ELSEVIER

Teaser This review highlights the latest findings regarding the role of TGF and BMPRII signalling in the pathogenesis of pulmonary arterial hypertension and emphasises new therapeutic strategies targeting TGF/BMPRII pathways.

TGF and BMPRII signalling pathways in the pathogenesis of pulmonary arterial hypertension

Birger Tielemans[1], Marion Delcroix[2], Catharina Belge[2] and Rozenn Quarck[2]

[1] Respiratory Division, Department of Chronic Diseases, Metabolism and Aging (CHROMETA), University of Leuven, Leuven, Belgium
[2] Respiratory Division, University Hospitals and Department of Chronic Diseases, Metabolism and Aging (CHROMETA), University of Leuven, Leuven, Belgium

Pulmonary arterial hypertension (PAH) is a severe condition characterised by remodelling of precapillary pulmonary arteries sometimes associated with mutations in the *bone morphogenetic protein receptor type 2* (*BMPR2*) gene. Even in the absence of *BMPR2* mutations, increased transforming growth factor (TGF)β receptor signalling and decreased BMPRII signalling have been shown to contribute to PAH pathogenesis. In this Keynote, we review the potential mechanisms by which the imbalance of BMP/TGFβ signalling contributes to endothelial dysfunction, vascular remodelling, inflammation and disordered angiogenesis in PAH. Additionally, we highlight how currently used drugs can influence BMP/TGFβ signalling. Finally, we browse the newly developed therapeutic approaches targeting BMPRII and TGFβ signalling pathways by focusing on preclinical studies and clinical trials and put them into perspectives.

Introduction

Pulmonary hypertension (PH) is characterised by increased blood pressure in the pulmonary arteries, veins or capillaries. Different pathological features and therapeutic approaches segregate five categories of PH: (i) pulmonary arterial hypertension (PAH); (ii) PH as a result of left heart disease; (iii) PH caused by lung disease and/or hypoxia; (iv) chronic thromboembolic PH (CTEPH); and (v) PH with unclear multifactorial mechanisms [1]. PAH is defined by a mean pulmonary arterial pressure (mPAP) ≥25 mmHg at rest, a normal capillary wedge pressure <15 mmHg and a pulmonary vascular resistance (PVR) >3 Wood units [1]. It is a progressive, severe and incurable disorder affecting the pulmonary vasculature, as well as the right ventricle (RV). The disease is characterised by an obstruction of the small precapillary pulmonary arteries resulting in increased PVR, RV hypertrophy, right heart failure and death if left untreated [2]. Idiopathic PAH (IPAH) corresponds to sporadic cases without any familial history or identified risk factors [1]. When PAH occurs in a hereditary context (HPAH), germline mutations are identified. In addition, PAH can be

Corresponding author: Quarck, R. (rozenn.quarck@kuleuven.be)

Birger Tielemans obtained his Master's degree in biochemistry & biotechnology at the University of Antwerp in 2015. He has performed his internship at the Institute of Molecular Medicine at the University of Denmark. He is currently a PhD student within the division of Respiratory Diseases at the University of Leuven under the supervision of Prof. Delcroix, Prof Belge and Dr Quarck. His research interests focus on cellular and molecular biology of the bone morphogenic protein receptor II (BMPRII) signalling pathway, angiogenesis and endothelial dysfunction in heritable and idiopathic pulmonary arterial hypertension.

Marion Delcroix obtained her medical doctor degree in 1987 and completed her PhD in 1995 at the Free University of Brussels. She is currently holding a position as Professor of Medicine and Respiratory Physiology at the University of Leuven. She is also the head of the Centre for Pulmonary Vascular Diseases at the University Hospitals of Leuven. Her research interests focus on the physiology of the pulmonary circulation and gas exchange, cardiac imaging and the pathogenesis of pulmonary hypertension. She has published >160 peer-reviewed articles in the fields of the physiology of the pulmonary circulation, cardiac imaging and pulmonary hypertension.

Rozenn Quarck completed her PhD in biomedical sciences at the University Denis Diderot in Paris (France) in 1996. Since 2015, she has held a position as a scientific research expert within the division of Respiratory Diseases at the University of Leuven and as Clinical Data Manager within the Centre of Pulmonary Vascular Diseases at the University Hospitals of Leuven. Her research interests focus on the biology of the pulmonary circulation, pulmonary vascular diseases, physiopathology and genetics of pulmonary hypertension using *in vitro* and animal models. She has published 40 peer-reviewed articles in the field of vascular biology and pulmonary hypertension.*et al. et al.*—

https://doi.org/10.1016/j.drudis.2018.12.001

associated with drug or toxin intake such as anorexigens (see Glossary), dasatinib or interferon, or connective tissue disease (CTD), congenital heart disease (CHD), portal hypertension, schistosomiasis and HIV [1]. According to the updated classification, PAH also includes pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis (PVOD/PCH) and persistent PH of the newborn [1].

PAH is a rare disease with a prevalence estimated between 15 and 50 subjects per million inhabitants in western countries, occurring more frequently in females (60–76% of IPAH patients are female) [3]. Although there is a significant increase in the awareness of the disease, there remains a diagnostic delay. Patients with PAH generally display unspecific symptoms including fatigue, syncope or dyspnoea and can be diagnosed at any age. In the absence of specific treatment, the average survival time is ~2.8 years [4]. Even with effective vasodilator therapies, mortality rates remain high with a 5-year survival rate of 49–67% [5]. Currently, there is no curative treatment available for PAH with lung transplantation being the only option in patients with severe PAH refractory to current medical therapies [6].

When PAH occurs in a hereditary context, germline mutations in the *bone morphogenetic protein receptor type 2* (*BMPR2*) gene, a member of the transforming growth factor-β (TGFβ) superfamily, are detected in 70% of cases. However, *BMPR2* mutations have also been detected in 11–40% of sporadic cases without any familial history [7]. The pathophysiological process of PAH that leads to increased PVR and RV hypertrophy involves vasoconstriction, vascular remodelling, inflammation and thrombosis, contributing to the obstruction of the precapillary arteries [8]. Pulmonary vascular remodelling is a complex process triggered by different stimuli-like hypoxia, shear stress, inflammation, drugs or toxins and genetic susceptibility [9]. Vascular remodelling involves thickening of intima, media and adventitia, attributable to hypertrophy and hyperplasia of pulmonary arterial smooth muscle cells (PASMCs), PA endothelial cells (PAECs) and fibroblasts, consequently contributing to vascular obstruction. Additionally, muscularisation of peripheral arteries, loss of precapillary arteries, neointima formation and formation of plexiform lesions are observed in severe PAH [10]. The presence of inflammatory cells such as T and B lymphocytes, macrophages and dendritic cells is frequently observed in remodelled pulmonary vessels, suggesting that the inflammatory process can contribute to the pathogenesis of PAH [11]. Altered angiogenesis is another important aspect of the pathophysiology of PAH; whether vascular endothelial growth factor (VEGF), one of the most important angiogenic factors, plays a mechanistic part in the development of PAH remains unclear. However, VEGF and VEGF receptor (VEGFR) expression levels are elevated within plexiform lesions from patients with PAH [12], and a combination of hypoxia with administration of the antiangiogenic VEGFR blocker SU5416 causes obliterative PAH in rodents [13]. Endothelial dysfunction is considered a hallmark of the pathophysiology of PAH and is defined by an imbalance between vasodilators and vasoconstrictors. This is an important aspect in PAH characterised by sustained reduction in vasodilators such as prostacyclin (PGI_2) or nitric oxide (NO) and upregulation of vasoconstrictors, such as endothelin (ET)-1, serotonin or thromboxane A_2. These pathways also exert, to a variable degree, effects on vascular remodelling and coagulation homeostasis [14]. The identification of molecules involved in the regulation of pulmonary vascular tone, including PGI_2, NO and ET-1, has led to the development of several approved PAH therapies targeting three major pathways (Fig. 1).

PGI_2 pathway

PGI_2 analogues include epoprostenol, iloprost and treprostinil and the selective agonist of the prostacyclin receptor (IP), selexipag. PGI_2 analogues activate the IP receptor and downstream adenylate cyclase (AC), resulting in the production of cyclic adenosine monophosphate (cAMP), which induces vasodilation and reduces vascular SMC proliferation [15].

NO pathway

Phosphodiesterase type 5 inhibitors (PDE_5I), including sildenafil and tadalafil, target the NO pathway by enhancing the production of cyclic guanosine monophosphate (cGMP), resulting in PASMC relaxation and vasodilation [16]. The activator of the soluble guanylate cyclase (sGC), riociguat, directly activates sGC in the absence of NO, resulting in the production of cGMP, PASMC relaxation and vasodilation [17].

ET-1 pathway

ET-1 receptor antagonists (ERAs) inhibit the vasoconstrictive effect of ET-1 by antagonising ET-1 receptor type A (ET_A) and/or B (ET_B). ET_A and ET_B receptors expressed by SMCs stimulate vasoconstriction, whereas ET_B receptors stimulate release of vasodilator agents by ECs. ERAs can be selective of ET_A (ambrisentan) or nonselective blockers of ET_A and ET_B (bosentan and macitentan) [18]. In pathological conditions like PAH, ET_B receptors are upregulated on PASMCs and downregulated on PAECs [19].

One important issue is that PAH patients can respond differently to treatment; currently, only acute vasoreactivity testing with NO or PGI_2 analogues is performed to orientate treatment towards low-priced calcium channel blockers. In addition, currently approved drugs exhibit vasodilator effects but do not reverse pulmonary vascular remodelling with further insight into the pathogenesis of PAH required to advance drug development and improve patient management. This review focuses on TGFβ signalling, its dysregulation in PAH and potential interventions.

TGFβ superfamily

TGFβ family classification

BMPR2 encodes for BMPRII, which is part of the TGFβ superfamily. This is a large family of structurally similar polypeptide growth factors and related receptors with >30 different ligands including TGFβs, activins, inhibins, Nodal, Lefty (left-right determination factors), bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and anti-Müllerian hormone (AMH) [20]. These ligands are known to signal via a heterocomplex of two groups of transmembrane serine/threonine kinase receptors (i.e., type I and type II receptors). This dimeric complex comprises two type I (ALK1-7) and two type II receptors (TGFβ receptor type II, BMPRII, ActRIIA, ActRIIB or anti-Müllerian hormone receptor II). Co-receptors to this complex include endoglin or betaglycan, involved in specific ligand–receptor binding and in regulating receptor signalling pathways. TGFβ signalling is further complicated by expression of endogenous antagonists (i.e., noggin,

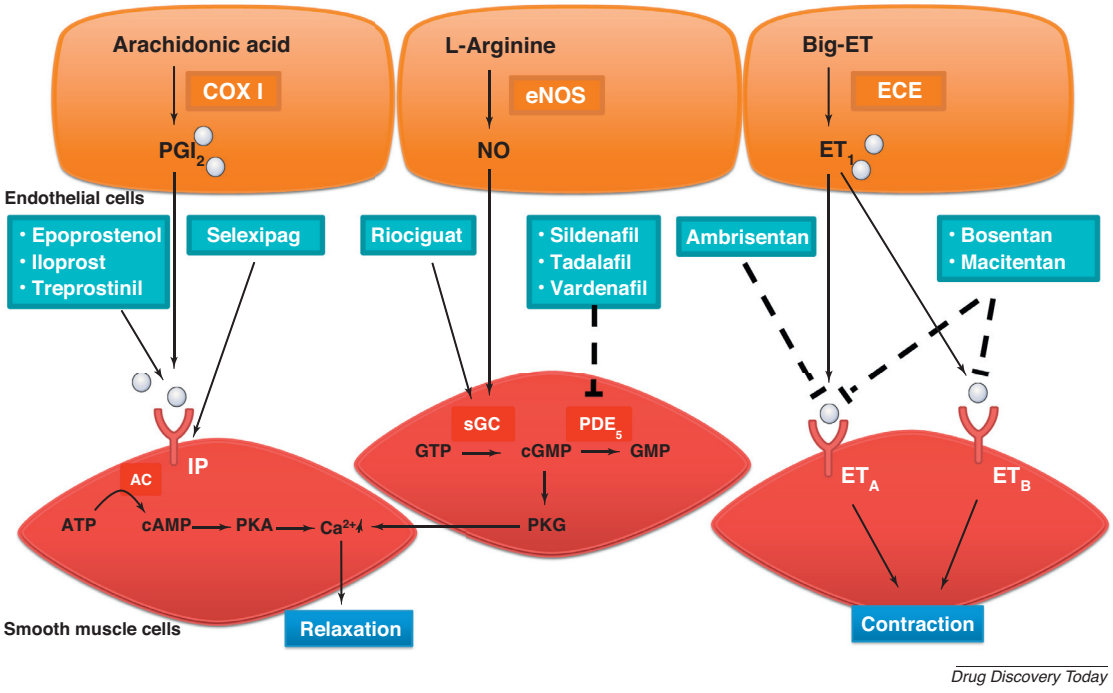

FIGURE 1

Overview of current therapeutic targets. Abbreviations: AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX 1, cyclooxygenase 1; ECE, endothelin-converting enzyme; eNOS, endothelial nitric oxide synthase; ET, endothelin; ET1, endothelin-1; ET_A, endothelin receptor A; ET_B, endothelin receptor B; GMP, guanosine monophosphate; GTP, guanosine triphosphate; IP, prostaglandin I2 receptor; NO, nitric oxide; PDE5, phosphodiesterase 5; PGI_2, prostacyclin; PKA, protein kinase A; PKG, protein kinase G; sGC, soluble guanosine cyclase.

gremlin-1, chordin or follistatin) that can modulate ligand–receptor interactions and signalling. Noggin and chordin bind and antagonise BMP2, 4 and 7; noggin, by competitively interacting with BMP4, inhibits its activity and prevents BMP4 from binding to cell-surface receptors [21] and chordin antagonises normal signalling by blocking receptor binding. Additionally, follistatin can inhibit the effect of BMP2, 4 and 7 by forming a trimeric complex with the receptors [22]. Gremlin-1 is part of the dead-enylating nuclease (DAN)/Cerberus protein family and can block BMP2, 4 and 7 *in vivo* and *in vitro* [23]. Altogether, this highlights the numerous combinations and complexity of TGFβ signalling (Fig. 2).

TGFβ signalling

Upon ligand binding, the type II receptor subunit phosphorylates type I receptors, which initiates two intracellular signalling cascades: the canonical (Smad-dependent) and noncanonical (Smad-independent) pathways (Fig. 3). Small mothers against decapentaplegic (Smad) protein is a transcription factor that transduces the signal into the nucleus [24]. Once phosphorylated, Smad1/5/8 or Smad2/3 proteins form a complex with Smad4 and translocate into the nucleus. It is commonly accepted that BMPs and GDFs signal through activation of Smad1, Smad5 and Smad8 by phosphorylation; and that TGFβs, activins and Nodal signal through Smad2 and Smad3. Activation of downstream noncanonical pathways include phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK), TGF-activated kinase-1 (TAK1) and protein kinase C (PKC) pathways. Regulation of these signalling cascades involves multiple partners like inhibitory Smad proteins (Smad6 and Smad7), protein ligases (SMURF1 and SMURF2), protein phosphatases (PPA1α) and miRNAs. Cross-communication with Wnt, Notch, MAPK, Rho/Rho-kinase, TGFβ, hypoxia-inducible factor (HIF) and nuclear factor-κB (NF-κB) signalling pathways could also contribute to the regulation of canonical and noncanonical TGFβ signalling [25].

The wide range of TGFβ signalling pathways implies that its effects on tissues are complex and opposing effects can often occur. Receptor dimerisation plays a major part in determining the activation of canonical or noncanonical signalling. Hence, preformed BMP receptor complexes, encompassing homodimers of type I and II receptors, would preferentially induce canonical Smad-dependent signalling. By contrast, ligand-induced oligomerisation of type I and type II receptors would predominantly activate noncanonical non-Smad signalling [26]. Therefore, the ligand will first bind a type I receptor complex and then recruit a type II receptor for phosphorylation and downstream signalling (Fig. 3). This might explain the increase in noncanonical signalling in *BMPR2* mutation carriers because decreased BMPRII expression favours *de novo* heterodimer formation.

TGFβ superfamily ligands modulate a wide range of developmental programmes, cellular processes and disease states. $TGF\beta_1$ is one of the three subtypes and is known to have key physiological roles in embryonic development, angiogenesis, wound healing, inflammation and immune cell function by T-cell regulation and differentiation. However, excessive $TGF\beta_1$ production is associated with lung fibrotic diseases [27]. All TGFβs are synthesised as

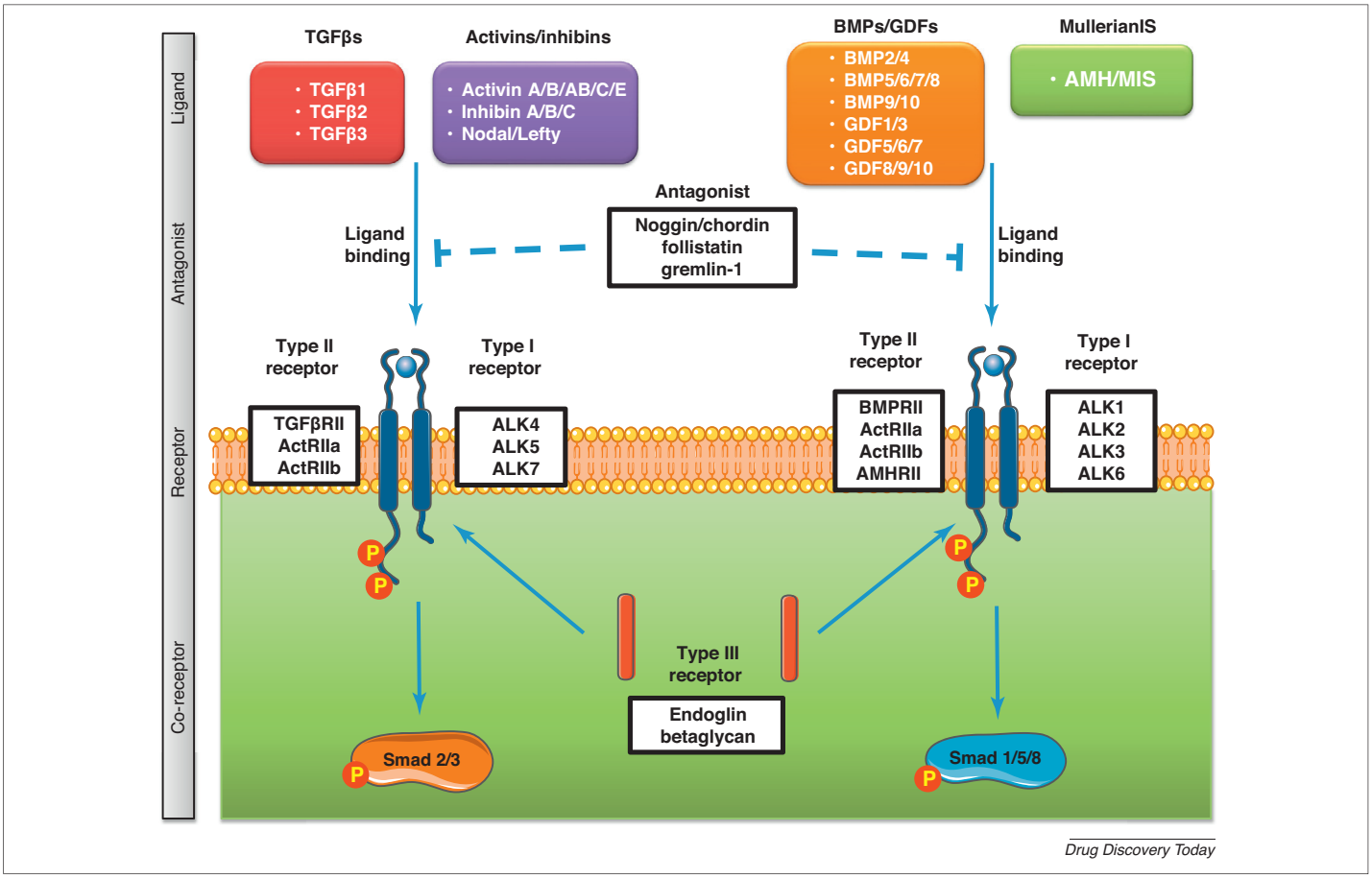

FIGURE 2

Schematic overview of the TGFβ superfamily. Abbreviations: ActRII, activin type 2 receptor; ALK, activin-like kinase; AMH, anti-Müllerian hormone; AMHRII, anti-Müllerian hormone receptor type II; BMP, bone morphogenetic protein; BMPRII, bone morphogenetic protein receptor type II; GDF, growth differentiation factor; IS, inhibiting substance; MIS, Müllerian-inhibiting substance; Smad, Small mothers against decapentoplegic; TGFβ, transforming growth factor beta; TGFβRII, transforming growth factor beta receptor type II.

precursor molecules that will form a latent TGFβ complex. Once bound to latent TGFβ-binding protein (LTBP), the complex will be secreted as a large latent complex (LLC), which can be activated by proteases, integrins, reactive oxygen species (ROS) or change in pH. Impairment of these activating factors can result in dysregulated TGFβ signalling, potentially inducing inflammation, autoimmune disorders, fibrosis, cancer or PAH [28].

TGFβ signalling in PAH

Mutations in the BMPR2 gene

BMPRII is known to be involved in osteogenesis and cell differentiation. Within the pulmonary circulation, the BMPRII pathway inhibits SMC cell proliferation, primarily within the small pulmonary arterioles. When mutated, BMPRII is associated with an increased susceptibility to develop PAH [29]; *BMPR2* mutation carriers develop PAH 10 years earlier than noncarriers and display a more severe haemodynamic profile [30]. In patients with a familial history of PAH, germline mutations in the *BMPR2* gene are detected in 70% of HPAH patients. *BMPR2* gene is located on chromosome 2q33 and consists of 13 exons encoding four functional domains: an N-terminal ligand-binding domain, a single transmembrane region, a serine/threonine kinase and a cytoplasmic domain. Lastly, a compilation of 384 distinct variants, including mutations and polymorphisms, has been identified across the *BMPR2* locus in different cohorts of patients worldwide (Fig. 4). Mutations are spread across the gene, with an exception in exon 13, and include missense mutations, nonsense-mutations, splice defects, deletions and duplications [31]. Whereas a large proportion of variants and polymorphisms are pathogenic, whether all mutations are disease-causing has yet to be elucidated [31].

The majority of *BMPR2* mutations are frameshift and nonsense mutations, triggering nonsense-mediated mRNA decay (NMD), resulting in degradation of the mutated mRNA and leading to haploinsufficiency [32]. Approximately 40% of *BMPR2* mutations are missense mutations affecting highly conserved amino acids that produce stable mRNA transcripts and mutant proteins. HPAH patients carrying these mutations harbour more-severe forms of PAH with lower age at diagnosis and reduced time to lung transplantation [33], suggesting dominant-negative effects on BMPRII function [32]. NMD-negative mutants can affect the ligand-binding domain, the kinase domain or the cytoplasmic tail [34]. Missense mutations located in the cytoplasmic tail result in disrupted interaction with signalling partners of Smad-independent pathways [32] but do not impair the capacity of the protein to be localised to the cell surface or its ability to activate BMP/Smad signalling [34].

Cysteine substitutions in the ligand-binding domain, missense mutations in the kinase domain or in-frame deletion of exon 2 are

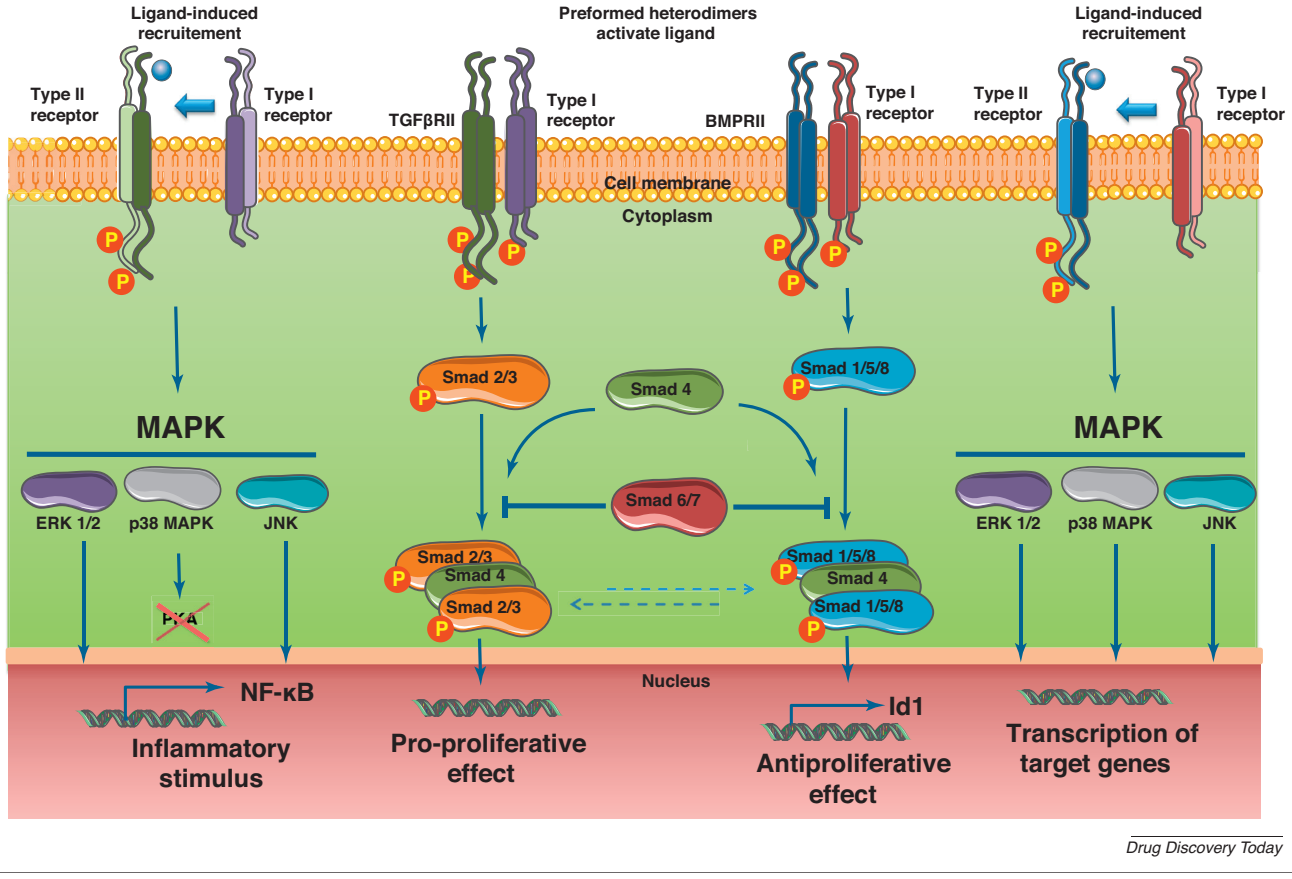

FIGURE 3

Schematic overview of different TGFβ and BMPRII signalling pathways. Abbreviations: BMPRII, bone morphogenetic protein receptor type II; ERK, extracellular-signal-regulated kinase; Id1, inhibitor of DNA binding; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; p38MAPK, p38-mitogen activated protein kinase; Smad, small mothers against decapentoplegic; TGFβRII, transforming growth factor beta receptor type II.

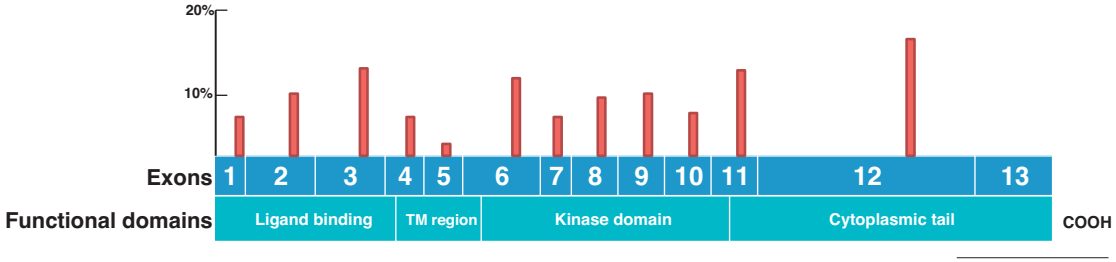

FIGURE 4

BMPR2 mutations associated with PAH. Red bars represent all different mutation categories and the relative occurrence in the exon. Abbreviation: TM region, transmembrane region.

associated with dysfunctional intracellular membrane trafficking. This results in retention of dysfunctional mutant receptors within the endoplasmic reticulum (ER) or Golgi compartments [35], without reduction in the global expression of BMPRII. The dominant-negative effects of intracellular retention could be attributed to chaperone proteins [36], or changes in protein-folding kinetics. Accordingly, chemical chaperones can restore the trafficking of mutated BMPRII protein to the cell surface and increase Smad1/5 signalling [36]. Therefore, enhancing protein trafficking offers a narrow window for therapeutic intervention in HPAH associated with mutations located within the ligand-binding domain; it also implies tailored individual analysis of *BMPR2* mutations to identify patients who could benefit from chaperone therapy.

Mutations in TGFβ family and partners of the BMP signalling pathway

Mutations in the *ACVRL1* gene encoding the activin-receptor-like kinase (ALK)1 protein and endoglin (ENG) have been identified in patients with PAH [37]. Interestingly, *ACVRL1* and *ENG* mutations have been identified in PAH patients with a history of hereditary

haemorrhagic telangiectasia [37], a disease characterised by abnormal blood vessel formation, suggesting that TGFβ signalling might have an important role in angiogenesis and vessel formation. *ALK-1* mutations are rare in PAH, mostly affect the functional kinase domain and result in a more severe phenotype compared with *BMPR2* mutations. ALK-1, which is a type I receptor, promotes proliferation and migration via the type II receptor Smad1/5/8 pathway in ECs. Mutations in *ACVRL1* result in decreased ALK1 expression, reduced Smad1/5/8 activation and increased Smad2/3 signalling through increased ALK5 activity [38]. Endoglin, predominantly expressed on ECs, acts as a co-receptor to stabilise the receptor complexes and ensure correct receptor signalling. Splice-site mutations cause haploinsufficiency, resulting in impaired receptor signalling, dysregulated angiogenesis, vascular malformations and aberrant EC proliferation [25].

Mutations in proteins that do not belong to the TGFβ family but are associated with TGFβ/BMP signalling have also been associated with PAH. Mutations in Smad8 protein (*smad9* gene), an intracellular transcription factor, are associated with a heritable form of PAH; however, the consequences of *smad9* mutations are currently poorly understood because Smad1 and Smad5 can compensate for the loss of Smad8 function [39]. In 2012, a novel genetic mutation in the *CAV1* gene was found in families in which no known mutations in the TGFβ signalling pathway were identified. *CAV1* encodes for caveolin-1, a membrane protein of caveolae, regulating endothelial function and PAEC permeability [40]. Caveolin-1 expression is reduced in remodelled pulmonary arteries from PAH patients, suggesting that loss of caveolin-1 reflects the proliferating and apoptosis-resistant phenotype of pulmonary vascular cells in severe PH. Its downregulation in mouse aortic SMCs results in impaired BMPRII membrane localisation and reduced BMP-dependent Smad signalling, suggesting a direct interaction of caveolin-1 with BMPRII [41]. Accordingly, elafin, an elastase-specific protease inhibitor, amplifies BMP signalling in a caveolin-dependent way in SU5416-hypoxic (SuHx) rats [42].

Biallelic mutations in the *eukaryotic translation initiation factor 2-alpha kinase 4 (EIF2AK4)* gene have been detected in patients with PVOD/PCH, and PAH patients with reduced diffusion capacities [43] who were probably misdiagnosed PVOD patients. *EIF2AK4* encodes a kinase termed general control nonderepressable-2 (GCN2), which phosphorylates the eukaryotic translation initiation factor 2α leading to a global protein synthesis downregulation in response to amino acid starvation, hypoxia or viral infection. EIF2AK4 can regulate the BMPRII/TGFβ pathway by activating Tribbles homolog 3 (TRB3), which interacts with the tail domain of BMPRII in human primary PASMCs [44]. However, the understanding of EIF2AK4 involvement in the pathogenesis of PAH remains incomplete.

Impaired BMPRII signalling in PAH

Despite the impact of *BMPR2* as a genetic factor in PAH, currently we do not completely understand the molecular mechanisms by which loss of BMP receptor function impairs pulmonary vascular cell function in PAH. Unravelling these uncertainties could explain why only 20% of *BMPR2* mutation carriers develop advanced PAH [45] and might predict which mutation carriers will further develop PAH. So far, *BMPR2* gene mutations result in impaired Smad signalling and activation of alternative pathways such as the p38 MAPK pathway [34].

BMP and TGFβ receptor pathways are involved in PAH pathogenesis [46]; consequently, a loss of homeostasis by decreased BMPRII and/or increased TGFβ signalling would contribute to PAH pathogenesis. TGFβ is involved in inflammation, angiogenesis, wound healing and immune cell function, and exerts potent effects on PASMC proliferation, extracellular matrix synthesis and differentiation *in vitro* [47]. Growth, migration and excess matrix deposition by pulmonary vascular cells contribute to vascular wall remodelling in PH [10]. Interestingly, PASMCs isolated from patients harbouring disease-causing mutations exhibit an enhanced mitogenic response to TGFβ [48]; accordingly, a loss of BMPRII in explant-derived PASMCs from PAH patients harbouring *BMPR2* mutations opposed the normal growth-inhibitory effect of TGFβ [49]. *BMPR2* transient silencing in human lung microvascular ECs (HLMVECs) did not affect total TGFβ concentration levels. However, addition of TGFβ causes an increase in downstream TGFβ signalling, even without *BMPR2* knockdown [50], suggesting the importance of the balance between BMPRII and TGFβ (Fig. 5). In addition, TGFβ signalling has been assessed *in vivo* by performing immunohistochemical detection of phosphorylated active downstream effectors of TGFβ receptors (Smad 1/5/8 and Smad2) in normal lungs and lungs with remodelled pulmonary arteries in chronic cigarette smokers or in patients with IPAH. This

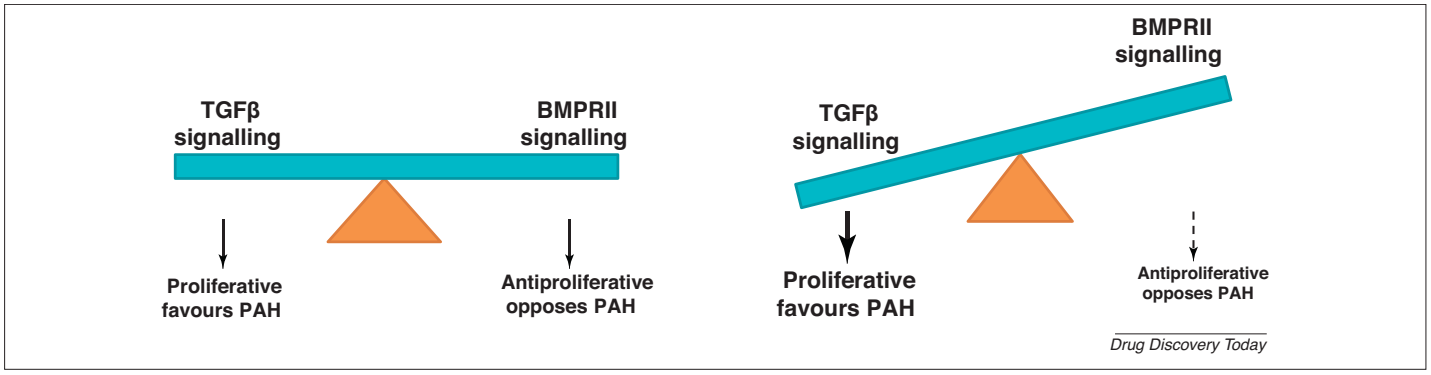

FIGURE 5

Equilibrium (left) and disequilibrium (right) between TGFβ and BMPRII signalling pathways. Disequilibrium is due to either increased TGFβ or decreased BMPRII signalling resulting in proliferative phenotype in favour of progression of PAH. Abbreviations: BMPRII, bone morphogenetic protein receptor type II; PAH, pulmonary arterial hypertension; TGFβRII, transforming growth factor beta receptor type II.

showed that ECs from remodelled pulmonary arteries expressed all TGFβ signalling molecules, whereas ECs from occlusive lesions displayed altered expression of downstream effectors of TGFβ receptors [51].

Animal models of PAH, such as monocrotaline (MCT) and the SuHx rat models, have highly contributed to unravelling the role of BMPRII in the pathogenesis of PAH. MCT, an alkaloid derived from *Crotalaria spectabilis*, induces an obliterative vasculitis, resulting in endothelial dysfunction, medial thickening, loss of peripheral arteries, increased PVR and RV hypertrophy [52]. Combination of chronic hypoxia with a single subcutaneous injection of VEGFR2 inhibitor SU5416 (SuHx) in rats results in severe PAH, progressive obstructive pulmonary vasculopathy characterised by intimal hyperplasia, fibrosis and plexiform lesion formation comparable with human lesions [52]. Homozygous *BMPR2*$^{(-/-)}$ knockout mice die *in utero* whereas heterozygous *BMPR2*$^{(+/-)}$ mice are viable but do not develop PH spontaneously [52]. By contrast, conditional smooth-muscle-specific transgenic mice, expressing a dominant-negative BMPRII, display a reproducible PAH phenotype characterised by increased RV systolic pressure (RVSP), higher Fulton index and pulmonary arterial muscularisation [53]. Similarly, conditional endothelial-specific expression of a truncated BMPRII resulted in increased RVSP and pulmonary arterial muscularisation in mice [54]. Most recently, rats with a monoallelic deletion of 71 bp in exon 1 of *BMPR2* were shown to display reduced BMPRII expression and downstream signalling; these rats also develop age-dependant PH with a low penetrance, are more susceptible to hypoxia-induced PH and display progressive pulmonary vascular remodelling. In addition, they exhibit intrinsic RV dysfunction. This study highlighted how *BMPR2* mutations can alter the pulmonary vasculature as well as the RV function, and the deleterious effect in patients bearing *BMPR2* mutations with compromised response to therapy [55].

Dysfunctional BMPRII/TGFβ signal transduction results in altered expression of transcription factors, including Smads and Id (inhibitor of DNA binding) and decreased expression of BMPRII in hypoxic and MCT rat models of PAH. By contrast, reduced BMP signalling and enhanced TGFβ$_1$ signalling is only observed in MCT rats. Additionally, ALK5 inhibition prevents disease progression in MCT rats, suggesting a shift from BMPRII/Smad1/5/8 signalling to TGFβ/Smad2/3 signalling as a potential mechanism for PAH development [47]. The attenuation of hypoxic PH in rats by adenoviral-mediated overexpression of BMPRII highlights the crucial role of BMPRII loss in the pathogenesis of PAH. Targeted *BMPR2* gene delivery to the endothelium also reduced PH in MCT and hypoxic rats, resulting in increased Smad1/5/8 phosphorylation, reduced Smad3 phosphorylation and changes in non-Smad pathways such as PI3K and p38MAPK [56]. The current hypothesis is that BMPRII and TGFβ work in a strict equilibrium in which BMPRII buffers the effects of TGFβ, with both cascades competing for Smad4 protein availability. When an imbalance occurs, either by decreased levels of BMPRII or increased TGFβ levels, the predominant effect shifts to TGFβ signalling owing to its increased binding with Smad4 [47] (Fig. 5).

TGFβ signalling and endothelial dysfunction

Considering endothelial dysfunction as a hallmark of PAH and the crucial role of TGFβ signalling in the pathogenesis of the disease, it is of major interest to investigate whether both pathways interact, taking into account that endothelial dysfunction could have effects on vascular remodelling and homeostasis [14]. Currently, there is clinical evidence that BMPRII dysfunction impairs pulmonary vasoreactivity: a large majority of PAH patients with a *BMPR2* mutation do not respond to acute vasodilators [57].

Firstly, decreased NO production observed in PAH could be caused by uncoupling of endothelial nitric oxide synthase (eNOS) or reduced eNOS activation. Interestingly, NO production by HLMVECs expressing different *BMPR2* mutants is significantly decreased and mutations in *BMPR2* resulted in impaired eNOS activation in human PAECs [58]. Consequently, eNOS uncoupling might explain the increased ROS production by transgenic mice overexpressing *BMPR2* mutants [59]. It is suggested that BMP2/4-induced eNOS activation is protein kinase A (PKA)-dependent and can be regulated by direct interaction with BMPRII [58]. Because BMPRII and eNOS closely interact with caveolin-1, which plays an important part in TGFβ signalling and pulmonary endothelial barrier function [60], increased TGFβ signalling inhibiting caveolin-1 expression would indirectly affect eNOS activation [61].

Secondly, transient *BMPR2* silencing in HLMVECs results in increased ET-1 secretion [50] and HLMVECs from PAH patients with *BMPR2* mutations produce significantly higher levels of ET-1 [62]. TGFβ is known to stimulate ET-1 production in ECs and, similarly, *BMPR2* silencing induced ET-1 production in HLMVECs but not TGFβ production. Reduced BMPRII expression or TGFβ stimulation in HLMVECs resulted in elevated ET-1 levels [50], suggesting that TGFβ activity could be counterbalanced by BMPRII. Accordingly, adenoviral-mediated BMPRII overexpression restores the imbalance between ET-1 and eNOS by increasing eNOS phosphorylation and limiting TGFβ$_1$-triggered ET-1 stimulation [63], such that disruption of the homeostasis between NO/eNOS and ET-1 could play an important part in PAH pathogenesis (Fig. 6). Besides the effect of ET-1 on NO release via the ET$_B$ receptor, NO can also inhibit the production of ET-1 by endothelin-converting enzyme inactivation depending on the sGC/cGMP pathway [64], fulfilling the potential feedback loop accounting for BMPRII-loss-induced endothelial dysfunction.

TGFβ signalling and inflammation

Endothelial dysfunction implies a loss of the endothelial barrier function. Interestingly, a loss of BMPRII results in impaired endothelial barrier function *in vitro* and *in vivo* [60,65]. Loss of endothelial barrier function increased permeability to circulating cytokines and growth factors, which could result in infiltration of inflammatory cells within the pulmonary vascular wall. Furthermore, chronic inflammation contributes to the pathogenesis of PAH and to the structural pulmonary vascular remodelling characterised by perivascular inflammatory infiltrates comprising T and B lymphocytes, macrophages, dendritic cells, mast cells and monocytes [66]. Circulating levels of several cytokines and chemokines are also elevated in PAH and could be associated with a worse clinical prognosis [66–69].

Whether impaired BMPRII function alters immune function or increases susceptibility to inflammatory triggers remains debateable. Circulating levels of inflammatory mediators, including interleukins or C-reactive protein (CRP), are similar in PAH patients with or without a *BMPR2* mutation [62,68]. Similarly,

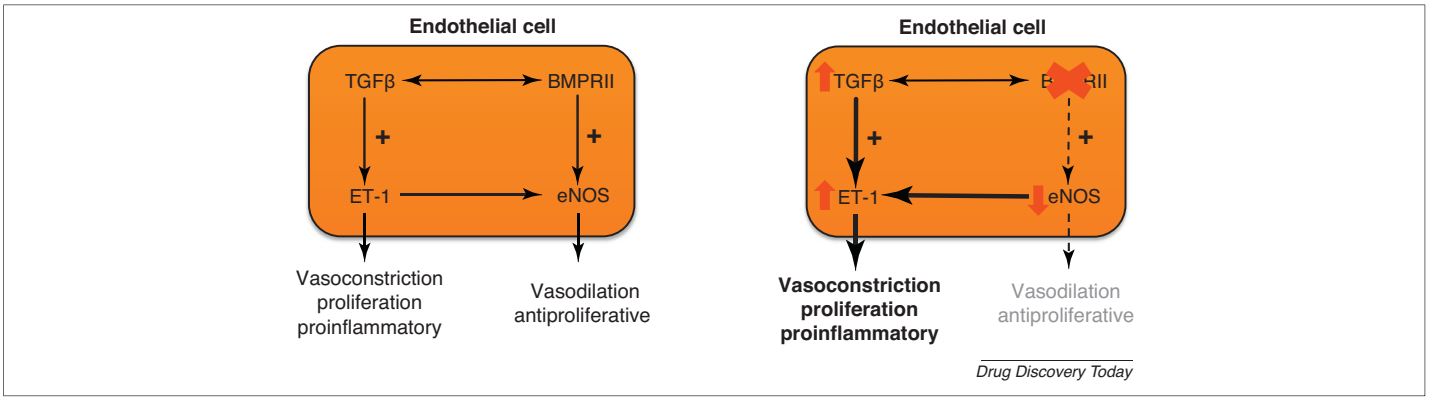

FIGURE 6

Overview of the cooperation of the BMPRII/TGFβ pathway with the NO and ET pathway. Whereas TGFβ and BMPRII are in homeostasis in normal conditions, loss of BMPRII breaks the equilibrium and shifts the predominant effect towards the TGFβ pathway, resulting in increased ET-1 production and in a defect in the homeostasis between ET-1 and eNOS. Abbreviations: BMPRII, bone morphogenetic protein receptor type II; eNOS, endothelial nitric oxide synthase; ET-1 endothelin-1; TGFβRII, transforming growth factor beta receptor type II.

BMPR2$^{+/-}$ knockout mice, which do not develop PAH spontaneously, do not elicit increased levels of interleukin (IL)-6 or keratinocyte chemoattractant (KC) – the mouse equivalent to IL-8 – compared with control mice [70]. However, endothelial-specific loss of BMPRII results in increased CXC chemokine receptor type 2 (CCR2) expression, enhanced leukocyte infiltration and circulating KC in mice [65]. In addition, endothelial-specific expression of a truncated BMPRII protein in mice induced infiltration of inflammatory cells including macrophages and CD3^{+} T cells in remodelled pulmonary vessels [54].

Considering the low penetrance of *BMPR2* mutations in PAH, a role for inflammation as a trigger has been suggested. Accordingly, *BMPR2*$^{+/-}$ knockout mice overexpressing 5-lipoxygenase are more susceptible to developing PH, wherein enhanced endothelial injury and inflammatory response could work in concert with *BMPR2* heterozygosity to promote persistent PH [71]. Similarly, acute and chronic exposure of *BMPR2*$^{+/-}$ knockout mice to lipopolysaccharide resulted in increased IL-6 and KC/IL-8 production and increased RVSP [70]. Another consequence of *BMPR2* mutations could be an enhanced vulnerability to an inflammatory second hit, as suggested by upregulated IL-6 in transgenic mice expressing an inducible dominant-negative *BMPR2* in smooth muscle [72]. Accordingly, a CCR1/2 antagonist has been shown to reduce pulmonary vascular leakage and leukocyte infiltration in mice with a specific EC loss of BMPRII [73]. Additionally, during aging, rats bearing a monoallelic deletion in *BMPR2* exon 1 display pulmonary perivascular leukocyte accumulation [55].

In vitro, PASMCs from *BMPR2*$^{+/-}$ knockout mice or from PAH patients with *BMPR2* mutations exposed to lipopolysaccharide, or from transgenic mice expressing a mutated *BMPR2* in smooth muscle, display an increased production of inflammatory mediators including IL-6, KC/IL-8, MCP-1 and TGFβ [70,72]. Moreover, in response to tumour necrosis factor (TNF)α, transient *BMPR2* silencing in human PAECs enhanced transmigration of leukocytes by disrupting barrier function and releasing proinflammatory cytokines including IL-6 and IL-8 [65] or production of the potent chemokine granulocyte macrophage colony-stimulating factor (GMCSF) [74]. In response to inflammatory mediators such as TNFα or CRP, HLMVECs from PAH patients with a *BMPR2* mutation displayed enhanced adhesiveness to monocytes and IL-6 release, supporting the hypothesis that reduced BMPRII expression in pulmonary endothelium elicits an exacerbated inflammatory response [62]. In human PASMCs, transient *BPMR2* silencing enhanced TNFα-induced cytokine mRNA levels, whereas BMP4, a ligand of BMPRII, reduced these levels through myocardin-related transcription factor A (MRTF-A) by inhibiting NF-κB expression [75]. CCL5 deficiency inhibited PASMC proliferation and migration, increased PAEC apoptosis *in vitro* and attenuated SuHx-induced PAH in rats *in vivo* by restoring BMPRII signalling [76]. This is in accordance with the previous demonstration of the CCL5/CCR5 pathway as a potential therapeutic target in PAH [69].

Proinflammatory effects elicited by TGFβ are illustrated by NF-κB-induced release of IL-6 and IL-8 in response to enhanced TGFβ signalling in PASMCs deficient in BMPRII [49]. Prolonged TGFβ expression reduced the activity of AC and increased cyclooxygenase-2, inactivating AC by increased expression of Smad6 [77], an inhibitor of Smad1/5 activation. Interestingly, a loss of BMPRII in ECs promoted inflammation and atherosclerosis *in vitro* and *in vivo* by increasing expression of ICAM and VCAM and consequently monocyte adhesion or infiltration through NF-κB signalling [78]. This is in favour of the potential anti-inflammatory role of BMPRII, compared with TGFβ. In accordance with the equilibrium between BMPRII and TGFβ, loss of BMPRII favours TGFβ and NF-κB signalling, resulting in reduced BMPRII signalling and eNOS uncoupling that lead to enhanced ROS production, adhesion molecule expression and monocyte adhesion and infiltration. Although various arguments favour inflammation or the increased expression of cytokines as a consequence, there are currently various clues demonstrating that a coincidence of *BMPR2* heterozygosity with an inflammatory insult is required to develop PAH. Nevertheless, targeting inflammatory pathways appears to be a promising therapeutic strategy, as recently highlighted [79].

TGFβ signalling and angiogenesis

PAH has been initially described as a proliferative disease triggered by endothelial cell injury and apoptosis and compared to a cancer-like disorder. Lately, this paradigm has shifted towards alternative hypotheses. It has been recently stipulated that microvascular

rarefaction could be attributed to a degenerative process and that angiogenesis could thereby be beneficial [80]. Although angioproliferative pulmonary vascular remodelling and microvascular rarefaction contribute to increased pulmonary vascular resistance, a paradox about the deleterious *vs* the beneficial role of angiogenesis in the pathogenesis of PAH remains a matter of debate. It is generally accepted that genetic factors and environmental stress contribute to the progression of PAH pathogenesis by inducing pulmonary EC apoptosis [80]. Although PAH patients display increased circulating VEGF levels [12], the combination of hypoxia with the administration of SU5416, a VEGF receptor inhibitor, resulted in severe PAH in rats [13]. In addition, anticancer drugs such as imatinib or dasatinib inhibit angiogenesis but only dasatinib was responsible for drug-induced PAH [81]. Despite the encouraging effects of imatinib as an add-on therapy in PAH, ~31% had to discontinue the long-term safety and efficacy study, owing to serious and unexpected adverse events further discouraging the use of imatinib to treat PAH [82].

VEGF is considered the major angiogenic factor, with five isoforms, termed A, B, C, D and placental growth factor, and three VEGF receptors: VEGFR1, 2 and 3. VEGF-A mainly binds to VEGFR2 and displays several splice variants with some exhibiting angiogenic ($VEGF_{xxx}$ isoforms) or antiangiogenic ($VEGF_{xxx}b$ isoforms) properties; $VEGF_{165}b$ being the major antiangiogenic isoform. VEGFB preferably binds to VEGFR1 and is weakly angiogenic in most tissues, except the heart. VEGFC, which binds VEGFR3, and VEGFD, which can bind to VEGFR2 and VEGFR3, are largely considered angiogenic [83].

Angioproliferative effects

Apoptosis and injury of pulmonary vascular ECs can have indirect consequences including the emergence of hyperproliferative apoptosis-resistant cells that further form occlusive lesions. Accordingly, increased expression of VEGF and its receptors is observed in occlusive lesions, accompanied by disordered angiogenesis [12]. In addition, upregulation of remodelling-associated genes, such as $TGF\beta_1$, HIF1a, VEGFα, VEGFR1/2, thrombospondin, angiopoietin-1 and their receptor Tie-2, as well as sprouting-associated markers, including NOTCH and matrix metalloproteinase, was found in human occlusive lesions [84]. Because BMPRII is mainly expressed within the endothelium of pulmonary arteries, it is involved in the progression of occlusive lesions. Accordingly, loss of functional BMPRII is associated with increased PAEC apoptosis and emergence of apoptosis-resistant vascular cells resulting in obliterative arterial remodelling [85].

Microvascular rarefaction

PAEC apoptosis can result in EC dropout resulting in precapillary regression and then microvascular rarefaction. There is clinical evidence that the amount of occlusive lesions poorly correlates with compromised haemodynamics in PAH patients [80]. Interestingly, impaired neovascularisation has been associated with adverse outcomes in CTEPH patients [86]. Considering that BMPRII deficiency results in PAEC apoptosis, one can hypothesise that impaired BMPRII signalling would contribute to disordered angiogenesis in PAH. Accordingly, circulating levels of $VEGF_{165}b$, known to inhibit migration and proliferation of ECs as well as revascularisation, are significantly increased in patients with PAH [87]. More recently, decreased VEGFR3 expression was observed in lung tissue and PAECs from PAH patients, although *BMPR2* mutations did not appear to determine VEGFR3 expression [88]. VEGFR3 is physically associated with BMPRII, thus facilitating ligand-induced endocytosis of BMPRII, Smad protein phosphorylation and transcription of Id genes. Deletion of endothelial VEGFR3 worsened hypoxia-induced PH in mice and impaired BMP signalling, whereas reconstitution of VEGF3 in PAECs from PAH patients with a *BMPR2* mutation partially restored BMP signalling [88]. Angiogenesis is significantly reduced in progenitor stem cells differentiated *in vitro* into ECs issued from *BMPR2* mutation carriers and rescuing BMPRII enhances angiogenesis [89]. FK506 or tacrolimus, known to activate BMPRII signalling, induced angiogenesis *in vitro* similarly to the angiogenic factor VEGF [90]. Reduced pulmonary microvascular density was observed in rats with a monoallelic deletion in *BMPR2* exon 1 [55].

Regarding TGFβ signalling, TGFβ can induce Smad2/3 phosphorylation through ALK5, which results in reduced angiogenesis by inhibiting EC proliferation, migration and tube formation [38]. An ALK5 kinase inhibitor can also counteract the antiangiogenic effect of TGFβ by enhancing growth and monolayer integrity of stem-cell-derived ECs from mouse embryos [91]. Consequently, proliferative and degenerative concepts contribute to the pathogenesis of PAH. Although a loss of BMPRII results in hyperproliferation of PASMCs, it promotes PAEC apoptosis. Similarly, although BMPRII signalling promotes angiogenesis, TGFβ displays antiangiogenic properties, strengthening the hypothesis that a fair balance between TGFβ and BMPRII signalling is required to maintain intact endothelium barrier function. A lack of equilibrium in the expression of pro- and anti-angiogenic isoforms of VEGF can contribute either to angioproliferative arterial remodelling or to regression of precapillary arterioles throughout the pathophysiological process of PAH. Thereby, angiogenesis could be considered detrimental in early pathogenesis of PAH by contributing to occlusive arteriopathy and beneficial at more-advanced stages of the disease to reduce microvascular rarefaction and promote revascularisation of existing obstructive lesions, thus restraining an increase in PVR.

Effects of current therapies on TGFβ pathway signalling

Throughout the current review, we aim to highlight the importance of the role of the TGFβ pathway in the pathophysiology of PAH. Consequently, unravelling the mechanisms by which current vasodilatory PAH-specific therapies could interact with the BMPRII/TGFβ pathway is highly relevant.

Endothelin pathway

Bosentan, a dual ET-1 receptor antagonist, can restore BMPRII expression, Smad1/5/8 protein activation and Id1 transcription in response to hypoxia and BMP2 in PASMCs [92]. Macitentan, a more efficient dual ET-1 receptor antagonist, can inhibit the TGFβ profibrotic action, by blocking the ETR–TGFβRI-complex-mediated signalling in systemic sclerosis dermal fibroblasts [93]. Accordingly, bosentan and macitentan can prevent endothelial–mesenchymal transition in microvascular ECs by interfering with ET-1/TGFβ signalling and restoring tube formation ability [94].

Nitric oxide pathway

Sildenafil potentiates the antiproliferative effects of BMP4 by enhancing canonical BMP signalling and restores the antiproliferative response to BMP4 in human PASMCs harbouring a *BMPR2* mutation [95]. In addition, sildenafil can restore connexin-40 levels in PASMCs from MCT rats [96]. Interestingly, addition of a BMPRII antagonist (LDN193189) prevents sildenafil from increasing connexin-40 levels, indicating that BMPRII signalling can regulate the expression of connexin-40, connexins being transmembrane proteins of communication channels between adjacent cells. Moreover, sildenafil can stimulate angiogenic properties in human umbilical vein ECs (HUVECs) through a protein kinase G/MAPK-dependent way [97]. Riociguat can inhibit TGFβ expression, Smad-independent TGFβ signalling and reduce fibroblast activation and collagen release [98].

Prostacyclin pathway

Prostanoids are known for their vasodilatory, antiproliferative and anticoagulant properties, mainly occurring through the AC/PKA pathway [15]. In addition, prostanoids can inhibit the TGFβ pathway [99]. Beraprost, a PGI_2 analogue, selectively inhibits proliferation of murine primary PASMCs harbouring a pathogenic *BMPR2* nonsense mutation in the presence or absence of $TGF\beta_1$ stimulation. More specifically, beraprost inhibits $TGF\beta_1$-induced Smad-dependent and Smad-independent signalling via a PKA-dependent pathway by reducing Smad2/3 and p38MAPK protein phosphorylation. Treprostinil inhibits the TGFβ pathway by reducing Smad3 phosphorylation in MCT rats [100]. By inhibiting TGFβ signalling, prostanoids favour the beneficial BMPRII pathway; beraprost and iloprost inhibit Smad3 activity and induce Id1/2 gene in a cAMP/PKA-dependent manner [100].

Drugs targeting the TGFβ pathway in PAH

Restoring the equilibrium between TGFβ and BMPRII signalling in pulmonary vascular cells could represent future potential therapeutic targets to reverse PAH. This could include suppression of TGFβ signalling and/or downstream pathways, upregulation of BMPRII signalling or a combination of both.

Therapeutics that target BMPRII signalling

Upregulation of BMPRII by adenoviral gene delivery showed beneficial effects in experimental chronic hypoxia and MCT rat models [56]. Interestingly, BMP9 binds ALK1 and BMPRII with high affinity and can reverse experimental PAH in mice bearing a heterozygous knock-in allele of a human *BMPR2* mutation, R899X, as well as in MCT and SuHx rats, by enhancing BMPRII signalling. Additionally, BMP9 prevents apoptosis and enhances monolayer integrity of PAECs from PAH patients bearing *BMPR2* mutations [101]. Because BMP9 can increase *BMPR2* gene expression, in addition to protecting PAECs from apoptosis and promoting vascular stability, it provides a proof of concept to treat PAH by directly enhancing BMPRII signalling using recombinant BMP9 protein, as an example. Recently, rescuing BMPRII in progenitor stem cells differentiated *in vitro* into ECs, issued from *BMPR2* mutation carriers, has been shown to improve endothelial function, enhance angiogenesis and ameliorate endothelial barrier function [89]. This suggests that rescuing BMPRII-driven endothelial function combining gene and cell therapy could be a novel treatment strategy for PAH in the future [102].

Activation of BMPRII signalling by tacrolimus, which reverses dysfunctional BMPRII signalling, enhances downstream Smad signalling and *Id1* gene expression and reverses experimental PAH in conditional *BMPR2* knockout mice and in MCT and SuHx rats [90]. Recent clinical trials using tacrolimus in PAH have shown an improved condition in a limited number of patients with end-stage PAH and improvement in exercise capacity and RV function in a subset of PAH patients [103]. More recently, the expression of a novel *BMPR2* gene modifier: fragile histidine triad (FHIT), was shown to be reduced in PAH patients; reduced FHIT expression was associated with impaired BMPRII signalling and endothelial and smooth muscle cell dysfunction. Interestingly, enzastaurin, a cancer drug that can prevent lymphoma relapse, can enhance FHIT and BMPRII expression, as well as prevent and reverse experimental PH in mice exposed to hypoxia and SuHx rats [104]. This suggests that direct or indirect pharmaceutical elevation of BMPRII expression could be considered a potential novel treatment strategy for PAH therapy and even prevention.

Baicalin, an organic compound known to inhibit the inflammatory response by blocking NF-κB expression, attenuated lung damage in MCT rats by inhibiting proliferation and inducing apoptosis of PASMCs. Baicalin decreases the expression of gremlin-1, a BMP antagonist, and increases the expression of the inhibitor of NF-κB, I-κBα, BMPRII, BMP-4, BMP-9 and Smad1/5/8. Additionally, Bcl2 antiapoptotic gene is downregulated by baicalin, suggesting its proangiogenic properties. Furthermore, baicalin inhibits SMC proliferation *in vivo* and *in vitro* and attenuates endothelial–mesenchymal transition in MCT rats [105].

An innovative experimental approach is improving the trafficking and restoring the signalling of mutated BMPRII. Accordingly, functional rescue of mis-trafficked BMPRII mutants resulted in increased membrane expression and the restoration of Smad signalling [36]. Similarly, improving BMPRII trafficking by administration of chaperone proteins seemed to be beneficial in PAH [106]. Ataluren, which induces ribosomal read-through of nonsense mutations, can restore BMP signalling [107]. Chloroquine, known to inhibit autophagy, prevented lysosomal degradation of expressed BMPRII, resulting in sustained expression of BMPRII at the cell surface and restored the proapoptotic, antiproliferative phenotype of PASMCs [108]. Recently, upregulation of chloride intracellular channel-4 (CLIC4) expression and ADP ribosylation factor (Arf)6 activity, regulators of endosomal trafficking, has been observed in endothelial-colony-forming cells from IPAH patients. As a result of Arf6-mediated reduction, CLIC4 reduced BMPRII expression and signalling, and increased lysosomal targeting of BMPRII. Lung-endothelium-targeted knockdown of CLIC4 attenuated development of PH and restored lung expression of BMPRII in SuHx mice. HecinH3, an inhibitor of Arf6, also prevented development of PH in SuHx mice and attenuated PH in MCT rats [109]. Thereby, pharmacological therapies that can restore the trafficking of BMPRII protein appear as promising strategies to improve the treatment of PAH.

The protease inhibitor, elafin, a caveolin-1-dependent inhibitor of elastase, can amplify BMPRII signalling and reverse experimental PH [42]. Statins, commonly used to reduce low density lipoprotein (LDL) circulating levels in humans, were shown to

significantly increase expression levels of BMPR2 mRNA and protein in HUVEC cells [78]. However, randomised placebo-controlled clinical trials failed to show any beneficial effect of statins on exercise capacity or haemodynamics in PAH patients [110]. Sepiapterin, a substrate of sepiapterin reductase with a role in tetrahydrobiopterin (BH_4) synthesis, can restore BMPRII-dependent eNOS uncoupling and induce angiogenesis *in utero* [111]. Dexamethasone, used as an anti-inflammatory drug, can improve haemodynamics and pulmonary vascular remodelling. Experiments in MCT rats show that early PH prevention was through increasing BMPRII mRNA levels, suggesting a BMPRII-induced negative feedback loop reducing IL-6 expression [112]. Loss of BMPRII results in eNOS uncoupling and decreased NO availability. Accordingly, eNOS overexpression attenuates PH in MCT rats [14,113]. Thereby, the Pulmonary Hypertension and Cell-Therapy (PHACeT) trial aimed to investigate the tolerability and safety of the administration of culture-derived endothelial progenitor cells (EPCs) transiently transfected with eNOS on haemodynamics, exercise capacity and immune surveillance in seven PAH patients and showed an improvement in exercise capacity [114]. Most recently, susceptibility to PAH can be conferred onto a wild-type animal by transplantation of *BMPR2*-deficient mouse bone marrow; in addition, transplantation of bone marrow from wild-type mice into *BMPR2*-deficient mice prevents the development of PAH. This therefore suggests that haematopoietic stem cell transplantation might be considered as a potential treatment strategy in genetic forms of PAH [115].

Therapeutics that target TGFβ signalling

Instead of upregulating BMPRII expression, inhibition of TGFβ activity is another potential therapeutic approach to treat PAH. The TGFβ-neutralising antibody 1D11 and SB431542, an inhibitor of ALK5, decrease RVSP and media thickness in a mouse model of Schistosoma-induced pulmonary vascular disease, although ALK5 blockade was less effective [116]. Accordingly, a selective TGFβ ligand trap was able to attenuate vascular remodelling, haemodynamics and improve survival using prophylactic and rescuing approaches in MCT and SuHx rats [117], suggesting that antagonism of TGFβ ligand could be an effective strategy to mitigate PAH. Sotatercept, another TGFβ ligand trap that can restore BMP signalling and was successful in a Phase I clinical trial, is currently being tested in a Phase II trial in PAH patients (PULSAR study; NCT03496207). As recently highlighted, whether a TGFβ ligand trap therapeutic strategy can translate to the clinic remains to be

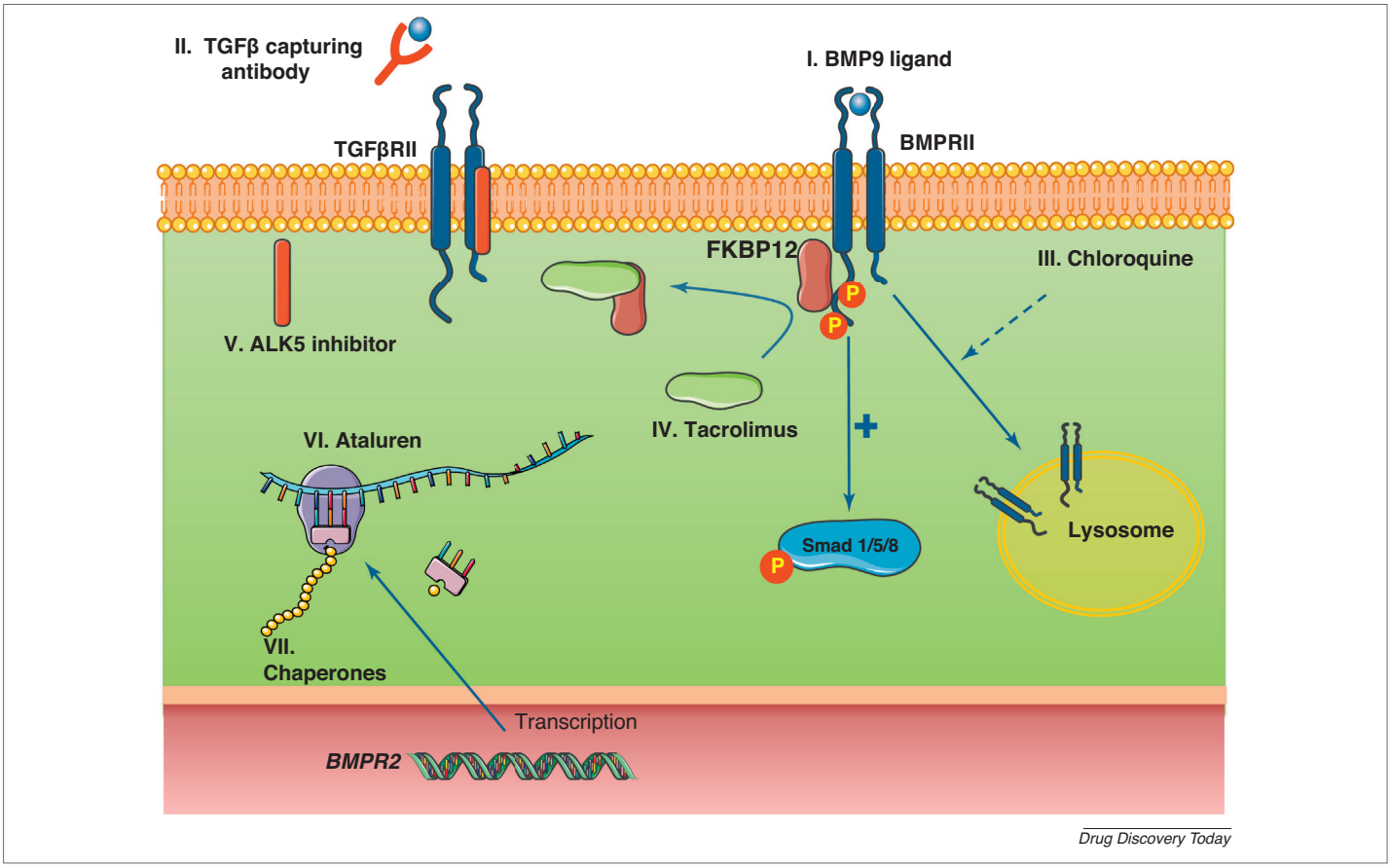

FIGURE 7

Potential therapeutic strategies targeting the TGFβ pathway. Inhibiting ALK5 (V) or using TGFβ capturing antibodies (II) can decrease TGFβ signalling. Increasing BMPRII signalling can be performed by adding BMPRII ligand, BMP9 (I) or chloroquine (III) to inhibit endocytosis and lysosomal degradation of BMPRII receptors, or tacrolimus (IV) to trap FKBP12 and increase downstream signalling pathway, or ataluren (VI) with ribosomal read-through of nonsense mutations, or chemical chaperones (VII) to assist correct folding of the BMPRII protein. Abbreviations: ALK5, activin-receptor-like kinase type 5; BMP9, bone morphogenetic protein 9; BMPRII, bone morphogenetic protein receptor type II; FKBP, FK506 binding protein 12; Smad, small mothers against decapentoplegic; TGFβ, transforming growth factor beta; TGFβRII, transforming growth factor beta receptor type II.

investigated; the recent experimental findings and the launch of the PULSAR study provide promising rationale to consider successful treatment of hereditary and idiopathic PAH [118].

Schisandrin B, a molecule isolated from *Schisandra chinensis,* commonly used in traditional Chinese medicine, inhibited hypoxia-induced arterial SMC proliferation and migration by attenuating TGFβ$_1$ levels and reversing activation of ERK and p38MAPK proteins [119]. Potential therapeutic approaches targeting BMPRII and TGFβ signalling pathways are summarised in Fig. 7.

Concluding remarks and future perspectives

This review highlights the importance of the TGFβ family signalling in the pathogenesis of PAH. Various preclinical data support the concept of restoring normal TGFβ signalling and enhancing the BMPRII axis as new therapeutic approaches for PAH. However, most strategies are not tissue specific and comprehensive investigation is required to better target the pulmonary vasculature and, importantly, the RV. Despite its complexity and involvement at several stages of the pathogenesis of PAH, the TGFβ signalling pathway remains an appealing therapeutic target.

PAH is a heterogeneous disorder with morphological similarities but also distinct genomic variations. The heterogeneous and/or opposing signalling effects of the downstream effectors of the TGFβ receptors can contribute to the heterogeneity observed in response to treatment in PAH patients. Accordingly, pharmacogenomic approaches have been implemented to better understand variable response patterns to these therapies and investigate how genetic variations could influence the response to drugs [120]. The real utility of pharmacogenomic approaches in the treatment of PAH remains to be defined in basing treatment selection and clinical trial inclusion on individual patient gene expression patterns and associated targeted therapies.

Rather than targeting pulmonary vasculature vasoconstriction, innovative therapeutic approaches could encompass broader aspects of the pathophysiology of PAH. The recent demonstration that reprogramming skin fibroblasts, from PAH patients with a *BMPR2* mutation, into ECs in combination with *in vitro* correction of the mutation [89], the recent identification of the potentially 'druggable' BMPR2 gene modifier [104], the current PULSAR clinical trial based on the use of the TGFβ ligand trap, sotatercept or the more recent targeting of regulators of endosomal trafficking [109] open new perspectives for the future treatment of PAH. It could become a reality to implement innovative and tailored therapeutic approaches by combining gene editing, cell therapy and oral medication to definitely cure PAH targeting vasoconstriction, TGFβ/BMPRII imbalance, pulmonary vascular remodelling and RV failure [102].

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to warmly thank Dr John McDonough for his critical reading and editing of the manuscript.

References

1 Galie, N. *et al.* (2016) 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS). *Eur. Heart J.* 37, 67–119
2 Humbert, M. *et al.* (2004) Treatment of pulmonary arterial hypertension. *N. Engl. J. Med.* 351, 1425–1436
3 Peacock, A.J. *et al.* (2007) An epidemiological study of pulmonary arterial hypertension. *Eur. Respir. J.* 30, 104–109
4 D'Alonzo, G. *et al.* (1991) Survival in patients with primary pulmonary hypertension. *Ann. Intern. Med.* 115, 343–349
5 Humbert, M. *et al.* (2010) Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. *Eur. Respir. J.* 36, 549–555
6 Benza, R.L. *et al.* (2012) An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the reveal registry. *Chest* 142, 448–456
7 Simonneau, G. *et al.* (2013) Updated clinical classification of pulmonary hypertension. *J. Am. Coll. Cardiol.* 62, D34–D41
8 Humbert, M. *et al.* (2004) Cellular and molecular pathobiology of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 43, 13–24
9 Montani, D. *et al.* (2013) Pulmonary arterial hypertension. *Orphanet J. Rare Dis.* 8, 1–28
10 Rabinovitch, M. (2012) Molecular pathogenesis of pulmonary arterial hypertension. *J. Clin. Invest.* 122, 4306–4313
11 Perros, F. *et al.* (2007) Dendritic cell recruitment in lesions of human and experimental pulmonary hypertension. *Eur. Respir. J.* 29, 462
12 Tuder, R.M. *et al.* (2001) Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. *J. Pathol.* 195, 367–374
13 Taraseviciene-Stewart, L. *et al.* (2001) Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *FASEB J.* 15, 427–438
14 Budhiraja, R. *et al.* (2004) Endothelial dysfunction in pulmonary hypertension. *Circulation* 109, 159–165
15 Gomberg-Maitland, M. and Olschewski, H. (2008) Prostacyclin therapies for the treatment of pulmonary arterial hypertension. *Eur. Respir. J.* 31, 891–901
16 Klinger, J.R. *et al.* (2013) Nitric oxide deficiency and endothelial dysfunction in pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 188, 639–646
17 Ghofrani, H.A. and Grimminger, F. (2009) Soluble guanylate cyclase stimulation: an emerging option in pulmonary hypertension therapy. *Eur. Respir. Rev.* 18, 35–41
18 Dupuis, J. and Hoeper, M.M. (2008) Endothelin receptor antagonists in pulmonary arterial hypertension. *Eur. Respir. J.* 31, 407–415
19 Clozel, M. (2016) Endothelin research and the discovery of macitentan for the treatment of pulmonary arterial hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 311, R721–R726
20 De Caestecker, M. (2004) The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev.* 15, 1–11
21 Zimmerman, L.B. *et al.* (1996) The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* 86, 599–606
22 Iemura, S.-I. *et al.* (1998) Direct binding of follistatin to a complex of bone-morphogenetic protein and its receptor inhibits ventral and epidermal cell fates in early *Xenopus* embryo. *Dev. Biol.* 95, 9337–9342
23 Hsu, D.R. *et al.* (1998) The *Xenopus* dorsalizing factor gremlin identifies a novel family of secreted proteins that antagonize BMP activities. *Mol. Cell.* 1, 673–683
24 Derynck, R. and Zhang, Y.E. (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425, 577–584
25 García de Vinuesa, A. *et al.* (2016) BMP signaling in vascular biology and dysfunction. *Cytokine Growth Factor Rev.* 27, 65–79
26 Morrell, N.W. (2006) Pulmonary hypertension due to BMPR2 mutation: a new paradigm for tissue remodeling? *Proc. Am. Thorac. Soc.* 3, 680–686
27 Coward, W.R. *et al.* (2010) The pathogenesis of idiopathic pulmonary fibrosis. *Ther. Adv. Respir Dis.* 4, 367–388
28 Pardali, E. and Ten Dijke, P. (2012) TGFβ signaling and cardiovascular diseases. *Int. J. Biol. Sci.* 8, 195–213
29 Machado, R.D. *et al.* (2009) Genetics and genomics of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 54, S32–S42
30 Girerd, B. *et al.* (2010) Absence of influence of gender and BMPR2 mutation type on clinical phenotypes of pulmonary arterial hypertension. *Respir. Res.* 11, 1–8
31 Machado, R.D. *et al.* (2015) Pulmonary arterial hypertension: a current perspective. *Hum. Mutat.* 36, 1113–1127

32 Machado, R.D. (2012) The molecular genetics and cellular mechanisms underlying pulmonary arterial hypertension. *Scientifica* 2012, 1–17
33 Austin, E.D. *et al.* (2009) Truncating and missense BMPR2 mutations differentially affect the severity of heritable pulmonary arterial hypertension. *Respir. Res.* 10, 87
34 Rudarakanchana, N. *et al.* (2002) Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. *Hum. Mol. Genet.* 11, 1517–1525
35 John, A. *et al.* (2015) Defective cellular trafficking of the bone morphogenetic protein receptor type II by mutations underlying familial pulmonary arterial hypertension. *Gene* 561, 148–156
36 Sobolewski, A. *et al.* (2008) Failure of bone morphogenetic protein receptor trafficking in pulmonary arterial hypertension: potential for rescue. *Hum. Mol. Genet.* 17, 3180–3190
37 Girerd, B. *et al.* (2010) Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. *Am. J. Respir. Crit. Care Med.* 181, 851–861
38 Goumans, M.J. *et al.* (2003) Activin receptor-like kinase (ALK) 1 is an antagonistic mediator of lateral TGF beta/ALK5 signaling. *Mol. Cell.* 12, 817–828
39 Shintani, M. *et al.* (2009) A new nonsense mutation of SMAD8 associated with pulmonary arterial hypertension. *J. Med. Genet.* 46, 331–337
40 Austin, E.D. *et al.* (2012) Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. *Circ. Cardiovasc. Genet.* 5, 336–343
41 Mathew, R. (2014) Pathogenesis of pulmonary hypertension: a case for caveolin-1 and cell membrane integrity. *Am. J. Physiol. Heart Circ. Physiol.* 306, H15–25
42 Nickel, N.P. *et al.* (2015) Elafin reverses pulmonary hypertension via caveolin-1-dependent bone morphogenetic protein signaling. *Am. J. Crit. Care Respir. Med.* 191, 1273–1286
43 Eyries, M. *et al.* (2013) EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. *Nat. Med.* 46, 65–69
44 Ma, L. and Bao, R. (2015) Pulmonary capillary hemangiomatosis: a focus on the EIF2AK4 mutation in onset and pathogenesis. *Appl. Clin. Genet.* 8, 181–188
45 Newman, J.H. *et al.* (2004) Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J. Am. Coll. Cardiol.* 43, S33–S39
46 Morrell, N.W. *et al.* (2009) Cellular and molecular basis of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 54, S20–S31
47 Upton, P.D. and Morrell, N.W. (2013) The transforming growth factor-β–bone morphogenetic protein type signalling pathway in pulmonary vascular homeostasis and disease. *Exp. Physiol.* 988, 1262–1266
48 Morrell, N. *et al.* (2001) Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta1 and bone morphogenetic proteins. *Circulation* 104, 790–795
49 Davies, R.J. *et al.* (2012) BMP type II receptor deficiency confers resistance to growth inhibition by TGF-β in pulmonary artery smooth muscle cells: role of proinflammatory cytokines. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 302, L604–L615
50 Star, G.P. *et al.* (2013) ALK2 and BMPR2 knockdown and endothelin-1 production by pulmonary microvascular endothelial cells. *Microvasc. Res.* 85, 46–53
51 Richter, A. *et al.* (2004) Impaired transforming growth factor-β signaling in idiopathic pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 170, 1340–1348
52 Maarman, G. *et al.* (2013) A comprehensive review: the evolution of animal models in pulmonary hypertension research; are we there yet? *Pulm. Circ.* 3, 739–756
53 West, J. *et al.* (2004) Pulmonary hypertension in transgenic mice expressing a dominant-negative BMPRII gene in smooth muscle. *Circ. Res.* 94, 1109–1114
54 Majka, S. *et al.* (2011) Physiologic and molecular consequences of endothelial Bmpr2 mutation. *Respir. Res.* 12, 84
55 Hautefort, A. *et al.* (2018) Bmpr2 mutant rats develop pulmonary and cardiac characteristics of pulmonary arterial hypertension. *Circulation* http://dx.doi.org/10.1161/CIRCULATIONAHA.118.033744 Available at:
56 Harper, R.L. *et al.* (2016) BMPR2 gene therapy for PAH acts via Smad and non-Smad signalling. *Respirology* 21, 727–733
57 Sztrymf, B. *et al.* (2008) Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am. J. Respir. Crit. Care Med.* 177, 1377–1383
58 Gangopahyay, A. *et al.* (2011) Bone morphogenetic protein receptor II is a novel mediator of endothelial nitric-oxide synthase activation. *J. Biol. Chem.* 286, 33134–33140
59 Lane, K. *et al.* (2011) Oxidative injury is a common consequence of BMPR2 mutations. *Pulm. Circ.* 1, 72–83
60 Prewitt, A.R. *et al.* (2015) Heterozygous null bone morphogenetic protein receptor type 2 mutations promote SRC kinase-dependent caveolar trafficking defects and endothelial dysfunction in pulmonary arterial hypertension. *J. Biol. Chem.* 290, 960–971
61 Del Galdo, F. *et al.* (2008) Caveolin-1, transforming growth factor-beta receptor internalization, and the pathogenesis of systemic sclerosis. *Curr. Opin. Rheumatol.* 20, 713–719
62 Vengethasamy, L. *et al.* (2016) BMPRII influences the response of pulmonary microvascular endothelial cells to inflammatory mediators. *Pflugers Arch.* 468, 1969–1983
63 Feng, F. *et al.* (2016) BMPR2 gene delivery reduces mutation-related PAH and counteracts TGF-β-mediated pulmonary cell signalling. *Respirology* 21, 526–532
64 Kelly, L.K. *et al.* (2004) Nitric oxide decreases endothelin-1 secretion through the activation of soluble guanylate cyclase. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 286, L984–L991
65 Burton, V.J. *et al.* (2011) Bone morphogenetic protein receptor II regulates pulmonary artery endothelial cell barrier function. *Blood* 117, 333–341
66 Huertas, A. *et al.* (2014) Immune dysregulation and endothelial dysfunction in pulmonary arterial hypertension. *Circulation* 129, 1332–1340
67 Quarck, R. *et al.* (2009) C-Reactive protein: a new predictor of adverse outcome in pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 53, 1211–1218
68 Soon, E. *et al.* (2010) Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* 122, 920–927
69 Amsellem, V. *et al.* (2014) CCR5 as a treatment target in pulmonary arterial hypertension. *Circulation* 130, 880–891
70 Soon, E. *et al.* (2015) Bone morphogenetic protein receptor type II deficiency and increased inflammatory cytokine production a gateway to pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 192, 859–872
71 Song, Y. *et al.* (2008) Inflammation, endothelial injury, and persistent pulmonary hypertension in heterozygous BMPR2-mutant mice. *Am. J. Physiol. Heart. Circ. Physiol.* 295, H677–H690
72 Hagen, M. *et al.* (2007) Interaction of interleukin-6 and the BMP pathway in pulmonary smooth muscle. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292, 1473–1479
73 Burton, V.J. *et al.* (2011) Attenuation of leukocyte recruitment via CXCR1/2 inhibition stops the progression of PAH in mice with genetic ablation of endothelial BMPR-II. *Blood* 118, 4750–4759
74 Sawada, H. *et al.* (2014) Reduced BMPR2 expression induces GM-CSF translation and macrophage recruitment in humans and mice to exacerbate pulmonary hypertension. *J. Exp. Med.* 211, 263–280
75 Wang, D. *et al.* (2012) Bone morphogenetic protein signaling in vascular disease: anti-inflammatory action through myocardin-related transcription factor A. *J. Biol. Chem.* 287, 28067–28077
76 Nie, X. *et al.* (2018) CCL5 deficiency rescues pulmonary vascular dysfunction, and reverses pulmonary hypertension via caveolin-1-dependent BMPR2 activation. *J. Mol. Cell Cardiol.* 116, 41–56
77 El-Haroun, H. *et al.* (2004) Interleukin-1beta, transforming growth factor-beta1, and bradykinin attenuate cyclic AMP production by human pulmonary artery smooth muscle cells in response to prostacyclin analogues and prostaglandin E2 by cyclooxygenase-2 induction and downregulation of adenylyl cyclase isoforms 1, 2, and 4. *Circ. Res.* 94, 353–361
78 Kim, C.W. *et al.* (2013) Anti-inflammatory and anti-atherogenic role of BMP receptor II in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 33, 1350–1359
79 Rabinovitch, M. *et al.* (2014) Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ. Res.* 115, 165–175
80 Chaudhary, K.R. *et al.* (2017) Proliferative versus degenerative paradigms in pulmonary arterial hypertension have we put the cart before the horse? *Circ. Res.* 120, 1237–1240
81 Montani, D. *et al.* (2012) Pulmonary arterial hypertension in patients treated by dasatinib. *Circulation* 125, 2128–2137
82 Frost, A.E. *et al.* (2015) Long-term safety and efficacy of imatinib in pulmonary arterial hypertension. *J. Heart Lung Transplant.* 34, 1366–1375
83 Evans, I. (2015) An overview of VEGF-mediated signal transduction. *VEGF Signal. Methods Mol. Biol.* 2015, 91–120
84 Jonigk, D. *et al.* (2011) Plexiform lesions in pulmonary arterial hypertension composition, architecture and microenvironment. *Am. J. Pathol.* 179, 167–179
85 Teichert-Kuliszewska, K. *et al.* (2006) Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. *Circ. Res.* 98, 209–217
86 Quarck, R. *et al.* (2015) Contribution of inflammation and impaired angiogenesis to the pathobiology of chronic thromboembolic pulmonary hypertension. *Eur. Respir. J.* 46, 431–443
87 Suzuki, S. *et al.* (2016) Association between levels of anti-angiogenic isoform of vascular endothelial growth factor A and pulmonary hypertension. *Int. J. Cardiol.* 222, 416–420
88 Hwangbo, C. *et al.* (2017) Modulation of endothelial bone morphogenetic protein receptor type 2 activity by vascular endothelial growth factor receptor 3 in pulmonary arterial hypertension. *Circulation* 135, 2288–2298
89 Gu, M. *et al.* (2017) Patient-specific iPSC-derived endothelial cells uncover pathways that protect against pulmonary hypertension in BMPR2 mutation carriers. *Cell Stem Cell* 20, 490–504

90 Spiekerkoetter, E. *et al.* (2013) FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J. Clin. Invest.* 123, 3600–3613
91 Watabe, T. *et al.* (2003) TGF-beta receptor kinase inhibitor enhances growth and integrity of embryonic stem cell–derived endothelial cells. *J. Cell Biol.* 129, 1303–1311
92 Maruyama, H. *et al.* (2016) Bosentan reverses the hypoxia-induced downregulation of the bone morphogenetic protein signaling in pulmonary artery smooth muscle cells. *Life Sci.* 159, 111–115
93 Cipriani, P. *et al.* (2015) Macitentan inhibits the transforming growth factor-beta profibrotic action, blocking the signaling mediated by the ETR/T beta RI complex in systemic sclerosis dermal fibroblasts. *Arthritis Res. Ther.* 17, 1–9
94 Corallo, C. *et al.* (2016) Bosentan and macitentan prevent the endothelial-to-mesenchymal transition (EndoMT) in systemic sclerosis: *in vitro* study. *Arthritis Res. Ther.* 18, 228
95 Yang, J. *et al.* (2012) Sildenafil potentiates bone morphogenetic protein signaling in pulmonary arterial smooth muscle cells and in experimental pulmonary hypertension. *Arter. Thromb. Vasc. Biol.* 33, 34–42
96 Yang, L. *et al.* (2014) Sildenafil increases connexin 40 in smooth muscle cells through activation of BMP pathways in pulmonary arterial hypertension. *Int. J. Clin. Exp. Med.* 7, 4674–4684
97 Pyriochou, A. *et al.* (2006) The phosphodiesterase 5 inhibitor sildenafil stimulates angiogenesis through a protein kinase G/MAPK pathway. *J. Cell Physiol.* 211, 197–204
98 Beyer, C. *et al.* (2015) Stimulation of the soluble guanylate cyclase (sGC) inhibits fibrosis by blocking non-canonical TGF β signalling. *Ann. Rheum. Dis.* 74, 1408–1416
99 Li, N. *et al.* (2014) Role of the prostaglandin E2/E-prostanoid 2 receptor signalling pathway in TGF-β-induced mice mesangial cell damage. *Biosci. Rep.* 34, 797–809
100 Ogo, T. *et al.* (2013) Inhibition of overactive transforming growth factor-β signaling by prostacyclin analogs in pulmonary arterial hypertension. *Am. J. Respir. Cell Mol. Biol.* 48, 733–741
101 Long, L. *et al.* (2015) Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat. Med.* 21, 777–785
102 Quarck, R. and Perros, F. (2017) Rescuing BMPR2-driven endothelial dysfunction in PAH: a novel treatment strategy for the future? *Stem Cell Investig.* 12, 4–6
103 Spiekerkoetter, E. *et al.* (2017) Randomised placebo-controlled safety and tolerability trial of FK506 (tacrolimus) for pulmonary arterial hypertension. *Eur. Respir. J.* 50, 1–12
104 Dannewitz Prosseda, S. *et al.* (2018) Fragile histidine triad (FHIT), a novel modifier gene in pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* http://dx.doi.org/10.1164/rccm.201712-2553OC
105 Luan, Y. *et al.* (2015) Therapeutic effects of baicalin on monocrotaline-induced pulmonary arterial hypertension by inhibiting inflammatory response. *Int. Immunopharmacol.* 26, 188–193
106 Frump, A.L. *et al.* (2013) Abnormal trafficking of endogenously expressed BMPR2 mutant allelic products in patients with heritable pulmonary arterial hypertension. *PLoS One* 8, 1–11
107 Drake, K.M. *et al.* (2013) Correction of nonsense BMPR2 and SMAD9 mutations by ataluren in pulmonary arterial hypertension. *Am. J. Respir. Cell Mol. Biol.* 49, 403–409
108 Long, L. *et al.* (2013) Chloroquine prevents progression of experimental pulmonary hypertension via inhibition of autophagy and lysosomal bone morphogenetic protein type II receptor degradation. *Circ. Res.* 112, 1159–1170
109 Abdul-Salam, V.B. *et al.* (2018) CLIC4/Arf6 pathway — a new lead in BMPRII inhibition in pulmonary hypertension. *Circ. Res.* http://dx.doi.org/10.1161/CIRCRESAHA.118.313705
110 Wang, L. *et al.* (2016) Statins have no additional benefit for pulmonary hypertension: a meta-analysis of randomized controlled trials. *PLoS One* 12, 1–11
111 Teng, R.J. *et al.* (2011) Sepiapterin improves angiogenesis of pulmonary artery endothelial cells with *in utero* pulmonary hypertension by recoupling endothelial nitric oxide synthase. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 301, L334–L345
112 Price, L.C. *et al.* (2011) Dexamethasone reverses monocrotaline-induced pulmonary arterial hypertension in rats. *Eur. Respir. J.* 37, 813–822
113 Liu, L. *et al.* (2006) Sleeping Beauty-mediated eNOS gene therapy attenuates monocrotaline-induced pulmonary hypertension in rats. *FASEB J.* 20, 2594–2596
114 Granton, J. *et al.* (2015) Endothelial NO-synthase gene-enhanced progenitor cell therapy for pulmonary arterial hypertension: the PHACeT trial. *Circ. Res.* 117, 645–654
115 Crosby, A. *et al.* (2018) Hematopoietic stem cell transplantation alters susceptibility to pulmonary hypertension in Bmpr2-deficient mice. *Pulm. Circ.* 8, 1–9
116 Graham, B.B. *et al.* (2013) Transforming growth factor-beta signaling promotes pulmonary hypertension caused by *Schistosoma mansoni*. *Circulation* 128, 1354–1364
117 Yung, L.-M. *et al.* (2016) A selective transforming growth factor-β ligand trap attenuates pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 194, 1140–1151
118 Roman, B.L. and St Hilaire, C. (2016) Catching a disease: a molecular trap as a therapy for pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* 194, 1047–1049
119 Wu, J. *et al.* (2017) Schisandrin B displays a protective role against primary pulmonary hypertension by targeting transforming growth factor β1. *J. Am. Soc. Hypertens.* 11 148–157.e1
120 Badlam, J.B. and Bull, T.M. (2017) Steps forward in the treatment of pulmonary arterial hypertension: latest developments and clinical opportunities. *Ther. Adv. Chronic Dis* 8, 47–64

GLOSSARY

Anorexigens Drugs used to suppress appetite
Hemangiomatosis A condition where a benign tumor made up of blood vessels is present in several parts of the body
Haploinsufficiency Mechanism of action to explain a phenotype when a diploid organism has lost one copy of a gene and is left with a single functional copy of that gene
Hereditary haemorrhagic telangiectasia Also known as Osler–Weber–Rendu syndrome, is a rare autosomal-dominant genetic disorder that leads to abnormal blood vessel formation
Vasculopathy Any disorder of the blood vessels
Arteriopathy Any disease of the arteries
Prostanoids Subclass of eicosanoids consisting of the prostaglandins, the thromboxanes and the prostacyclins
Tacrolimus An immunosuppressive drug used mainly after allogeneic organ transplant to lower the risk of organ rejection
Ataluren Pharmaceutical drug for the treatment of Duchenne muscular dystrophy