Inhibition of endothelin-1 with darusentan: a novel antihypertensive approach for the treatment of resistant hypertension

Darusentan is an ET_A-selective endothelin receptor antagonist that is currently in Phase III clinical development for the treatment of resistant hypertension (RHTN). Current hypertension treatment guidelines describe RHTN as the failure to achieve goal blood pressure despite treatment with full or adequate doses of an appropriate three-drug regimen that includes a diuretic. The exact prevalence of RHTN in the hypertensive community is unknown; however, recent clinical trials suggest that as many as a third of enrolled subjects may demonstrate treatment resistance. While the mechanisms responsible for the development of RHTN are also unknown, one of the few vasoconstrictive signaling pathways that remain unopposed, even in the presence of aggressive treatment with currently available antihypertensive agents, is the endothelin pathway. The efficacy and safety of darusentan, added on to full-dose background therapy including three or more medications, has recently been demonstrated in a randomized, placebo-controlled study in patients with guideline-defined RHTN. The ongoing development program will provide additional insight into the characteristics of patients with RHTN, in addition to providing key results on the efficacy and safety of darusentan in this difficult-to-treat patient population.

KEYWORDS: darusentan, endothelin, endothelin receptor antagonist, resistant hypertension

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Resistant hypertension (RHTN) is the failure to achieve goal blood pressure (BP) despite treatment with optimized doses of an appropriate three-drug antihypertensive regimen, according to contemporary hypertension treatment guidelines [1–4]. Historically, hypertension that is resistant to treatment has been commonly referred to as a pseudo-condition – one that can be successfully managed through additional effort on the part of the clinician, to identify and treat potential secondary causes of resistance or to more rigorously optimize antihypertensive therapy, as well as the patient, to be more compliant with prescribed treatments. However, more recently, it has been suggested that due to increasing rates of diabetes, chronic kidney disease (CKD), ischemic heart disease and obesity in the aging population, treatment-resistant hypertension exists, it is not uncommon and its prevalence is on the rise [4].

Guideline-recommended BP goals are currently less than 140/90 mmHg for the general population, and less than 130/80 mmHg for subjects with diabetes, CKD or other comorbid conditions [1–3]. In recent prospective studies, these targets have proven to be difficult to achieve, even when participants were receiving multidrug antihypertensive therapy. Results from the National Health and Nutrition Survey (NHANES), as well as the Framingham Heart Study, have indicated that the proportion of patients with BP not at goal according to current guidelines was sizeable at 50% or more [5,6]. Rates of BP control in the elderly and among patients with comorbidities, such as diabetes or CKD, were particularly low (<40%) [5–7]. Similar findings have been reported from large hypertension outcome studies completed in the last decade. In the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), after roughly 5 years of follow-up, approximately 34% of subjects had not achieved goal BP while receiving treatment with an average of two antihypertensive medications (27% were receiving three or more drugs) [8,9]. The Controlled Onset Verapamil Investigation of Cardiovascular End Points Trial (CONVINCE) demonstrated that after a mean follow-up of 3 years, 33% of subjects did not achieve goal BP, and approximately 18% were receiving treatment with at least three or more medications [10,11]. Likewise, in the International Verapamil-Trandolapril Study (INVEST) and the Losartan Intervention for Endpoint Reduction in Hypertension Study (LIFE), approximately 29 and 53% of participants, respectively, did not have their BP controlled below 140/90 mmHg, despite the use of intensive therapy with three or more antihypertensive drugs [12,13]. Not surprisingly, the

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lack of BP control observed in the above studies was most often due to persistent elevations in systolic BP (SBP).

While the exact prevalence of RHTN is unknown, the above results suggest that despite best efforts, a considerable number of hypertensive patients, especially those with diabetes and/or CKD, may still be at risk for significant progressive cardiovascular and renal complications due to inadequate BP control. These data also highlight the importance of identifying effective treatment options for patients with RHTN, in order to adequately address this potential unmet medical need.

Endothelin antagonism as an approach for the treatment of RHTN

The exact mechanisms responsible for the development of RHTN in individual patients are unknown. However, the involvement of endothelin-1 (ET-1), one of the most potent and long-acting endogenous vasoconstrictors known, has been implicated in the pathophysiology of a variety of cardiovascular and renal diseases, including hypertension. Most of the classical mechanisms known to promote vasoconstriction are addressed by currently available antihypertensive drugs; however, one of the few potentially relevant signaling pathways that remains unopposed, even in the presence of multidrug antihypertensive therapy, is the ET-1 pathway.

ET-1 is released from vascular endothelial cells in response to a variety of factors, including angiotensin II, catecholamines, growth factors, free radicals and mechanical stress (**Figure 1**). ET-1 is synthesized *de novo* as a pre-pro-protein (pre-pro-ET-1) that undergoes stepwise cleavage in the cytoplasm to form big ET-1, which is further cleaved to form the 21-amino-acid peptide ET-1 primarily by endothelin-converting enzymes (ECE). In the vasculature, secreted ET-1 exhibits autocrine and paracrine effects on the vascular endothelium and neighboring smooth muscle cells [14,15]. The effects of ET-1 are mediated via two receptor subtypes, ET_A and ET_B receptors, which belong to the G-protein-coupled receptor superfamily. In human vascular smooth muscle cells and cardiac myocytes, activation of endogenous ET_A or ET_B receptors results in phospholipase C-mediated vasoconstriction and protein kinase C-mediated cell proliferation [14,15]. In vascular endothelial cells, activation of ET_B receptors stimulates nitric oxide and prostacyclin production, resulting in vasodilation. The ET_B receptor also mediates ET-1 clearance via endocytosis, primarily in the lung [15].

Of note, elevated ET-1 plasma concentrations have been reported in patients with essential hypertension [16], and several hypertensive subgroups, including patients with hypertension and diabetes, patients with salt-sensitive hypertension, Black patients, the elderly and the obese [17–21]. The pathophysiological significance of these ET-1 elevations is not fully understood; however, it is well known that BP control can be particularly difficult to achieve in these patients. These data suggest that ET-1 signaling may play an important role in hypertension that is difficult-to-treat or refractory to existing treatments. Significant overlap also likely exists between the above hypertensive subgroups and patients with guideline-defined RHTN. Consequently, inhibition of ET-1 may represent a novel treatment approach to further reduce BP in patients already receiving multidrug therapy with traditional antihypertensive medications.

Darusentan, an ET_A-selective endothelin receptor antagonist

Darusentan ([S]-2-[4,6-dimethoxy-pyrimidine-2-yloxy]-3-methoxy-3,3-diphenyl-propanoic acid; **Figure 2**) is an orally active, propanoic acid-based endothelin receptor antagonist (ERA) that selectively blocks activity of ET-1 at the ET_A receptor. Clinical development of darusentan was originally initiated by Knoll Pharmaceuticals in the 1990s. Within Knoll's development program, several Phase I studies with darusentan were completed, including single- and multiple-dose pharmacokinetics in healthy volunteers, drug interaction studies with warfarin and digoxin, a food effect study, a hepatic impairment study and mass balance. In addition, darusentan was evaluated for the treatment of advanced chronic heart failure (CHF) in several Phase II studies, including a 642-patient left ventricular remodeling study conducted in Europe and the USA [22]. Darusentan was also evaluated as a monotherapy in subjects with moderate essential hypertension in a Phase II dose-ranging study conducted in Germany and Israel [23]. Gilead Sciences, Inc. (CA, USA; then, Myogen, Inc.) acquired the compound in 2003 and began clinical development of darusentan in the RHTN indication in 2004. The Phase III pivotal studies that will evaluate the efficacy and safety of darusentan in RHTN (DAR-311/DORADO and DAR-312/DORADO-AC) are currently ongoing.

Few details on the clinical pharmacology of darusentan are currently described in the literature. Darusentan is orally bioavailable and, when administered to humans, maximum plasma

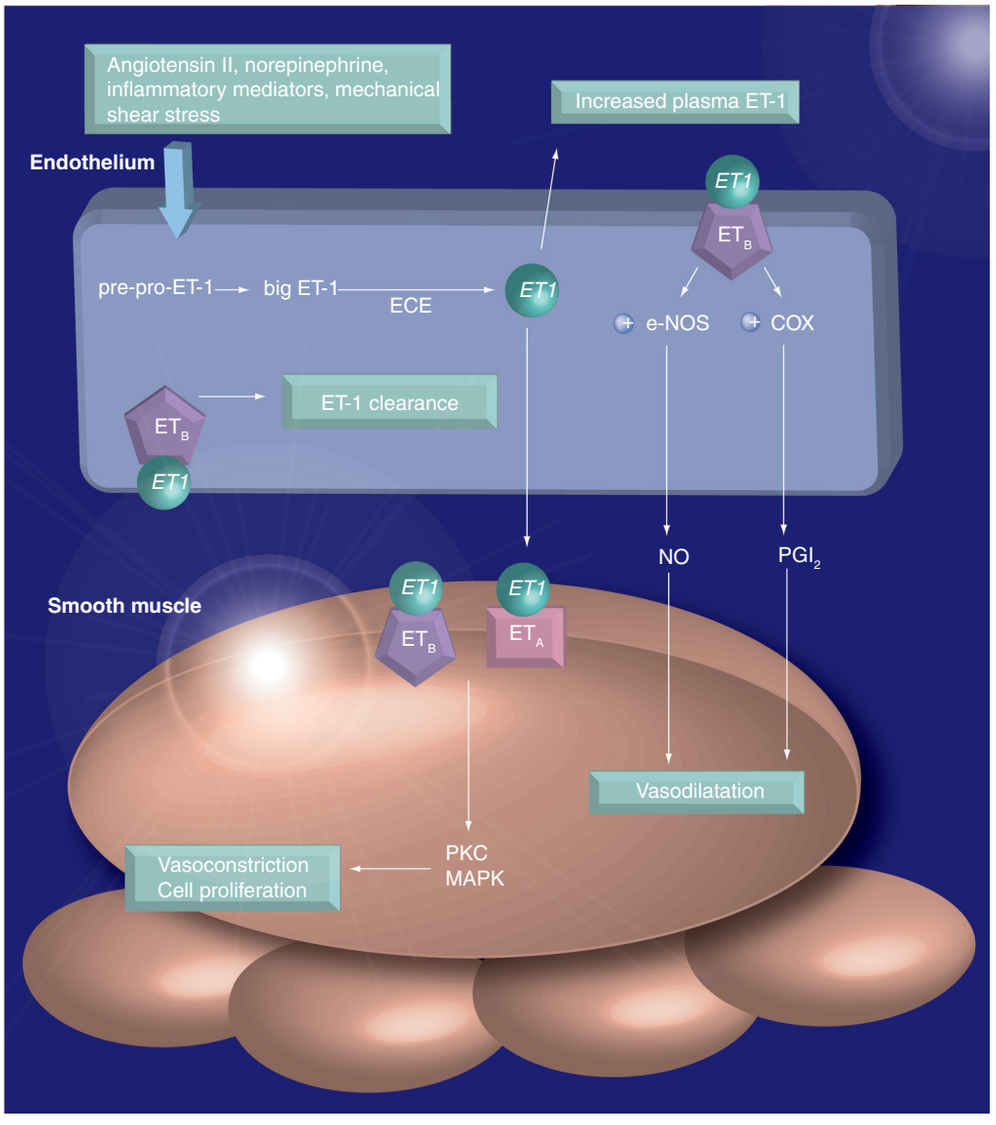

Figure 1. Endothelin signaling in the vasculature. A variety of circulating vasoactive peptides (Ang II, NE), inflammatory mediators (cytokines, NF-κB and TNFα), and growth factors stimulate pre-pro-endothelin gene expression in human vascular endothelial cells. Stepwise cleavage of the peptides pre-pro-ET-1 and big ET-1 result in production of the endothelin-1 (ET-1) peptide. ET-1 acts through two receptor subtypes, ET_A and ET_B. In human vascular smooth muscle cells, ET_A and ET_B receptors mediate vasoconstriction and cell proliferation, whereas in the vascular endothelium, ET_B receptors mediate smooth muscle vasodilation and ET-1 clearance.
COX: Cyclooxygenase; ECE: Endothelin converting enzyme; e-NOS: Endothelial nitric oxide synthase; MAPK: Mitogen-activated protein kinase; NO: Nitric oxide; PGI_2: Prostacyclin; PKC: Phosphokinase C.

concentrations are observed within 1–2 h post-dosing [24,25]. The mean elimination half-life is relatively long (>15 h), which is consistent with once-daily dosing [24,25]. Darusentan is primarily glucuronidated by Phase II enzymes in the liver, and the major route of elimination of darusentan and its metabolites is via the bile. Some glucuronidated metabolites of darusentan are also excreted in the urine [24]. One circulating hydroxyl metabolite has been identified [24], but its contribution to the efficacy and safety of darusentan has not been reported.

The *in vitro* binding affinity of darusentan for endothelin receptors has been reported in several systems. In Chinese hamster ovary (CHO) cells transiently expressing recombinant human ET_A or ET_B receptors, binding has been investigated at short incubation times and at steady-state with differing results (Table 1) [26–29]. Steady-state binding to endogenous receptors has also been examined using human left ventricular cardiac myocytes. Steady-state binding results are reasonably consistent between cell systems. Under these conditions, darusentan (a chiral molecule in the

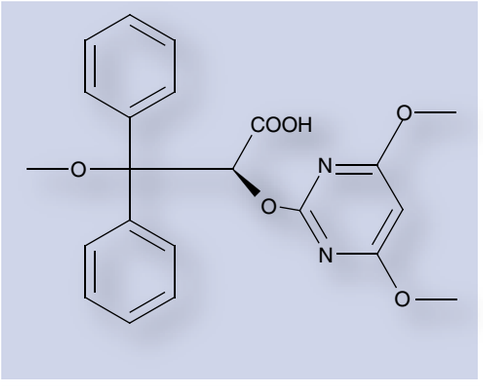

Figure 2. Darusentan. The chemical name of darusentan is (*S*)-2-(4,6-dimethoxy-pyrimidine-2-yloxy)-3-methoxy-3,3-diphenyl-propanoic acid ($C_{22}H_{22}N_2O_6$; 410.43 g/mol).

S-configuration; FIGURE 2) exhibited sub-nanomolar binding affinity and approximately 1000-fold selectivity for the ET_A receptor. Experiments to examine the binding affinity of darusentan and its known metabolites in vascular endothelial and smooth muscle cells, as compared with other ERAs, are currently ongoing.

Darusentan efficacy in patients with resistant hypertension

Results from a Phase II randomized, double-blind, placebo-controlled study examining the efficacy and safety of darusentan in patients with guideline-defined RHTN (DAR-201) were recently published [30]. All participating subjects were required to have a SBP above goal (i.e., >130 mmHg for subjects with diabetes or CKD, and >140 mmHg for all others) at three consecutive visits prior to randomization. Inclusion criteria for diastolic BP (DBP) were not specified. Background antihypertensive therapy must have included a diuretic and at least two other drugs from different antihypertensive classes (i.e., angiotensin-converting enzyme inhibitors [ACEIs]/angiotensin receptor blockers [ARBs], calcium-channel blockers [CCBs] or β-blockers). All antihypertensive drugs were required to be administered at full dose, which was defined in the study protocol according to JNC 7 guidelines. Patients with a BP of greater than 180/110 mmHg, estimated glomerular filtration rate (eGFR) of less than 30 ml/min/1.73 m^2, a history of CHF or liver enzyme elevations of more than two-times the upper limit of normal (ULN), and women who were pregnant or nursing were excluded. Other inclusion and exclusion criteria for this study were reviewed elsewhere [30]. Eligible subjects were randomized 2:1 to darusentan or placebo for 10 weeks, and stratified by race (black/non-black) and comorbidity status (present/absent). Subjects randomized to darusentan were initiated at a dose of 10 mg/day for 2 weeks, followed by forced-titration through doses of 50, 100 and 150 mg/day every 2 weeks until a target dose of 300 mg/day was reached. Co-primary efficacy end points were the changes from baseline to week 8 and week 10 in SBP, which assessed the 150 and 300 mg doses, respectively, as compared with placebo. Secondary end points examined changes in mean 24-h ambulatory pressures, the proportion of subjects who met SBP goals and changes in urinary albumin excretion rate (UACR).

DAR-201 was the first prospective, placebo-controlled study to enroll patients with guideline-defined RHTN. Of the 115 randomized subjects (76 darusentan and 39 placebo), the majority were male (59%) and Caucasian (71%); however, a relatively high proportion of Black subjects were also enrolled (28%). The mean age of the study population was 62 ± 10 years, and a large proportion of the population was obese (median body mass index [BMI] was approximately 32 kg/m^2). A majority of subjects (61%) had either diabetes or CKD at baseline, with 75% of the population exhibiting reduced eGFR (i.e., <90 ml/min/1.73 m^2). The baseline mean (± SD) sitting SBP and DBP measured by sphygmomanometry were 149.4 ± 13.1 mmHg and 81.5 ± 13.0 mmHg, respectively. Consistent with entry criteria, all subjects were on a diuretic, most often a thiazide, and 93% of subjects were taking an ACEI/ARB, 67% a CCB and 47% were on a β-blocker at full dose. Approximately 17% of subjects were also receiving drugs from other antihypertensive classes [30]. Overall, 56.5% were on exactly three medications, and 43.5% received four or more medications [31].

On top of full-dose antihypertensive therapy with three or more medications, darusentan treatment resulted in clinically and statistically significant placebo-corrected decreases in trough, sitting and ambulatory BP. Results for the co-primary end points are depicted in FIGURE 3. Statistically significant reductions in trough, sitting SBP of 7.4 ± 3.0 mmHg (p = 0.048) and 11.5 ± 3.1 mmHg (p = 0.015) were observed at darusentan doses of 150 and 300 mg/day, respectively. Placebo-corrected changes in DBP at the 150 and 300 mg doses were -5.0 ± 1.9 mmHg (p = 0.003) and -6.3 ± 2.0 mmHg (p = 0.004), respectively [30,31]. Clinically significant reductions in SBP after 10 weeks of darusentan treatment (reflective of the 300 mg dose) were also observed within predefined subgroups (FIGURE 4).

The SBP response to darusentan across subgroups was generally consistent, ranging from a decrease of approximately 15 mmHg to almost 20 mmHg; however, the observed placebo response was more variable and quite large in some groups (e.g., Blacks, women), which may be due in part to the relatively small sample sizes. Consequently, these data should be interpreted with caution. The ongoing Phase III program, which is expected to enroll more than 1100 patients with RHTN, is anticipated to provide a more robust assessment of the efficacy and safety of darusentan in various subgroups.

Ambulatory blood pressure monitoring (ABPM) was also performed in DAR-201 at baseline and after 10 weeks of darusentan treatment. Changes from baseline in mean 24-h ambulatory SBP and DBP were -9.2 ± 2.2 and -7.2 ± 1.6 mmHg, respectively ($p < 0.001$) [30]. At the 300 mg dose, blood pressure reductions were sustained throughout the dosing interval [30,32]. The effectiveness of lower doses throughout the dosing interval has yet to be established.

Consistent with the efficacy described above, treatment with darusentan in DAR-201 also resulted in an increase in the proportion of subjects who were able to achieve guideline-recommended SBP goals. Approximately 43% of patients on 150 mg/day darusentan and 28% of patients on placebo (week 8) achieved SBP goal ($p = 0.054$); the percentage of patients meeting the SBP goal increased to 51% on 300 mg/day darusentan, as compared with 33% on placebo (week 10) ($p = 0.069$) [30]. Lastly, a greater proportion of darusentan-treated subjects experienced large reductions in trough SBP during study participation (FIGURE 5) [33]. Almost twice as many darusentan subjects had a drop of trough SBP of 20 mmHg or more as compared with placebo following treatment with 50 or 300 mg/day, and the overall proportion of subjects in this response group increased with increasing darusentan dose.

While the sample size investigated in DAR-201 was small, the results of this proof-of-concept study were intriguing. If the magnitude and scope of effects were able to be reproduced in the pivotal studies, accompanied by an acceptable population safety profile, the results would provide compelling evidence in support of darusentan as a novel add-on therapy for the treatment of RHTN.

Safety profile of darusentan

Darusentan doses up to 300 mg/day were well tolerated and associated with a manageable safety profile in patients with RHTN in DAR-201 [30]. A majority of subjects (87%) in each treatment group completed the study, and 78% of darusentan-treated subjects successfully up-titrated to the maximum target dose of 300 mg/day. The most frequently reported adverse events in darusentan-treated subjects were peripheral edema (17%) and headache (11%), which were mostly mild or moderate in

Table 1. Summary of darusentan binding to endothelin receptors *in vitro*.

Study	K_i (nM)		ET_B:ET_A K_i ratio	Ref.
	ET_A	ET_B		
CHO cells				
Raschack *et al.* (1995), racemic darusentan, 30-min incubation	6	1000	161	[26]
Riechers *et al.* (1996), racemic darusentan, 30-min incubation	6 ± 0.87	371 ± 106	~62	[27]
Greene *et al.* (2006), (*S*)-darusentan, steady-state incubation	0.312 ± 0.064	551 ± 47	1766 ± 299	[29]
Greene *et al.* (2007), (*S*)-darusentan, steady-state incubation	0.187–0.399	442 – 602	1508–2363	[28]
Human left ventricular myocytes				
Greene *et al.* (2006), (*S*)-darusentan, steady-state incubation	0.178 ± 0.055	216 ± 85	1159 ± 159	[29]
Greene *et al.* (2007), (*S*)-darusentan, steady-state incubation	0.080–0.500	73–700	912–1400	[28]

CHO: Chinese hamster ovary cells; ET_A: Endothelin type A receptor; ET_B: Endothelin type B receptor; Ki: Dissociation binding constant.

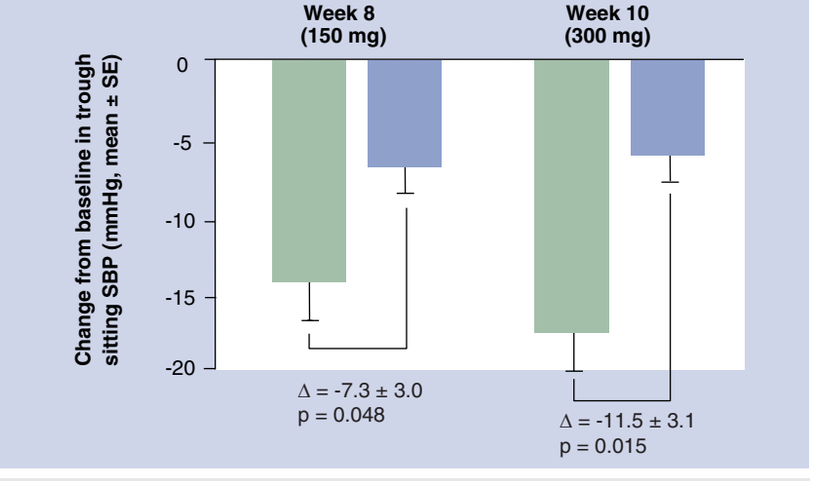

Figure 3. Darusentan treatment significantly reduced systolic blood pressure in patients with RHTN in DAR-201. Co-primary end points of mean change from baseline in trough, sitting SBP measured by standard sphygmomanometry after 8 and 10 weeks of treatment (representative of the 150 and 300 mg doses, respectively) are displayed.
RHTN: Resistant hypertension; SBP: Systolic blood pressure; SE: Standard error.

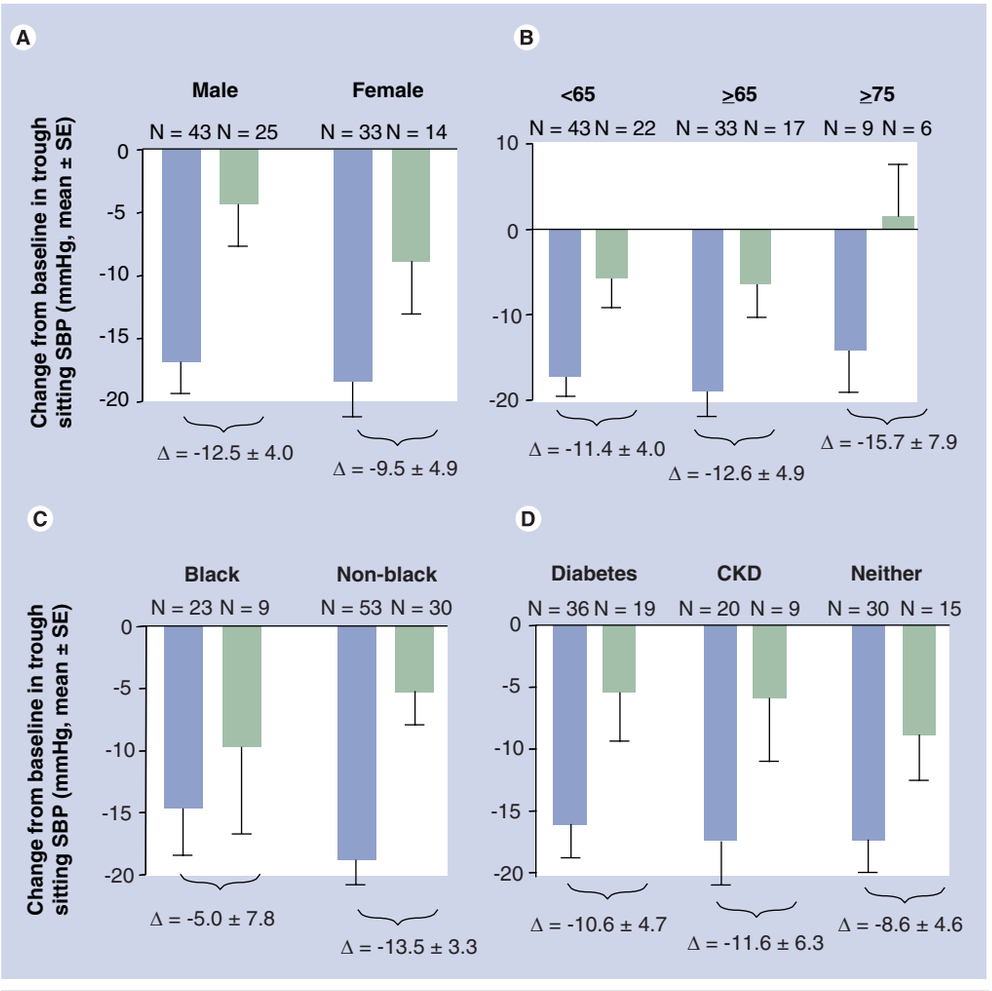

Figure 4. Darusentan-mediated systolic blood pressure reductions were maintained across subgroups.
An efficacy analysis in predefined subgroups of the DAR-201 RHTN population revealed that the blood pressure lowering effects of darusentan after 10 weeks of treatment (300 mg) were maintained across gender **(A)**, age **(B)**, race **(C)** and co-morbid status **(D)**.
SBP: Systolic blood pressure; SE: Standard error.

severity. These side effects were not reported to be dose-dependent in DAR-201; however, the forced up-titration study design may have complicated any analysis of dose-relatedness. Other commonly reported adverse events in the darusentan treatment group were sinusitis (8%), dizziness (7%), nasopharyngitis (7%), upper respiratory tract infection (5%) and gastroenteritis (5%). These common adverse events were also reported by placebo-treated subjects, but each of the events occurred in 5% or less of the placebo group. Interestingly, the incidence of arthralgia or arthritis was lower in the darusentan group (2%) as compared with the placebo group (6%) in DAR-201.

Fluid retention, in the form of peripheral edema and/or clinically significant weight gain, is a common side effect associated with ERA administration. A higher rate of edema in ERA-treated subjects as compared with placebo has been reported in the prescribing information for the two ERAs that are currently approved for the treatment of pulmonary arterial hypertension, bosentan (Tracleer®; nonselective) and ambrisentan (Letairis™; ET_A-selective) [34,35]. As a more severe example, the avosentan Phase III trial in Type 2 diabetic nephropathy (avosentan on doubling of serum creatinine, end stage renal disease and death in diabetic nephropathy [ASCEND]) was terminated early due to an excess of fluid retention in avosentan-treated subjects as compared with placebo, which compromized patient safety [101]. In other studies of darusentan (e.g., essential hypertension and CHF) peripheral edema was also a commonly reported and dose-dependent adverse

event [22,23]. Of note, changes to concomitant antihypertensive medications, including diuretics, were prohibited in DAR-201; therefore, the rate of edema in patients with RHTN was somewhat higher than observed in other darusentan studies. Anecdotally, the fluid retention associated with ERA treatment has been reported to be diuretic-responsive.

The mechanisms by which ERAs cause fluid retention are not completely understood, particularly for the ET_A-selective ERAs. Taken simply, it has been suggested that more fluid retention may result from treatment with a nonselective ERA, due to blockade of the beneficial action of ET-1 at the ET_B receptor in the kidney to promote diuresis and natriuresis [36]. The same may be hypothesized for modestly ET_A-selective agents, if doses high enough to exceed the threshold for selectivity were administered. Another interesting theory involves the effects of ERAs on endothelin receptors in veins. ET_A-receptor activation by ET-1 in venous smooth muscle causes contraction, just as in arteries [37,38]. Therefore, if administration of an ERA results in both arteriolar dilation and venodilatation, a shift in blood volume to the venous side of the circulation could result in a compensatory response by the kidneys to retain salt and water. Lastly, an increase in microvascular permeability due to increased capillary and venous pressures in the periphery, similar to that observed with CCBs and other vasodilators, may also play a role. Data in support of the above hypotheses are currently limited, although avosentan (SPP301), an ET_A-selective ERA, was recently reported to cause sodium retention in normal healthy volunteers at high doses, an effect that was accompanied by evidence of hemodilution [102].

Reversible dose-dependent reductions in hemoglobin (Hb) concentrations and hematocrit (Hct) have been observed in all Phase II studies of darusentan. After 10 weeks of treatment in DAR-201, darusentan-treated subjects demonstrated placebo-corrected reductions of Hb and Hct of 1.3 g/dl and 3.4%, respectively [30]. These reductions did not appear to be clinically significant in most patients, as changes in Hb or Hct were reported by investigators as mild adverse events in only two subjects [30]. Of note, if ERA-induced reductions in Hb/Hct are related to increased blood volume, these effects may stabilize or even reverse over time as volume-control mechanisms and/or hematopoiesis are activated or upregulated to compensate. In order to further examine the mechanisms responsible for ERA-mediated fluid retention and its relationship to decreases in Hb/Hct, clinical studies will require early and extended time courses of hematology parameters and renal function measures following acute and chronic ERA administration.

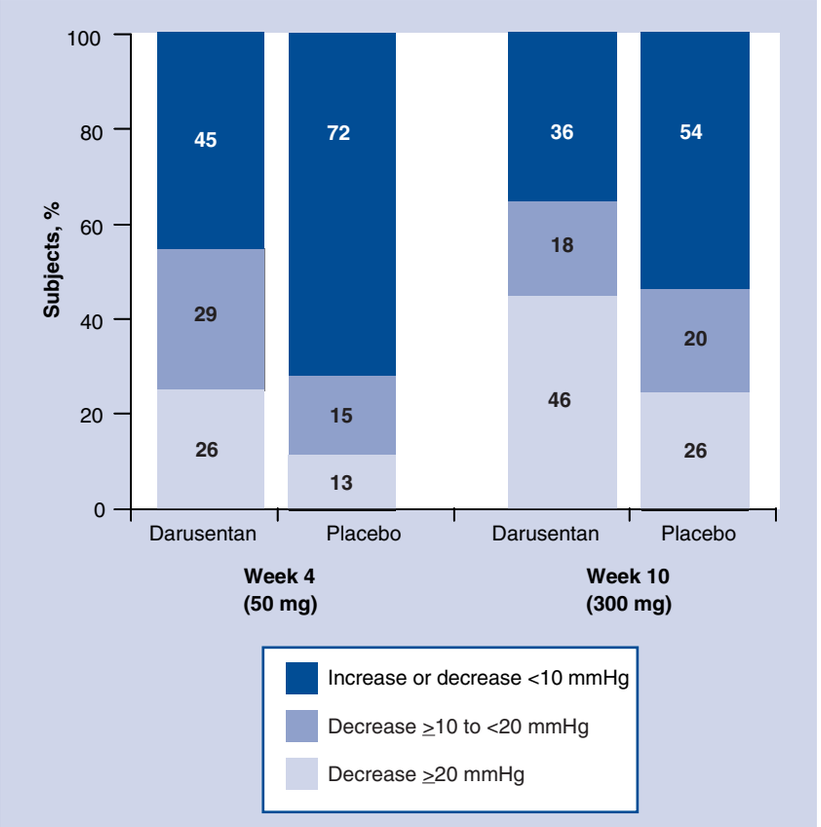

Figure 5. Magnitude of response in trough, sitting systolic blood pressure in DAR-201. At study weeks 4 and 10 (50 and 300 mg/day, respectively), a greater proportion of darusentan-treated subjects demonstrated large systolic blood pressure decreases as compared with placebo.

Teratogenicity is another reported class effect of ERAs. In animal models, endothelin inhibition resulted in a high rate of fetal malformations involving the face, thyroid and heart, and the period of greatest risk of exposure appeared to be early in development [39,40]. Consequently, currently approved ERAs are contraindicated in women who are pregnant or nursing, and women of child-bearing potential have been excluded from the ongoing Phase III studies of darusentan in RHTN. Given that the mean age of patients with RHTN as observed in DAR-201 was over 60 years, the latter exclusion is not anticipated to adversely affect study enrollment or otherwise significantly limit the RHTN patient population represented in these trials.

Aminotransferase elevations have also been reported during treatment with some ERAs. According to the literature and applicable prescribing information, nonselective and ET_A-selective

ERAs that contain a sulfonamide functional group in their molecular structure are associated with larger incidences of aminotransferase elevations of more than three-times the ULN as compared with placebo. These effects may also be accompanied by signs or symptoms of liver dysfunction, and can be treatment-limiting. Liver failure, and in some cases death, have been reported. In contrast, ERAs that are nonsulfonamide, in particular those that contain a propanoic acid backbone (e.g., ambrisentan and darusentan), have reported fewer treatment-related aminotransferase elevations or events of liver dysfunction as compared with placebo in randomized trials [30,34,35,41]. In DAR-201, no aminotransferase elevations of more than two-times the ULN were observed during the 10-week treatment period [30]. Events involving abnormal liver function parameters were also reportedly low in the essential hypertension and CHF trials (less than 1% in nearly 1000 patients) [22,23,42,43]. While these results are encouraging, clinical experience with darusentan remains limited at present. Therefore, in order to further assess the potential for drug-induced liver injury resulting from darusentan treatment, routine liver function testing has been incorporated into the Phase III program in RHTN, at least monthly over the first 28 weeks and every 3 months thereafter.

Finally, it should be noted that while darusentan offers a potentially promising combination of efficacy and safety as a novel treatment for RHTN patients, it holds less therapeutic promise in essential hypertension and heart failure. In patients with moderate essential hypertension in the Hypertension Endothelin Agonist Treatment study (HEAT-HTN), all darusentan doses (10, 30 and 100 mg) demonstrated clinically significant BP reductions when administered as monotherapy. Placebo-corrected mean changes from baseline (with 95% confidence interval) in SBP and DBP for the 100 mg treatment group were -11.3 mmHg (-16.3, -6.2) ($p = 0.0001$) and -8.3 mmHg (-11.1, -5.5) ($p = 0.0001$), respectively [23]. However, it may be difficult for a novel antihypertensive agent to compete as first-line therapy with other commonly used antihypertensives that are effective, well-tolerated and available in generic formulations. With regard to CHF, darusentan was studied in patients with moderate-to-severe disease in three Phase II clinical studies and a long-term follow-on [22,42,43]. Early evidence suggested that darusentan treatment may improve hemodynamic parameters in these patients. However, in studies of at least 6-months duration in patients with severely dilated cardiomyopathy, darusentan treatment provided no clinical benefit with regard to cardiac function or chamber characteristics. Moreover, darusentan-treated subjects reported more frequent hospitalizations for heart failure as compared with placebo in these studies, which were likely related to the fluid retention-promoting effects of darusentan treatment in some subjects. As selective ET_A receptor antagonism provided no clinical benefit to heart failure patients in this setting, and may have actually worsened heart failure, patients with CHF have been excluded from subsequent clinical studies of darusentan in RHTN.

Conclusion & future perspective

Inhibition of the effects of ET-1 represents a novel therapeutic approach for the treatment of RHTN. Darusentan is an orally active, ET_A-selective ERA that demonstrated clinically relevant and statistically significant blood pressure reductions in patients with RHTN when administered on top of full-dose background therapy with three or more antihypertensive drugs [30]. The safety profile of darusentan in RHTN appears favorable, with edema and headache being the most commonly reported side effects. The edema and/or fluid retention is anticipated to be manageable with the inclusion of loop diuretic therapy. In addition, the incidence of liver enzyme elevations associated with darusentan treatment has been low (<1%) in Phase II studies across several indications involving approximately 1000 darusentan-treated subjects [22,23,30,43]. The ongoing darusentan Phase III program is the first pivotal program to prospectively evaluate a novel add-on therapy in patients with guideline-defined RHTN. The Phase III program consists of two placebo-controlled clinical studies (DAR-311/DORADO and DAR-312/DORADO-AC) that will evaluate darusentan (50, 100 and 300 mg) in more than 1100 subjects with RHTN. In DAR-312/DORADO-AC, darusentan efficacy and safety will also be compared with an active control (guanfacine). Primary end points of the trials will evaluate changes in SBP and DBP, measured by sphygmomanometry, at trough darusentan concentrations. Other end points include changes from baseline in 24-h ambulatory BP, the percentage of subjects who achieve guideline-recommended BP goals and changes in eGFR. The long-term efficacy and safety of darusentan will be evaluated in two extension studies (DAR-311-E and DAR-312-E), available to patients who complete the pivotal trials, and

in DAR-312-E, results from the darusentan arm will be compared with long-term treatment with guanfacine. The results from this program will provide important insight into the characteristics of patients with RHTN, as well as establish the safety and effectiveness of darusentan in this setting.

Results from the above studies are eagerly awaited, as effective treatment options for patients with RHTN are currently limited. Triple therapy for most patients is typically comprised of: a diuretic, as recommended by treatment guidelines; an ACEI or ARB; and either a CCB or β-blocker. Drugs from other antihypertensive classes may instead be prescribed in the third-line space; however, this option must be individually tailored to the patient, taking severity of hypertension, presence of concomitant disease, medication side-effect profiles and other factors into account. The same challenge exists when considering which fourth antihypertensive drug should be added to the regimen of a patient with RHTN. In addition to CCBs and β-blockers, depending upon which drug class was selected as third-line, other available options include peripheral α-1 antagonists, central α-2 agonists, direct vasodilators and aldosterone antagonists. While most of these choices are effective antihypertensives when used as monotherapy, many are associated with adverse effects that may be treatment limiting, such as dizziness, fatigue/malaise, dry mouth, weakness and somnolence, particularly in patients with RHTN who are generally more complicated to manage as compared with patients with typical essential hypertension. Moreover, none of these agents have been prospectively studied in a RHTN patient population; therefore, it is difficult to predict if their efficacy as monotherapy will persist when they are added on to three or more background medications.

Few studies have been conducted to evaluate the efficacy and safety of antihypertensive drugs added on to dual or triple background therapy. Recent reports have indicated that aldosterone antagonists may be beneficial in patients with uncontrolled BP despite treatment with multidrug regimens [44–46]. However, few of these studies included a placebo control, and most have enrolled relatively small numbers of subjects. Hyperkalemia can also be an issue with the use of these agents; therefore, aldosterone antagonists may not be well suited for many patients with RHTN due to the high incidence of renal dysfunction anticipated in this population. Interestingly, a recent study of the new renin inhibitor aliskiren in patients with concomitant hypertension, Type 2 diabetes and nephropathy (Aliskiren in the Evaluation of Proteinuria in Diabetes [AVOID]) demonstrated that only a small difference in BP (-2/1 mmHg) was observed after 24 weeks as compared with placebo when 300 mg aliskerin was added to background therapy that included a full-dose ARB (100 mg losartan) [47]. Approximately 80% of subjects were receiving three or more antihypertensive medications during the treatment period (including losartan), in addition to aliskerin or placebo. While not the purpose of the trial, these results suggest that aliskerin may have limited utility for the treatment of RHTN due to the minor decrease in BP that was observed in this setting. Lastly, a clinical study assessing the efficacy of valsartan/hydrochlorothiazide/amlodipine (320/25/10 mg) triple combination therapy as compared with three dual therapies, comprised of different combinations of the same three drugs, was recently reported [48,103]. Results suggested that, when added-on to dual therapy, valsartan or amlodipine may provide an additional 8 mmHg drop in SBP (-5 mmHg for DBP). This decrease was smaller than anticipated, given the efficacy of these drugs as monotherapy, suggesting that the observed efficacy of a drug used as monotherapy or second-line treatment may not necessarily be retained if the same drug is used to treat a patient with sustained elevations in BP despite background therapy with two or more drugs.

In conclusion, it should be noted that in addition to its involvement in regulating vascular tone and promoting vasoconstriction, ET-1 is also a mediator of proinflammatory and profibrotic processes. In particular, ET-1 has been suggested to play a role in atherosclerosis, the development of fibrosis (in blood vessels and solid organs, such as the kidney) and tissue hypertrophy. Consequently, treatment with darusentan may be associated with additional clinical benefits beyond BP lowering. In an interesting study in a rat model of isolated systolic hypertension, darusentan treatment was able to reverse calcium deposition in large arteries by approximately 50% concomitant with a significant drop in BP [49,50]. In addition, a recent clinical study examining the effects of the ERA atrasentan in patients with multiple cardiovascular risk factors and coronary artery disease demonstrated that atrasentan treatment for 6 months significantly reduced central aortic pressure and improved fasting metabolic parameters and lipids [51]. These results imply

that treatment with an ERA may be beneficial in patients with hypertension and metabolic syndrome or hyperlipidemia.

In the kidney, it is well documented that ET-1 antagonism is renoprotective, resulting in improvements in glomerular function and reduced proteinuria [52–54]. In the DAR-201 RHTN population, approximately 75% of subjects had reduced renal function, and 25% had CKD. Approximately 15% had proteinuria at baseline, despite full-dose treatment with an ACEI or ARB. Darusentan treatment reduced albuminuria in these subjects by more than 40% after 10 weeks [55]. Reduction in albuminuria will be further examined in the ongoing Phase III program in RHTN. Results from this program will also provide insight into the effectiveness of darusentan in patients with RHTN and metabolic syndrome, the impact of darusentan on central aortic pressures, and the ability of darusentan to improve fasting glucose, cholesterol, lipids and glycosylated hemoglobin levels.

Financial & competing interests disclosure

R Weiss is a consultant to Gilead Sciences, Inc., and an investigator in clinical studies of darusentan in resistant hypertension. CA Graybill, J Linseman, and M Warren are employees of Gilead Sciences, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

- Darusentan is an orally active, propanoic acid-based endothelin receptor antagonist (ERA) that selectively blocks endothelin-1 (ET-1) binding to the endothelin type A (ET_A) receptor. Darusentan exhibited subnanomolar binding affinity and approximately 1000-fold selectivity for the ET_A receptor in binding experiments conducted *in vitro* under steady-state conditions.
- Darusentan is currently in Phase III clinical development for the treatment of resistant hypertension (RHTN). RHTN is defined by contemporary hypertension treatment guidelines as the failure to achieve goal blood pressure in patients treated with full or adequate doses of an appropriate three-drug antihypertensive regimen that includes a diuretic. The Phase III pivotal program will enroll more than 1100 patients with RHTN, and results will establish the efficacy and safety of darusentan as compared with placebo or an active control (guanfacine). Key end points include changes from baseline in systolic blood pressure (SBP) and diastolic blood pressure (DBP), the proportion of subjects who meet guideline-recommended BP goals, and changes in estimated glomerular filtration rate.
- In a Phase II randomized, double-blind, placebo-controlled study (DAR-201), darusentan treatment resulted in clinically relevant and statistically significant blood pressure reductions in patients with guideline-defined RHTN, receiving full-dose background therapy with three or more antihypertensive medications. Doses of 150 mg/day and 300 mg/day were associated with placebo-corrected reductions in systolic blood pressure of approximately 7.3 mmHg ($p = 0.048$) and 11.5 mmHg ($p = 0.015$), respectively.
- The risk/benefit profile of darusentan as a treatment for RHTN is potentially favorable. In DAR-201, no aminotransferase elevations of more than two-times the upper limit of normal (ULN) were observed during the 10-week treatment period. The incidence of aminotransferase elevations with darusentan have also been low in Phase II studies in essential hypertension and chronic heart failure (less than 1% in almost 1000 patients). Peripheral edema was a common adverse event in Phase II studies, including DAR-201; however, fluid retention associated with darusentan treatment is likely to be manageable through the use of diuretic therapy.
- The clinical benefits of darusentan in patients with RHTN have the potential to extend beyond blood pressure lowering, given that ET-1 has been implicated in the pathogenesis of a variety of cardiovascular and renal diseases. Additional results from the RHTN program will include information on the effects of darusentan in patients with RHTN and concomitant metabolic syndrome, on central aortic pressures, and on fasting metabolic parameters, cholesterol and lipids.

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