



# Endothelin: setting the scene in PAH

J. Dupuis

**ABSTRACT:** Endothelin (ET), a 21-amino acid peptide secreted by the vascular endothelial cells, is frequently reported to be one of the most potent vasoconstrictors. ET induces endothelial cell, smooth muscle cell and fibroblast dysfunction and is now well recognised as a contributor to the complex pathogenesis of pulmonary arterial hypertension, a devastating chronic disease characterised by progressive vascular remodelling and occlusion of the pulmonary arterioles.

ET produces its effects through the stimulation of two receptor subtypes, ET<sub>A</sub> and ET<sub>B</sub>, both of which are expressed on smooth muscle cells of pulmonary arterioles. Only the ET<sub>B</sub> receptor is expressed on vascular endothelial cells. In pre-clinical studies, dual blockade of both receptor subtypes was shown to produce greater inhibition of ET-induced contraction compared with blockade of either of the two receptor subtypes alone. There is also evidence that both the ET<sub>A</sub> and ET<sub>B</sub> receptors contribute to pulmonary artery smooth muscle cell proliferation. Pre-clinical research in various models of pulmonary arterial hypertension suggests that simultaneous blockade of the two receptor subtypes would be an effective therapeutic approach in the management of patients with pulmonary arterial hypertension. This has been shown to be the case in a number of randomised controlled clinical trials in pulmonary arterial hypertension due to a number of aetiologies.

**KEYWORDS:** Bosentan, endothelin, dual endothelin receptor antagonism, pulmonary arterial hypertension

**P**ulmonary arterial hypertension (PAH) is a chronic disease that is characterised by progressive vascular remodelling and occlusion of the pulmonary arterioles. Remodelling of the pulmonary vasculature includes intimal proliferation, medial hypertrophy, adventitial fibrosis, fibrinoid necrosis and development of pathognomonic plexiform lesions, all of which lead to elevated pulmonary vascular resistance (PVR) and development of symptoms and signs associated with PAH.

Understanding of the pathogenesis of PAH has progressed considerably over the past few years. Some individuals are genetically predisposed to develop the vascular injury that ultimately progresses to PAH through the possession of mutations in key molecules, such as the BMPR2 gene, which encodes for a transforming growth factor (TGF)- $\beta$  type II receptor [1]. This may be associated with a secondary trigger, such as the presence of underlying diseases, including connective tissue disease [2], congenital heart disease [3] or HIV infection [4]. Whatever the origins of the initial vascular insult, the end result is vascular smooth muscle cell dysfunction and endothelial cell dysfunction. Endothelial cell dysfunction results in an imbalance of molecules that control vascular homeostasis and maintain vascular tone. Levels of pro-vasodilatory and

antiproliferative molecules, such as nitric oxide (NO) and prostacyclin, are decreased, while pro-vasoconstrictive and pro-proliferative molecules, such as thromboxane A<sub>2</sub> and endothelin (ET)-1 are increased. The focus of the present article is on the actions of this latter molecule.

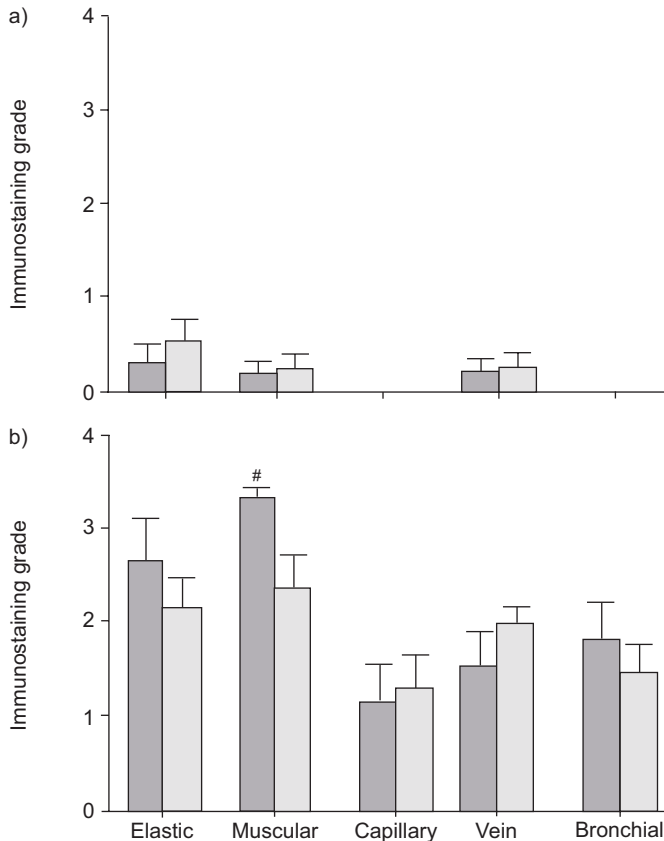
ET, a 21-amino acid peptide secreted by the vascular endothelial cells and frequently reported as one of the most potent vasoconstrictors, was discovered in 1988 [5]. ET-1 induces endothelial cell, smooth muscle cell and fibroblast dysfunction, as well as cytokine release, and is thus well recognised as playing a central role in the complex pathogenesis of PAH. Supporting evidence of the involvement of ET-1 and activation of the ET system in PAH pathogenesis came from observations that plasma levels of ET-1 are three to four times the upper limit of normal in patients with several types of PAH, including idiopathic PAH (iPAH) [6], PAH related to connective tissue disease [7], PAH secondary to congenital heart disease [8] and PAH secondary to HIV infection [9]. More important than absolute levels of ET-1 in the plasma, however, is the observation that ET-1 levels are elevated locally in all vessels of the pulmonary vasculature (fig. 1) [10]. In this *in vitro* evaluation of lung specimens from 15 control subjects and 11 patients with plexogenic pulmonary arteriopathy, ET-1-like

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## STATEMENT OF INTEREST

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**FIGURE 1.** Mean ± SE level of endothelin (ET)-1-like immunoreactivity in vascular endothelium of lung tissue from a) two groups of controls and b) two groups of patients with pulmonary arterial hypertension (PAH). ET-1-like immunoreactivity was assessed in the endothelium of elastic and muscular pulmonary arteries, capillaries, pulmonary veins and bronchial vessels. The level of ET-1-like immunoreactivity was significantly greater in the patients with PAH than in the controls in all vessels. a) ■: lung disease; □: normal lung; b) ■: idiopathic PAH (n=17); □: associated PAH (n=15). #: p=0.003 for the comparison between groups. Reproduced and modified from [10] with permission from the publisher.

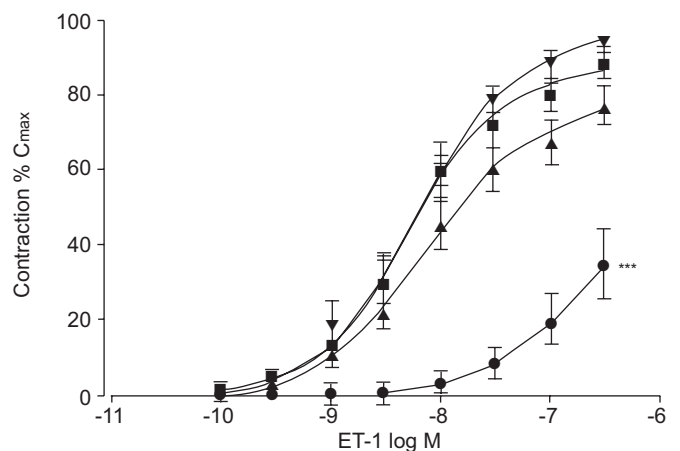
immunoreactivity was rarely observed in vascular endothelial cells. However, in samples from patients with PAH, ET-1-like immunoreactivity was abundant, predominantly in the endothelial cells of pulmonary arteries with medial thickening and intimal fibrosis, and there was a strong correlation between the intensity of ET-1-like immunoreactivity and PVR in the patients with plexogenic pulmonary arteriopathy. Moreover, elevated ET-1 levels have been shown to correlate with haemodynamic variables, such as increased PVR, 6-min walk distance (6MWD) and survival [6, 11].

**ET-1 MEDIATES ITS EFFECTS THROUGH BOTH THE ETA AND ETB RECEPTORS**

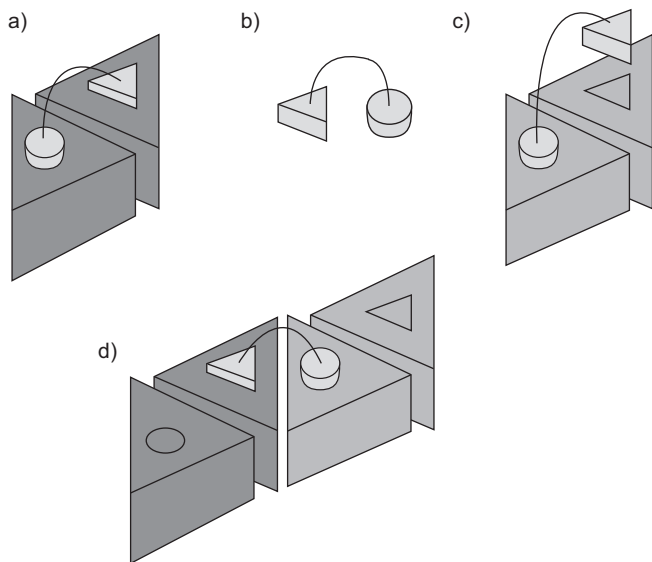
The effects of ET-1 are transmitted upon its binding to both its cognate receptors, ETA and ETB. These receptors are both highly expressed in the media of pulmonary arteries and on smooth muscle cells, where they mediate vasoconstriction and proliferation. Only the ETB receptor is found on the endothelium. Stimulation of the endothelial ETB receptor, whilst causing mild, transient vasodilation, has also been shown to result in the release of the potent vasoconstrictor thromboxane A2.

There is substantial evidence to suggest that both the ETA and ETB receptors contribute to pulmonary vasoconstriction. One *in vitro* study in isolated human pulmonary arteries [12] has demonstrated that stimulation of pulmonary arteries with ETB-selective agonists (sarafotoxin 6c) resulted in ~25% of the constriction caused by native ET-1. In agreement with this, animal studies using selected concentrations of antagonists to the two receptor subtypes in small pulmonary vessels were able to show that, while single receptor antagonists to each of the two receptors produced some inhibition of ET-1-induced contraction, use of their combination to achieve comprehensive dual ETA and ETB receptor antagonism significantly inhibits ET-1-induced constriction (52 ± 5.3%, p<0.001; fig. 2) [13]. The synergistic inhibition achieved with dual receptor antagonists that, at the concentrations used, are only mildly inhibitory when used on their own indicates that significant cooperation or “cross-talk” may exist between the two receptor subtypes.

One potential explanation for the synergistic effect of blocking both ETA and ETB receptors comes from the recent observation in a number of studies that these two receptors can exist as functional heterodimers (fig. 3) [14, 15]. It is known that ET-1 binds bivalently to the endothelin receptors through an address-recognition subdomain and a message subdomain. One hypothesis is that whereas both the ETA and ETB receptors have a message subdomain site, only the ETA receptor has an address-recognition site and ETB may not independently recognise ET-1, requiring the presence of the ETA receptor [14]. If this theory is correct, one model of how endothelin receptor heterodimerisation could result in synergistic inhibition of ET-1-induced vasoconstriction might be that an ET-1 molecule could bind to the functional site on ETB and the



**FIGURE 2.** Endothelin (ET)A and ETB contribute to pulmonary vasoconstriction in small-resistance human pulmonary arteries. This figure shows the examination of the respective contribution of ETA and ETB receptor subtypes to ET-1-induced constriction in small-resistance pulmonary arteries (~150 µm in diameter) of rats. Cumulative concentration–response curves to ET-1 were performed in the presence of an ETA-specific receptor antagonist (A-147627.1; 10 nM), an ETB-specific receptor antagonist (A-192621.1; 1 µM) and both antagonists together. Cmax: maximum contraction; R ant: receptor antagonist. ■: control; ▲: + ETA-R ant (10 nM); ▼: + ETB-R ant (1 µM); ●: + ETA-R ant + ETB-R ant. \*\*\*: p<0.001 versus control. Reproduced and modified from [13] with permission from the publisher.



**FIGURE 3.** Endothelin (ET)<sub>A</sub> and ET<sub>B</sub> receptors can exist as heterodimers. A hypothetical model of ET-1 (b) binding to ET<sub>A</sub> receptors (a), ET<sub>B</sub> receptors (c) and ET<sub>A</sub>–ET<sub>B</sub> receptor heterodimers (d). Adapted from [14].

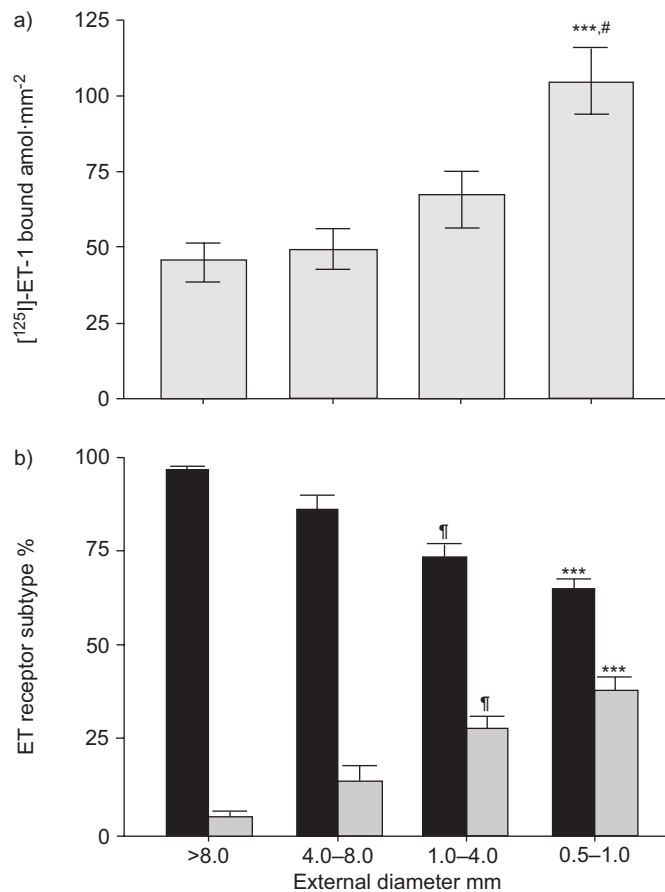
recognition site on ET<sub>A</sub>, thus bridging between the two receptors to form a functional ET<sub>A</sub>–ET<sub>B</sub> receptor heterodimer.

The current situation with endothelin biology is that most of the ET-1 produced by the endothelial cells from the cleavage of its precursor, “big ET-1”, is directed towards the underlying smooth muscle cells and fibroblasts, where it can interact with ET<sub>A</sub> and ET<sub>B</sub> receptors to promote vasoconstriction, proliferation and fibrosis. A smaller amount of ET-1 is released into the lumen of the pulmonary vasculature where it can stimulate the endothelial ET<sub>B</sub> receptors, resulting in the production of vasodilatory molecules, such as NO and prostacyclin, but also pro-constrictory molecules, such as thromboxane A<sub>2</sub>.

#### DISTRIBUTION OF ET<sub>A</sub> AND ET<sub>B</sub> RECEPTOR SUBTYPES

ET binding sites in the lungs increase with decreasing vessel diameter. Importantly, in a study in pulmonary arteries from pulmonary hypertensive patients and matched control subjects, the relative distribution of the two ET receptor subtypes was found to differ in relation to vessel diameter. More distal arteries were found to have a greater proportion of ET<sub>B</sub> receptors than larger proximal arteries ( $36 \pm 3$  versus  $3 \pm 1\%$ ;  $p < 0.001$ ; fig. 4), so that in smaller vessels with an external diameter of 0.5–1 mm, the ratio of ET<sub>A</sub>–ET<sub>B</sub> receptors is in the region of 70:30 [16]. Receptor density in distal arteries and lung parenchyma was also two-fold greater ( $p < 0.05$ ) in pulmonary hypertensive patients than in control subjects. These pathological modifications in ET receptor expression may have significant pharmacological implications.

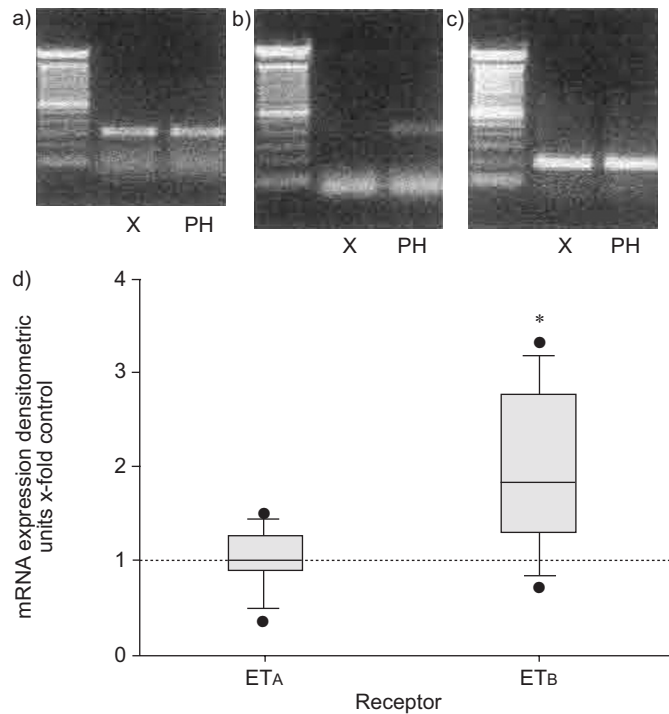
Another important concept is that the relative distribution of the ET<sub>A</sub> and ET<sub>B</sub> receptors may be disease dependent. This has been shown to be the case in the pulmonary arteries of chronic thromboembolic pulmonary hypertension (CTEPH) patients [17]. In the study by BAUER *et al.* [17], changes in plasma levels of big ET-1 and pulmonary artery ET<sub>A</sub> and ET<sub>B</sub> receptor gene



**FIGURE 4.** The changing distribution of endothelin (ET)<sub>A</sub> and ET<sub>B</sub> subtypes with decreasing vessel diameter. a) ET-1 binding and b) distribution of ET<sub>A</sub> and ET<sub>B</sub> receptors in pulmonary arteries from human subjects (n=18) are shown. Distal arteries possessed more medial [<sup>125</sup>I]-ET-1 binding sites ( $105 \pm 10$  versus  $45 \pm 6$  amol·mm<sup>-2</sup>;  $p < 0.001$ ) and a greater proportion of ET<sub>B</sub> receptors than proximal arteries ( $36 \pm 3$  versus  $3 \pm 1\%$ ;  $p < 0.001$ ). b) ■: ET<sub>A</sub>; □: ET<sub>B</sub>. \*\*\*:  $p < 0.001$  versus  $> 8.0$  mm external diameter; #:  $p < 0.05$  versus  $1.0$ – $4.0$  mm external diameter; †:  $p < 0.05$  versus  $> 8.0$  mm external diameter. Reproduced and modified from [16] with permission from the publisher.

expression were assessed in 14 consecutive patients undergoing pulmonary thromboendarterectomy for CTEPH. As reported in other aetiologies of PAH, plasma big ET-1 levels were found to be higher in patients with CTEPH (median  $2.2$  pg·mL<sup>-1</sup>; 25th–75th percentile  $1.5$ – $3.0$  pg·mL<sup>-1</sup>) compared with age-matched controls (median  $1.2$  pg·mL<sup>-1</sup>; 25th–75th percentile  $1.0$ – $1.4$  pg·mL<sup>-1</sup>;  $p = 0.002$ ). In addition to increased plasma big ET-1 levels, selective upregulation of ET<sub>B</sub> receptor mRNA transcripts and immunoreactive protein in the pulmonary artery was observed in patients relative to controls. In contrast, ET<sub>A</sub> receptor gene expression was unaffected (fig. 5). Together, these data suggest that changes in the ET signalling system in CTEPH are not limited to an increased production of ET-1: they can also affect endothelin receptor gene expression.

Given the expression of the ET<sub>B</sub> receptor in both the vessel media and the endothelium, it would be of interest to know whether alterations in ET<sub>B</sub> expression are due to changes in receptor expression in one or both of these locations. A recent

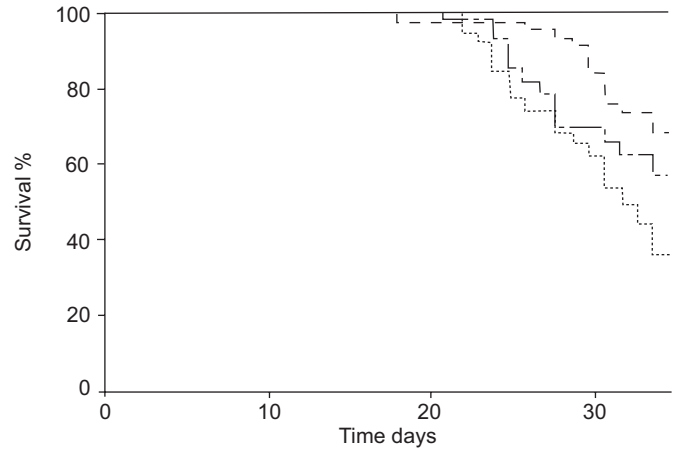


**FIGURE 5.** Endothelin (ET)<sub>B</sub> receptor gene expression is selectively upregulated in chronic thromboembolic pulmonary hypertension patients compared with age-matched controls. a) ETA, b) ET<sub>B</sub> and c) reduced glyceraldehyde phosphate dehydrogenase. a–c) mRNA concentration of transcripts encoding ETA and ET<sub>B</sub> receptors in biopsies of pulmonary arteries, as assessed by RT-PCR. RNA was isolated from pulmonary arteries obtained during pulmonary thromboendarterectomy for thromboembolic pulmonary hypertension (PH) or from harvested donor lungs (control; lung transplantation). d) Densitometric analysis of steady-state transcripts encoding ETA and ET<sub>B</sub> receptors. Data are presented as median and interquartile range (■), 10th and 90th percentiles (error bars), and 5th and 95th percentiles (●). X: transplant. \*: p<0.05. Reproduced and modified from [17] with permission from the publisher.

study evaluated ET-1 clearance in human PAH [19]. Since the endothelial ET<sub>B</sub> receptor is responsible for the clearance of ET-1, the study by LANGLEBEN *et al.* [19] was able to demonstrate that relative to controls, ET-1 clearance was reduced in iPAH, PAH secondary to connective tissue disease and CTEPH, suggesting that endothelial expression of ET<sub>B</sub> is not increased in these disease states.

**ENDOTHELIN BIOLOGY: IMPLICATIONS FOR PAH THERAPY**

The ET biology discussed so far would suggest that effective clinical manipulation of the ET system can be achieved by simultaneous blockade of both the ETA and ET<sub>B</sub> receptor subtypes. Bosentan is currently the only orally active dual ET receptor antagonist competitively antagonising the binding of ET-1 to both ETA and ET<sub>B</sub>. In all animal models of PAH, bosentan prevents and in some cases can reverse the development of PAH, vascular remodelling and right ventricular hypertrophy [20–23] and dual endothelin receptor antagonism has been shown to positively influence survival [18]. In the study by JASMIN *et al.* [18], in an established model of PAH, selective (ETA) or dual ET receptor antagonists were



**FIGURE 6.** Dual receptor antagonism improves survival in the monocrotaline (MCT)-induced pulmonary arterial hypertension model in rats. Two weeks after injection of saline or 60 mg·kg<sup>-1</sup> MCT, rats received 50 mg·kg<sup>-1</sup>·day<sup>-1</sup> of a selective (LU135252) or dual (BSF420627) endothelin receptor antagonist for 3 weeks. This resulted in four groups: control (—; n=15), MCT (·····; n=60), MCT+endothelin (ETA) (---; n=39) and MCT+ETA+ET<sub>B</sub> (- - -; n=40). p=0.0015, for the comparison between MCT and MCT+ETA+ET<sub>B</sub>. Reproduced and modified from [18] with permission from the publisher.

administered 2 weeks after injection of saline or 60 mg·kg<sup>-1</sup> monocrotaline (MCT). After a further 3 weeks, survival was 35% in the MCT group. This was increased to 56% in the MCT+ETA group (p=0.10) and to 67% in the MCT+ETA+ET<sub>B</sub> group (p=0.0015; fig. 6).

**CONCLUSIONS**

Investigations into the physiological and pathophysiological roles of the endothelin system, including altered expression of endothelin receptors and their roles in health and disease, have revealed a key pathogenic role of endothelin-1 in pulmonary arterial hypertension. As a result, the dual endothelin receptor antagonist bosentan has emerged as an effective treatment option for this patient population.

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