2007; Said et al., 2011) known agents with extensive data in cancer patients and good safety profile thus saving millions of dollars in development; 3) Given the rapid course of metastasis development in the selected population, the trial could be completed in record time; and 4) The lack of standard alternatives or competing trials in the clinical situation described here indicating an acute need for new therapeutics in the field.

It would be an ironic twist of fate that our scientific advances have now likely found one Achilles heel of metastatic colonization process only to find out we have neither the funding nor the small molecules to test this hypothesis in patients. Is it possible that we have effective drugs to prevent metastatic colonization that we can't now develop? Are we victims of our past failures? Hopefully we can come together as a scientific community to find a solution to this problem and should not be hesitant to design and conduct the appropriate studies to test the therapeutic promise – as possible adjuvant therapeutics in cancer patients – that these drugs still hold.

Current perspectives for ERA therapy in clinical medicine

Above, we have summarized the current state of ERA therapy and problems encountered during clinical development of ERAs during the past twenty years. As mentioned, most of these trials were conducted when much of biology of ET and its receptors – particularly in humans – was largely unknown. At the time when studies were conducted, newly developed ERAs were given to very sick patients – regardless of diagnoses were heart failure, cancer, or pulmonary arterial hypertension, or renal disease – at very high doses, resulting in edema and fluid retention, worsening their clinical outcome. In particular, the only recent discovery that ET_A receptor-mediated fluid retention/plasma volume expansion appears to be an ERA class effect, that – if uncontrolled – will importantly determine any ERA-associated health risk, as was observed in prostate cancer trials (Nelson et al., 2008) and in patients with advanced proteinuric kidney disease (Mann et al., 2010) receiving very high ERA doses. Until we have fully understood the mechanism and time course of this ERA-inherent effect and until we have developed appropriate therapeutic measures to circumvent this clinically relevant problem caution is advised. However, it appears that careful and early diuretic therapy can alleviate ERA mediated fluid retention (Andress et al., 2012; Kohan et al., 2011a,b,c).

The past decade, particularly through cell-specific or tissue-specific manipulation of genes encoding for ET and its receptors has provided important insights into ET biology in health and disease (Ahn et al., 2004; Gariepy et al., 2000; Ge et al., 2005; Ivy et al., 2001; Kisanuki et al., 2010; Shohet et al., 2004; Widyantoro et al., 2010; Zhao et al., 2006). The fact that most of the clinical trials of ERAs have been negative or neutral, and the recent withdrawal of sitaxentan at the end of 2010 (Galie et al., 2011) (which had been approved for PAH three years earlier by European agencies) has slowed down clinical research activity in this area. Opportunities might have been missed since recent clinical ERA trials were discontinued due to problems in patient recruitment, while the short remaining patent life of certain ERAs might have influenced discontinuation of clinical trials (Barton and Kohan, 2011).

Currently, only two diseases (pulmonary arterial hypertension and scleroderma-related digital ulcers (Dhillon, 2009)) have been approved for ERA therapy. Orally active ERAs have also been successfully used as snake venom antidotes for Atractaspis snake bites (Abd-Elsalam, 2011) given the similarity of ET with viper venoms (Kloog et al., 1988). ERAs could also become drug treatments for proteinuric renal disease (Barton, 2008; Kohan et al., 2011a,b,c), therapy-resistant arterial hypertension (Lazich and Bakris, 2011), cancer, chronic inflammatory and auto-immune diseases such as systemic sclerosis and rheumatoid arthritis (Kuryliszyn-Moskal et al., 2008; Muller-Ladner et al., 2009), and possibly heart failure in carefully selected patients with mild-to-moderate disease treated with the right doses of the right drug (Fig. 1). Optimization of ERA dosing to minimize side effects appears to be possible, e.g.

by carefully adding diuretics during the initial phase of treatment (Andress et al., 2012; Kohan et al., 2011a,b,c)., also alleviating hemodynamic side effects including changes in blood pressure or GFR, effects which are even greater in very sick patients suffering from CHF or CKD that are already on a number of vasoactive drugs, particularly ACEIs and ARBs which share some of the mechanisms of actions of ERAs and endothelin production, respectively (Lariviere et al., 1998). Changes of hemodynamics are more difficult to cope for elderly patients, who also experience a gradual decline of GFR by 1% per year starting at age 45. Novel pharmacological approaches to block either binding or formation of ET by inhibiting ET converting enzymes (Nelissen et al., 2012; Seed et al., 2012), or by combining ERAs with drugs targeting other G protein-coupled receptors (Kowala et al., 2004; Kurtz and Klein, 2009; Mohanan et al., 2011; Murugesan et al., 2005; Neutel et al., 2008), may prove effective to block the ET pathway in disease.

One of the major drawbacks in the field remains the lack of access to many of the results obtained in clinical trials in the 1990s and early 2000s (Clozel, 2011; Kelland and Webb, 2007); this information might provide valuable insights. Recently identified race- and sex differences in the effects of ERA therapy in PAH patients (Gabler et al., 2012), ERA-drug interactions (Venitz et al., 2012) (Pulido et al., 2009; Srinivas, 2009; Walker et al., 2009; Harrison et al., 2010; Spangler and Saxena, 2010), and epigenetic regulation in PAH (Xu et al., 2011) require further clinical research.

The previous disappointments in clinical development of ERAs should not prevent us from exploring the potential of this class of drugs using carefully designed and conducted clinical trials. There is a limited amount of money to invest in new drugs, and every failure of potential drug candidates implies a substantial loss of investment. Provided that the now known side effects of plasma volume expansion can be successfully controlled for, ERAs are promising drugs since they are clearly antiproteinuric and hold potential for slowing CKD progression (Barton, 2008), for improving the clinical course of patients with PAH as suggested by the recently announced results of the SERAPHIN trial (Reuters.com), and might have therapeutic potential in selected patients with CHF or cancer. In addition, ERAs have been found to reduce formation of new digital ulcers related to scleroderma, but had no effect on healing existing ulcers (Korn et al., 2004; Kuhn et al., 2010) (Fig. 1), effects that may be related to the anti-inflammatory effects of ERAs in patients in with scleroderma (Bellisai et al., 2011). Possibly, ERAs – via inhibiting the direct, pro-inflammatory effects of endothelin-1 (Yang et al., 2004) – may also be of therapeutic benefit for other autoimmune diseases. There still is the possibility that ERAs might be effective as therapeutics in a variety of diseases, either alone or in combination with other drugs, however for any clinical application of ERAs we still require more data (and access to the substantial amount of unpublished data (Kelland and Webb, 2007)), as well as outcome studies with defined and reasonable clinical endpoints. Using the lessons we have learned, it should be possible to design and conduct successful trials using these agents.

Conflict of interest statement

Consultant to Actelion, Gilead, Pfizer, United Therapeutics, GeNO, and AIRES (Dr. Rubin); Consultant to Abbott (Dr. Kohan).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2012.07.034.

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