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REVIEW

Translational medicine

Pulmonary arterial hypertension in congenital heart disease: translational opportunities to study the reversibility of pulmonary vascular disease

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Pulmonary arterial hypertension (PAH) is a progressive and lethal pulmonary vascular disease (PVD). Although in recent years outcome has improved by new treatments that delay disease progression, a cure has not yet been achieved. In PAH associated with congenital heart disease (CHD), remodeling of the pulmonary vasculature reaches an irreversible phenotype similar to all forms of end-stage PAH. In PAH-CHD, however, also an early stage is recognised, which can be completely reversible. This reversible phase has never been recognised in other forms of PAH, most likely because these patients are only diagnosed once advanced disease has developed. We propose that the clinical model of PAH-CHD, with an early reversible and advanced irreversible stage, offers unique opportunities to study pathophysiological and molecular mechanisms that orchestrate the transition from reversible medial hypertrophy into irreversible plexiform lesions. Comprehension of these mechanisms is not only pivotal in clinical assessment of disease progression and operability of patients with PAH-CHD; specific targeting of these mechanisms may also lead to pharmacological interventions that transform 'irreversible' plexiform lesions into a reversible PVD: one that is amenable for a cure. In recent years, significant steps have been made in the strive to 'reverse the irreversible'. This review provides an overview of current clinical and experimental knowledge on the reversibility of PAH, focussing on flow-associated mechanisms, and the near-future potential to advance this field.

Keywords Congenital heart disease • Reversible/irreversible•Operability • Vascular remodelling • Pulmonary blood flow • Neointimal/plexiform lesions

Introduction

Pulmonary arterial hypertension (PAH) is an obstructive arterial pulmonary vascular disease (PVD) that is progressive, irreversible and usually fatal, despite current treatment options.[1–3] PAH in congenital heart disease (PAH-CHD) is unique in this respect, as it also knows an early phase in which the arteriopathy can generally be reversed.[4] The potential for reversibility in PAH-CHD was first identified in the 1950's,[5] but mechanisms, timing, and identification of such reversibility are still obscure today.

We know that in PAH-CHD, systemic-to-pulmonary shunting can ultimately remodel the pulmonary vasculature to a characteristic irreversible phenotype similar to other forms of PAH.[6–8] We also know that *timely* closure of the shunt will generally result in normalisation of pulmonary hemodynamics and vascular morphology, i.e. cure PAH.[9] Yet, it appears that closure beyond the reversible phase relates to accelerated disease progression, as these patients have a prognosis substantially worse than those with uncorrected PAH-CHD.[10]

Our knowledge is still limited regarding the factors that distinguish reversible from irreversible PVD.[11] Identification of these factors would not only be pivotal to improve assessment of operability in PAH-CHD, it could also give clues to comprehend the mechanisms that orchestrate the

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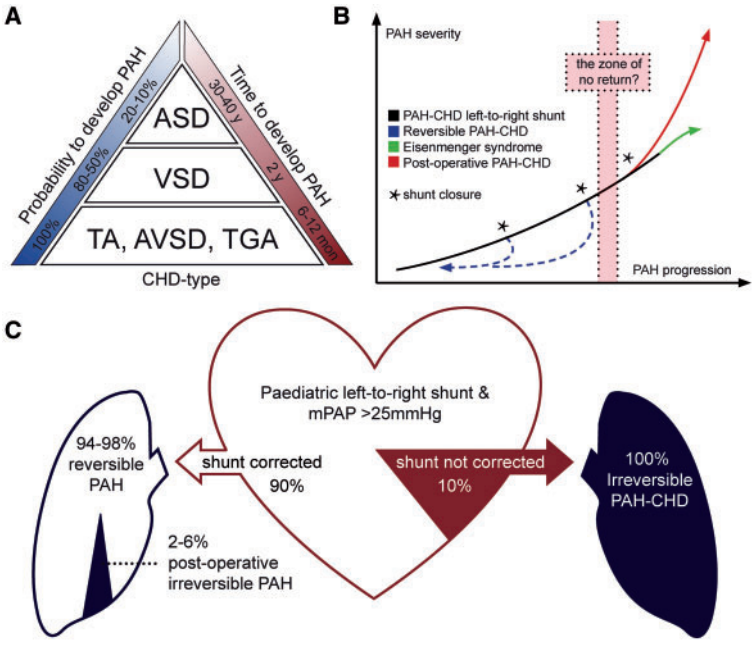

Figure 1 (*A*) CHD-type and shunt determine the progression of PAH. TA: Truncus Arteriosus, AVSD: Atrio-verntricular septal defect; TGA: Transposition of the great arteries.[18,20] (*B*) Correction of a left-to-right shunt may lead to full disease regression, but not after a certain zone of no return. Correction beyond this 'zone' is associated with accelerated deterioration of PAH-related symptoms. (*C*) When diagnostic criteria for PAH are met in paediatric left-to-right shunt, 90% is evaluated eligible for shunt correction. In a portion of corrected CHD, PAH will progress due to misjudged irreversible disease. When shunt correction is contra-indicated (PAH is evaluated as irreversible or other co-morbidities do not permit surgery), PAH will progress.

development of irreversible PVD. Specific targeting of the mechanisms involved could help to *reverse the irreversible*, also in other forms of PAH.

This review will discuss features and putative mechanisms associated with reversibility of PAH-CHD.

Reversibility in PAH-CHD: concepts and observations

To normalise vs. to stabilise

The current literature generally refers to *survival* in PAH as disease stability rather than disease reversal. Targeted pharmacotherapy may stabilise or decelerate disease progression, and improve clinical condition, but still does not cure the disease. Here, disease reversibility is defined as complete and permanent normalisation of both pulmonary hemodynamics and vascular morphology. Reversible PAH was first described in children with PAH associated with congenital cardiac shunts.[5] Timely correction of the shunt, removing the disease trigger, was found to result in full disease regression.[12,13] This unique feature distinguishes PAH-CHD from other forms of PAH. In idiopathic, heritable or PAH associated with connective tissue disease, the trigger cannot be removed, and a normalisation of vascular morphology has never been described. In PAH induced by suppressible triggers, like HIV- or schistosomiasis-associated PAH, treatment may stabilise progression, but complete normalisation remains debated.[14] Still, recent cases suggest that reversibility may not be exclusive to PAH-CHD. For instance, dasatinib, used in the treatment of leukaemia, can induce near systemic rises in pulmonary arterial pressure that may fully reverse after discontinuation of the drug.[15] PAH complicating bone marrow transplantation has also shown potential to completely normalise over time.[16] These cases support the clinical and histological concepts of reversibility that have been reported extensively in PAH-CHD.

Epidemiology of regressive and progressive PAH-CHD

All children born with a systemic-to-pulmonary shunt are at risk for PAH (*Figure 1A*). CHD-type and shunt size are considered principal determinants for the progression of PAH-CHD and the development of irreversible disease.[17,18] Increased pulmonary blood flow is considered a prerequisite, combination with high pressure an accelerator.[19] Large post-tricuspid shunts (high flow, high pressure) more frequently and quickly induce irreversible PAH than restrictive or pre-tricuspid shunts (high flow, normal pressure) (*Figure 1A*). In contrast, CHD-types associated with normal flow and high pressure, like congenital mitral stenosis, generally do not induce PAH.[19]

Improved monitoring of CHD has enabled paediatric cardiologists to identify and treat patients at risk for PAH early in life. Currently,

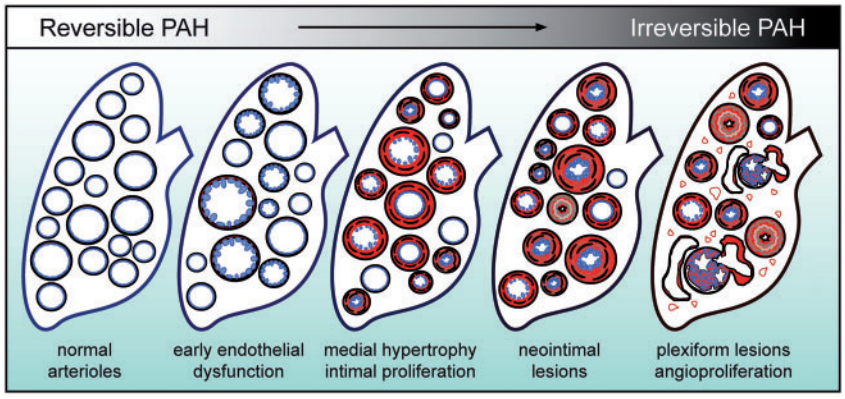

Figure 2 Schematic representation of the progression of vascular remodeling in PAH-CHD. As the disease advances, the morphological spectrum of vascular lesions also becomes more heterogeneous.

around 90% of all children with PAH-CHD are considered surgically correctable.[21] In the majority of these children, shunt closure completely normalises the disease: reversible PAH. Unfortunately, the portion of children with disease progression despite early surgery is still significant (2–6%, *Figure 1C*).[21] This is 5–13% for adults.[22,23] While in PAH-CHD, older age at correction is a well-recognised risk factor for irreversible disease, the occurrence of post-operative PAH in infants suggests that also other factors than timing of correction are involved.[22] Conditions such as disturbed perinatal transition or co-morbidities like Down syndrome are often associated with, and may contribute to progression of PVD after correction of the cardiac defect in these children.[24] Genetic susceptibility could also modify progression of PVD, albeit not through the gene-mutations identified in 'idiopathic' or heritable PAH, since these are rarely found in paediatric PAH-CHD.[25]

Whatever the reason for disease progression after correction, its prognosis is detrimental. Post-operative PAH is associated with lower survival rates bot at short-[26] and long-term follow up: 36 vs. 87% for non-operated PAH-CHD (*Figure 1B*).[10,20,27] This difference might be explained by the loss of potential for unloading of the right ventricle (RV) after correction of the defect. Whereas RV failure is recognised a major risk factor for mortality in adults,[28] RV function in children is generally better preserved at diagnosis.[29] Interestingly, pressure unloading of the RV in PAH (e.g. by lung transplantation) is generally associated with a marked reversal of RV function and remodeling, even when severe dysfunction and hypertrophy was present.[30]

Of all children with CHD who present with PAH, ±10% is regarded not eligible for shunt closure,[20] mostly due to concerns of irreversible PVD in the context of complex CHD and/or co-morbidities that prohibit invasive surgery. The most ominous clinical sign of irreversible PAH is the Eisenmenger syndrome (ES). In ES, pulmonary vascular resistance (PVR) has exceeded systemic values, which reverses the direction of the shunt, causing cyanosis. The mean survival of ES when developed during childhood is 11.4 years,[31] with a 4-year survival rate of 77%.[32] Currently, indexed PVR > 8 Woods Units m^2 is regarded a contra-indication for shunt closure, and 6–8 WU m^2 a '*grey zone*'.[33] Unfortunately, these cut-off values are mainly based on expert opinions and are not supported by prospective data.

The vascular morphology in PAH-CHD: which lesions reverse?

In end-stage disease, all forms of PAH share a common histology, typically hallmarked by the occurrence of neointimal lesions such as concentric laminar intimal fibrosis and plexiform lesions.[1] Plexiform lesions are present in 90% of biopsies in various forms of advanced PAH.[1] In early-stage PAH-CHD, these neointimal lesions are generally still absent.[9] Early-stage PAH is predominantly characterised by medial hypertrophy of pre-acinar vessels and muscularisation of normally non-muscularised arterioles: the first visible structural change in the vascular remodeling process. This may be accompanied by mild proliferation of intimal cells (*Figure 2*).[1,7]

The link of vascular histopathology to disease reversibility dates back to the studies of Wagenvoort, and Heath & Edwards published in the 1950's. They postulated that PAH-CHD might be reversible when histology shows medial hypertrophy only.[34,35] In fact, all healthy new-borns display medial hypertrophy directly after birth, which reverses in the first 8 weeks of life. Children with a ventricular septal defect (VSD), however, maintain this medial hypertrophy postnatally and often display proliferative intimal changes within 1 year, and plexiform lesions within 2.5 years after birth.[35] The number and size of acinar arterioles in these children were also reported to be reduced at this age,[36] suggesting developmental rarefaction that may contribute to disease progression.

Heath & Edwards classified concentric laminar intimal fibrosis, plexiform lesions, dilatation lesions, and necrotizing arteriitis as severe lesions, conjoined to irreversible, progressive disease.[34,35] Wagenvoort used a descriptive analysis of these vascular lesions to predict outcome after cardiac surgery in 137 PAH-CHD cases. Patients with medial hypertrophy without severe intimal fibrosis

were likely to improve after surgery, whereas those with more severe intimal fibrosis or plexiform lesions were likely to deteriorate.[12] The concept of vascular reversibility was confirmed in study that assessed morphological change of various lesions in consecutive lung biopsies prior to and several years after pulmonary artery banding: medial hypertrophy reversed, whereas neo-intimal lesions and plexiform lesions did not.[4]

Despite these opportunities, pre-operative morphologic evaluation has largely disappeared from clinical practice since the eighties, due to the procedural risks of open lung biopsy (13% morbidity and 20% mortality in children with PAH-CHD).[37]Furthermore, it should be noted that absence of neointimal lesions in a lung biopsy does not ensure post-operative regression of PAH, since the advanced lesions may not be distributed equally through the lungs.[13,38] Still, vascular morphology has been confirmed as a useful tool to understand reversibility and should be considered the gold standard when phenotyping PAH in experimental models.

Reversibility in animal models

The first animal study that investigated the reversibility of PAH by manipulating pulmonary blood flow, used a rat model that induced over circulation in the left lung by right lung excision. Isolated medial hypertrophy developed in the remaining left lung, which reversed after unloading by lung transplantation into a healthy recipient rat.[39] In piglets with shunt-induced medial hypertrophy, shunt closure also decreases medial thickness and PVR.[40] These studies confirm the clinical observation that isolated medial hypertrophy has the biological potential to reverse. This also warrants a critical appraisal towards studies that claim reversibility by pharmacological compounds in P(A)H-models characterised by muscularisation only. These animal models have led to a great number of potential therapies that failed to translate to clinical application and should therefore be considered inadequate to investigate the PVD in PAH.[42] To more closely resemble human disease, animal models have been developed that lead to rapid progression of the characteristic neointimal lesions associated with human disease: the monocrotalin + increased-pulmonary-flow (MCT + Flow) and Sugen5146 + Hypoxia (SuHx) model, both in rats.[6,41,42] At present, the effects of hemodynamic unloading in neointimal flow-induced PAH models are unknown. Investigating these effects could help to answer why and how increased pulmonary flow leads to progression in PAH, and why medial hypertrophy may reverse and why neointimal lesions may not. Approaches to mimic hemodynamic unloading in (neointimal) PAH models are by pulmonary artery banding, shunt closure or transplantation of a lung with flow-induced PAH into a healthy recipient.

Mechanisms of disease progression in PAH-CHD

Triggers for plexogenic remodelling and the unique opportunities of PAH-CHD

Plexiform lesions have a variety of etiological backgrounds, including genetic mutations, infections, connective tissue diseases or certain drugs.[43] In PAH-CHD, increased pulmonary blood flow is regarded the essential trigger for disease development.[6] Several factors make PAH-CHD an ideal 'human model' to study the mechanisms involved in *early* disease progression, disease reversal and transition to irreversibility: (1) the trigger is known, (2) the onset and magnitude of the trigger can be estimated, (3) the trigger can be removed, (4) *timely* removal of the trigger potentiates disease reversal, and (5) persistence of the trigger leads to progressive PVD that shares many characteristics with other forms of PAH. Moreover, (6) the subgroup of patients that do not reverse despite trigger removal allows to identify conditions and mechanisms specifically associated with (the transition towards) irreversible disease.

Comparison of human reversible to irreversible PAH-CHD could help to identify pathways related to (ir)reversibility (*Figure 3A*).[9,44] The 'liquid biopsy' could be an innovative, minimally-invasive alternative to 'solid' lung biopsy to investigate human PAH. The concept of liquid biopsy originated from oncology and utilises the fact that tumours shed cells and DNA directly into the bloodstream, and tumour-RNA into platelets, which can be analysed to determine stage and treatment response.[45] Liquid biopsy may also be applicable in PAH, for instance by performing high-throughput screening on platelets and circulating endothelial cells, which have already been shown detectable in blood samples of PAH(-CHD) patients (*Figure 3B*).[46] Specifically, new markers such as Micro-RNAs may be identified in these samples. This knowledge can be confirmed (bedside-to-bench) in animal models that show normalisation of *established* neointimal lesions, either by hemodynamic unloading or pharmacotherapy. We advise the use of multiple, complementary animal models to increase robustness of the data.

The next section describes potential pathways that may be involved in the transition from reversible to irreversible PAH. A proposed mechanistic overview of the relationship of increased flow to neointimal remodeling is presented in *Figure 4*.

Pathobiology: what could make PAH irreversible?

Apoptosis and apoptosis-resistance

The balance between endothelial cell (EC) apoptosis and apoptosis-resistance appears to shift during disease progression. In lung biopsies obtained during shunt correction in paediatric PAH-CHD, the pro-apoptotic marker p53 dominated the tissue of patients that had reversed one year after closure, whereas Bcl-2 (associated to apoptosis-resistance) was expressed only in patients with progressive disease.[9] Survivin, another marker for apoptosis-resistance, is expressed abundantly in patients with end-stage PAH and nearly absent in CHD without PAH.[47] These observations were confirmed both in vitro and in vivo. Experiments on human pulmonary ECs confirm that apoptosis-induction combined with high shear stress, ultimately results in hyper proliferation of ECs that are apoptosis-resistant and express survivin.[48] In MCT + Flow rats, pro-apoptotic factors peak in early disease, directly after the induction of flow.[49] In SuHx rats, anti-apoptotic factors appear late in the disease, specifically in neointimal lesions.[50] Recently, in the SuHx model, inhibition of Bromodomain-containing protein 4, an indirect stimulator of survivin and blc-2, has shown to reverse established neointimal lesions.[50] These data underline the role of disturbed apoptosis in the formation of neointimal lesions and suggest potential for drugs that target apoptosis-resistance in the reversal of *irreversible* PAH.

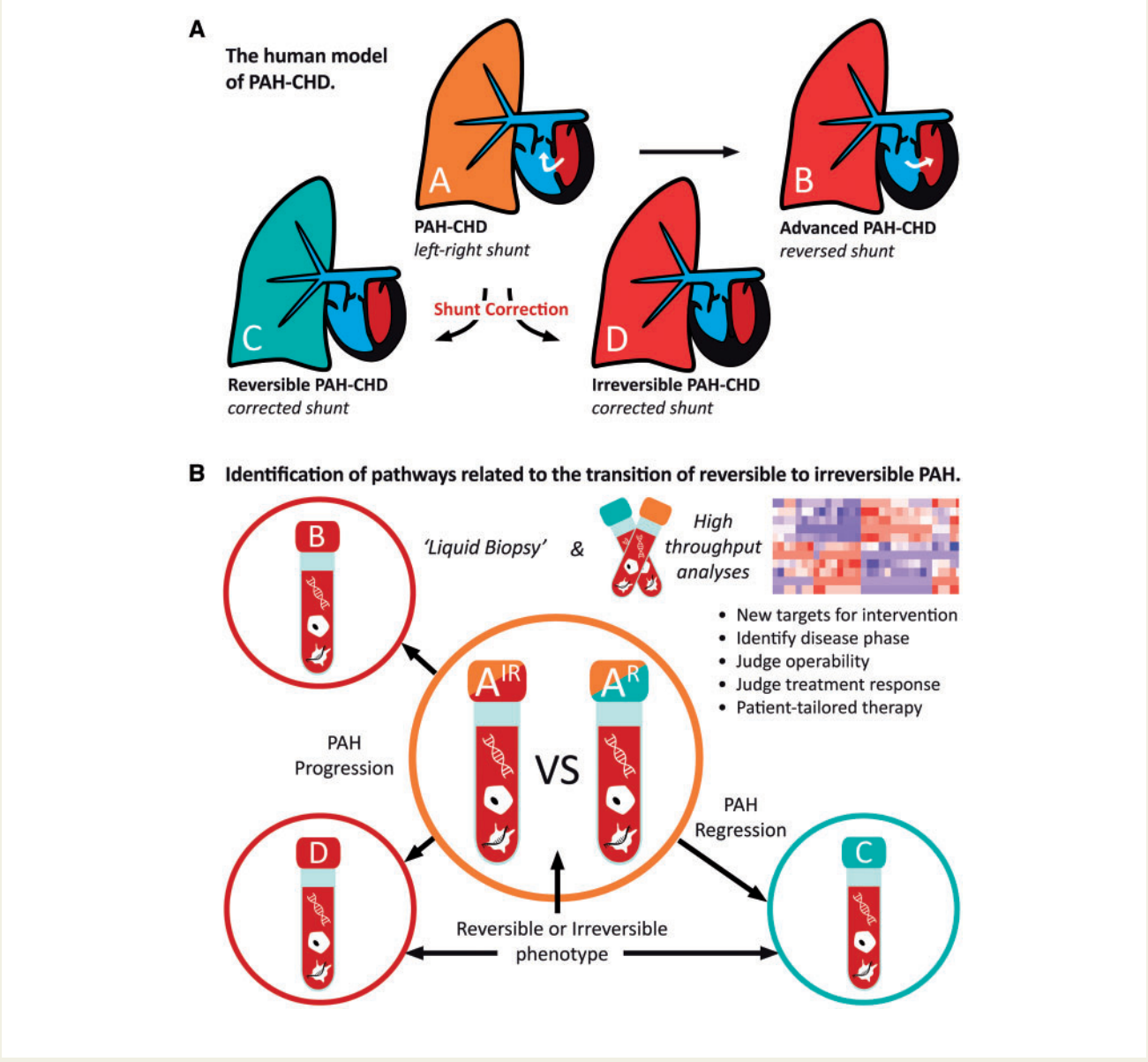

Figure 3 (*A*) The human model of PAH in CHD. A shunt initiates disease progression up to stage A, in which shunt correction either leads to regression of the disease (stage C) or to progression (stage D). Without shunt correction, stage A will progress to stage B: the Eisenmenger Syndrome. (*B*) $A^{R\ or\ IR}$: *PAH-CHD that becomes reversible or irreversible*. Using liquid biopsy combined with high throughput screening, multiple profiles can be compared with investigate mechanisms associated with (1) flow-dependent progression (A^{IR} to B), (2) flow-independent progression (A^{IR} to D), (3) Reversible or Irreversible phenotype before correction, e.g. to judge operability (A^{IR} to A^{R}), or disease course (C to D), and (4) disease reversal (A^{R} to C).

Inflammation

Inflammatory cytokines (IL-6, IL-1β, MCP-1, and TNF-α most importantly) and macrophages have been associated with disease progression not only in inflammation-associated PAH, but also in PAH-CHD[51] and IPAH.[52,53] Plexiform lesions are found both in HIV and schistosomiasis-associated PAH (sch-PAH) and in auto-inflammatory conditions like scleroderma and systemic lupus erythematosus.[54] The inflammation hypothesis (reviewed in[54,55]) therefore proposes that inflammation can initiate and sustain vascular remodeling up to the formation of neointimal lesions. Indeed, transgenic mice that over-express IL-6 spontaneously develop neointimal lesions,[56] which is particularly remarkable since pulmonary neointimal lesions rarely occur in mice.[42] In severe murine sch-PAH, associated with overwhelming vascular inflammation, established neointimal lesions were reversed by anti-parasitic treatment that inhibited the cytokine response.[57] In MCT + Flow rats, inhibition of mast cells or chymase, both attenuate vascular remodeling, but do not lead to reversal.[58] Considering the emerging evidence for an inflammatory component

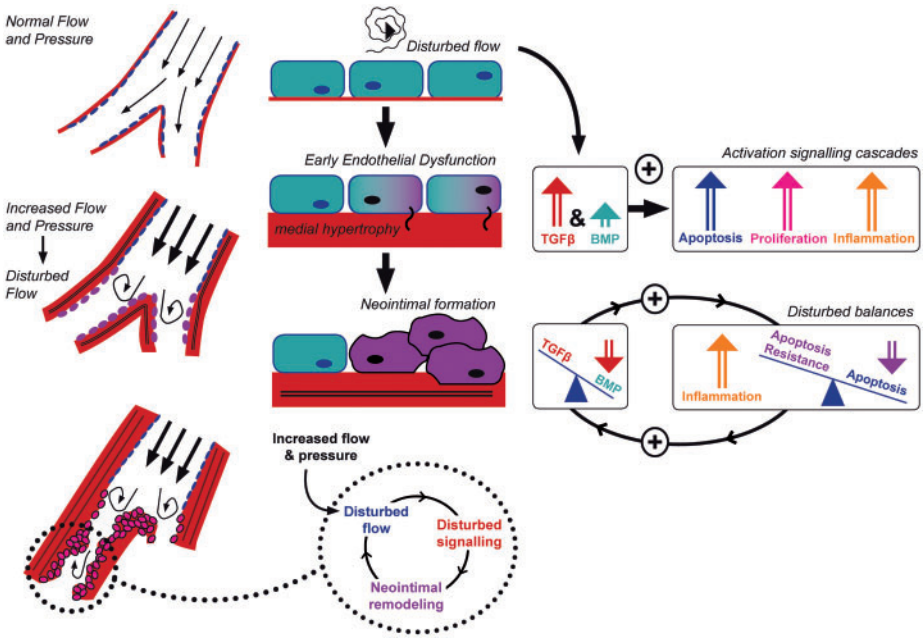

Figure 4 Flow in the normal circulation is laminar. In the situation of a large left-to-right shunt, flow and pressure increase. This causes disturbances in local flow, especially at the branching points. Disturbed flow causes endothelial dysfunction, which, together with increased pressure, causes medial hypertrophy. Disturbed flow also induces a disturbance in TGF-β-BMP balance, which up regulates apoptosis, proliferation, and inflammation. Misbalance in these cascades cause the endothelial cells to proliferate, which eventually become resistant to apoptosis and form a neointimal layer. This irregular neointimal layer further disrupts flow patterns, which further disturbs signalling balances, causing an irreversible vicious cycle.

in PAH and neointimal formation, anti-inflammatory drugs may have potential as (adjuvant) therapy to reverse irreversible PAH.

Tgf-β/BMP-signalling: a common denominator?

It is intriguing that a disease with such diverse triggers, or associated conditions, leads to a rather uniform end-stage vascular phenotype, regardless of aetiology. So could there be a common denominator? Increasing evidence points towards Tgf-β and BMP signalling as respective orchestrators of plexogenic remodeling, or protection and repair of the pulmonary vasculature (reviewed in[59]).

In 2000, mutations in BMP-receptor 2 (BMPR2) were first identified as a cause for hPAH.[60] BMPR2-mutations impair vaso-protective BMP signalling and account for 80% of hPAH cases.[61] Disturbed Tgf-β/BMP signalling was further identified in IPAH,[62] sch-PAH,[63] CTD-PAH,[64] PAH-CHD,[65] drug (cocaine) and HIV-induced PAH[66] and PAH associated with hereditary haemorrhagic telangiectasia.[67] Vice versa though, rats with deficient BMPR2-function[68] or mice with a BMPR2-knock-out[69,70] only develop an inconsistent pattern of mild pulmonary hypertension and medial hypertrophy without neointimal lesions. These observations signify that these models may still lack a second hit, or display an early (reversible) phase of BMPR2-deficiency related PAH.

Mechanistically, BMPR2-deficiency can elicit pro-proliferative and anti-apoptotic responses in murine pulmonary smooth muscle cells;[71] factors downstream of Tgf-β also induce these responses in human PAECs.[72] Also, both BMPR2-deficiency and increased Tgf-β are able to promote the progression of PAH by a hyper inflammatory response through cytokines such as IL-6 and -8.[73,74]

Therapeutically, BMP-9, an endogenous stimulator of BMP-signalling, has shown to reverse medial hypertrophy in BMPR2-deficient mice and MCT rats, but also neointimal lesions in SuHx rats.[70] Similarly, FK506 (Tacrolimus) showed to (1) restore disturbed BMPR2-signalling and endothelial function in PAECs from IPAH patients, (2) prevent PAH progression in BMPR2-deficient mice, and (3) reverse established neointimal lesions in SuHx rats.[69] As a first clinical observation, low-dose Tacrolimus (blood level 1.5–2.5 ng/mL) was associated with relieved symptoms of right heart failure and improved WHO functional class in 3 end-stage IPAH patients.[75] Finally, Elafin, an endogenous serine protease inhibitor that enhances BMPR2 signalling, has been shown to reverse neointimal lesions in SuHx rats and reduce neointimal thickness of pulmonary arteries in cultured sections from lung explants of patients with PAH.[76] These recent experimental studies serve proof of the concept that reversibility of advanced PAH, considered irreversible, is feasible in animals and cultured human pulmonary arteries. This concept holds promise for future therapeutic potential in patients.

Conclusion

PAH is considered a progressive and irreversible arteriopathy and is still lethal. Although advances in the last decade have led to an

improvement in outcome, mainly by delaying disease progression, reversal or cure has not yet been achieved.

PAH in congenital heart disease has a vascular end-stage that is universal to other forms of PAH, but also knows a reversible phase. This unique clinical model of PAH-CHD, with an early reversible and advanced irreversible stage, offers opportunities to study pathophysiological and molecular mechanisms that orchestrate the transition from reversible medial hypertrophy into irreversible neointimal and plexiform lesions. Comprehension of these mechanisms is not only pivotal in clinical assessment of disease progression and operability of patients with PAH-CHD; specific targeting of these mechanisms may also lead to pharmacological reversal of '*irreversible*' plexiform lesions into a reversible arteriopathy: one that is amenable for a cure. The recent manipulation of the BMPR2 signalling pathway is an example of the steps to make in the strive for '*reverse remodeling*'.

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References

1. Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, Jessup M, Grizzle WE, Aldred MA, Cool CD, Tuder RM. Modern age pathology of pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012;**186**:261–272.
2. Hoeper MM, Bogaard HJ, Condliffe R, Frantz R, Khanna D, Kurzyna M, Langleben D, Manes A, Satoh T, Torres F, Wilkins MR, Badesch DB. Definitions and diagnosis of pulmonary hypertension. *J Am Coll Cardiol* 2013;**62**(25Suppl):D42–D50.
3. Galie N, Corris PA, Frost A, Girgis RE, Granton J, Jing ZC, et al. Updated treatment algorithm of pulmonary arterial hypertension. *J Am Coll Cardiol* 2013;**62**(25Suppl):D60–D72.
4. Wagenvoort CA, Wagenvoort N, Draulans-Noe Y. Reversibility of plexogenic pulmonary arteriopathy following banding of the pulmonary artery. *J Thorac Cardiovasc Surg* 1984;**87**:876–886.
5. Heath D, Edwards JE. The pathology of hypertensive pulmonary vascular disease: a description of six grades of structural changes in the pulmonary arteries with special reference to congenital cardiac septal defects. *Circulation* 1958;**18**(4Part1):533–547.
6. Dickinson MG, Bartelds B, Borgdorff MA, Berger RM. The role of disturbed blood flow in the development of pulmonary arterial hypertension: lessons from preclinical animal models. *Am J Physiol Lung Cell Mol Physiol* 2013;**305**:L1–L14.
7. Tuder RM, Archer SL, Dorfmuller P, Erzurum SC, Guignabert C, Michelakis E, Rabinovitch M, Schermuly R, Stenmark KR, Morrell NW. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol* 2013;**62**(25 Suppl):D4–D12.
8. Hoffman JI, Rudolph AM, Heymann MA. Pulmonary vascular disease with congenital heart lesions: pathologic features and causes. *Circulation* 1981;**64**:873–877.
9. Levy M, Maurey C, Celermajer DS, Vouhe PR, Danel C, Bonnet D, Israel-Biet D. Impaired apoptosis of pulmonary endothelial cells is associated with intimal proliferation and irreversibility of pulmonary hypertension in congenital heart disease. *J Am Coll Cardiol* 2007;**49**:803–810.
10. Manes A, Palazzini M, Leci E, Bacchi Reggiani ML, Branzi A, Galie N. Current era survival of patients with pulmonary arterial hypertension associated with congenital heart disease: a comparison between clinical subgroups. *Eur Heart J* 2014;**35**:716–724.
11. D'alto M, Romeo E, Argiento P, Correra A, Santoro G, Gaio G, Sarubbi B, Calabro R, Russo MG. Hemodynamics of patients developing pulmonary arterial hypertension after shunt closure. *Int J Cardiol* 2013;**168**:3797–3801.
12. Wagenvoort CA. Open lung biopsies in congenital heart disease for evaluation of pulmonary vascular disease: predictive value with regard to corrective operability. *Histopathology* 1985;**9**:417–436.
13. Haworth SG. Pulmonary vascular disease in ventricular septal defect: structural and functional correlations in lung biopsies from 85 patients, with outcome of intracardiac repair. *J Pathol* 1987;**152**:157–168.
14. dos Santos Fernandes CJ, Jardim CV, Hovnanian A, Hoette S, Dias BA, Souza S, Humbert M, Souza R. Survival in schistosomiasis-associated pulmonary arterial hypertension. *J Am Coll Cardiol* 2010;**56**:715–720.
15. Hong JH, Lee SE, Choi SY, Kim SH, Jang EJ, Bang JH, Park JE, Jeon HR, Oh YJ, Yi JE, Jung HO, Youn HJ, Kim DW. Reversible pulmonary arterial hypertension associated with dasatinib for chronic myeloid leukemia. *Cancer Res Treat* 2015;**47**:937–942.
16. Limsuwan A, Pakakasama S, Hongeng S. Reversible course of pulmonary arterial hypertension related to bone marrow transplantation. *Heart Vessels* 2011;**26**:557–561.
17. van Albada ME, Schoemaker RG, Kemna MS, Cromme-Dijkhuis AH, van Veghel R, Berger RM. The role of increased pulmonary blood flow in pulmonary arterial hypertension. *Eur Respir J* 2005;**26**:487–493.
18. Haworth SG. Pulmonary vascular disease in different types of congenital heart disease: implications for interpretation of lung biopsy findings in early childhood. *Br Heart J* 1984;**52**:557–571.
19. Kulik TJ. Pulmonary blood flow and pulmonary hypertension: is the pulmonary circulation flowophobic or flowophilic?. *Pulm Circ* 2012;**2**:327–339.
20. van Albada ME, Berger RM. Pulmonary arterial hypertension in congenital cardiac disease–the need for refinement of the Evian-Venice classification. *Cardiol Young* 2008;**18**:10–17.
21. van Loon RL, Roofthooft MT, Hillege HL, ten Harkel AD, van Osch-Gevers M, Delhaas T, Kapusta L, Strengers JL, Rammeloo L, Clur SA, Mulder BJ, Berger RM. Pediatric pulmonary hypertension in the Netherlands: epidemiology and characterization during the period 1991 to 2005. *Circulation* 2011;**124**:1755–1764.
22. van Riel AC, Schuuring MJ, van Hessen ID, Zwinderman AH, Cozijnsen L, Reichert CL, Hoorntje JC, Wagenaar LJ, Post MC, van Dijk AP, Hoendermis ES, Mulder BJ, Bouma BJ. Contemporary prevalence of pulmonary arterial hypertension in adult congenital heart disease following the updated clinical classification. *Int J Cardiol* 2014;**174**:299–305.
23. Engelfriet PM, Duffels MG, Moller T, Boersma E, Tijssen JG, Thaulow E, Gatzoulis MA, Mulder BJ. Pulmonary arterial hypertension in adults born with a heart septal defect: the Euro Heart Survey on adult congenital heart disease. *Heart* 2007;**93**:682–687.
24. van Loon RL, Roofthooft MT, van Osch-Gevers M, Delhaas T, Strengers JL, Blom NA, Backx A, Berger RM. Clinical characterization of pediatric pulmonary hypertension: complex presentation and diagnosis. *J Pediatr* 2009;**155**:176–182.e1.
25. Levy MM. Genetic analyses in a cohort of children with pulmonary hypertension. *Eur Resp J* 2016 (10);**48**:1118–1126.
26. Roofthooft MT, Bergman KA, Waterbolk TW, Ebels T, Bartelds B, Berger RM. Persistent pulmonary hypertension of the newborn with transposition of the great arteries. *Ann Thorac Surg* 2007;**83**:1446–1450.
27. Haworth SG, Hislop AA. Treatment and survival in children with pulmonary arterial hypertension: the UK Pulmonary Hypertension Service for Children 2001–2006. *Heart* 2009;**95**:312–317.
28. van de Veerdonk MCMC. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. *J Am Coll Cardiol* 2011(12-6);**58**:2511.
29. Berger RM, Beghetti M, Humpl T, Raskob GE, Ivy DD, Jing ZC, Bonnet D, Schulze-Neick I, Barst RJ. Clinical features of paediatric pulmonary hypertension: a registry study. *Lancet* 2012;**379**:537–546.
30. Moulton MJ, Creswell LL, Ungacta FF, Downing SW, Szabó BA, Pasque MK. Magnetic resonance imaging provides evidence for remodeling of the right ventricle after single-lung transplantation for pulmonary hypertension. *Circulation* 1996;**94**:II312–II319.
31. Zijlstra WM, Douwes JM, Rosenzweig EB, Schokker S, Krishnan U, Roofthooft MT, Miller-Reed K, Hillege HL, Ivy DD, Berger RM. Survival differences in pediatric pulmonary arterial hypertension: clues to a better understanding of outcome and optimal treatment strategies. *J Am Coll Cardiol* 2014;**63**:2159–2169.
32. Barst RJ, Ivy DD, Foreman AJ, McGoon MD, Rosenzweig EB. Four- and seven-year outcomes of patients with congenital heart disease-associated pulmonary arterial hypertension (from the REVEAL Registry). *Am J Cardiol* 2014;**113**:147–155.
33. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M, Aboyans V, Vaz Carneiro A, Achenbach S, Agewall S, Allanore Y, Asteggiano R, Paolo Badano L, Albert Barbera J, Bouvaist H, Bueno H, Byrne RA, Carerj S, Castro G, Erol C, Falk V, Funck-Brentano C, Gorenflo M, Granton J, Iung B, Kiely DG, Kirchhof P, Kjellstrom B, Landmesser U, Lekakis J, Lionis C, Lip GY, Orfanos SE, Park MH, Piepoli MF, Ponikowski P, Revel MP, Rigau D, Rosenkranz S, Voller H, Luis Zamorano J. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J* 2016;**37**:67–119.
34. Dammann JF Jr, Ferencz C. The significance of the pulmonary vascular bed in congenital heart disease. I. Normal lungs. II. Malformations of the heart in which there is pulmonary stenosis. *Am Heart J* 1956;**52**:7–17.

35. Wagenvoort CA, Neufeld HN, Dushane JW, Edwards JE. The pulmonary arterial tree in ventricular septal defect: a quantitative study of anatomic features in fetuses, infants and children. *Circulation* 1961;**23**:740–748.
36. Haworth SGSG. A morphometric study of regional variation in lung structure in infants with pulmonary hypertension and congenital cardiac defect: a justification of lung biopsy. *Br Heart J* 1978-8;**40**:825–831.
37. Wilson NJ, Seear MD, Taylor GP, LeBlanc JG, Sandor GG. The clinical value and risks of lung biopsy in children with congenital heart disease. *J Thorac Cardiovasc Surg* 1990;**99**:460–468.
38. Egito ES, Aiello VD, Bosisio IB, Lichtenfels AJ, Horta AL, Saldiva PH, Capelozzi VL. Vascular remodeling process in reversibility of pulmonary arterial hypertension secondary to congenital heart disease. *Pathol Res Pract* 2003;**199**:521–532.
39. O'blenes SB, Fischer S, McIntyre B, Keshavjee S, Rabinovitch M. Hemodynamic unloading leads to regression of pulmonary vascular disease in rats. *J Thorac Cardiovasc Surg* 2001;**121**:279–289.
40. Mercier O, Sage E, de Perrot M, Tu L, Marcos E, Decante B, Baudet B, Herve P, Dartevelle P, Eddahibi S, Fadel E. Regression of flow-induced pulmonary arterial vasculopathy after flow correction in piglets. *J Thorac Cardiovasc Surg* 2009;**137**:1538–1546.
41. Taraseviciene-Stewart L, Kasahara Y, Alger L, Hirth P, Mc Mahon G, Waltenberger J, Voelkel NF, Tuder RM. Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *Faseb J* 2001;**15**:427–438.
42. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009;**297**:L1013–L1032.
43. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, Gomez Sanchez MA, Krishna Kumar R, Landzberg M, Machado RF, Olschewski H, Robbins IM, Souza R. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2013;**62**(25 Suppl):D34–D41.
44. van der Feen DE, Dickinson MG, Bartelds B, Borgdorff MAJ, Sietsma H, Lévy M, Sietsma H, Berger RMF. Egr-1 Identifies neointimal remodeling and relates to progression in human pulmonary arterial hypertension. *J Heart Lung Transplant* 2016;**35**(4):481–490.
45. Best MG, Sol N, Kooi I, Tannous J, Westerman BA, Rustenburg F, Schellen P, Verschueren H, Post E, Koster J, Ylstra B, Ameziane N, Dorsman J, Smit EF, Verheul HM, Noske DP, Reijneveld JC, Nilsson RJ, Tannous BA, Wesseling P, Wurdinger T. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell* 2015;**28**:666–676.
46. Smadja DM, Gaussem P, Mauge L, Israel-Biet D, Dignat-George F, Peyrard S, Agnoletti G, Vouhe PR, Bonnet D, Levy M. Circulating endothelial cells: a new candidate biomarker of irreversible pulmonary hypertension secondary to congenital heart disease. *Circulation* 2009;**119**:374–381.
47. McMurtry MS, Archer SL, Altieri DC, Bonnet S, Haromy A, Harry G, Bonnet S, Puttagunta L, Michelakis ED. Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. *J Clin Invest* 2005;**115**:1479–1491.
48. Sakao S, Taraseviciene-Stewart L, Lee JD, Wood K, Cool CD, Voelkel NF. Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. *Faseb J* 2005;**19**:1178–1180.
49. Dickinson MG, Kowalski PS, Bartelds B, Borgdorff MA, van der Feen D, Sietsma H, Molema G, Kamps JA, Berger RM. A critical role for Egr-1 during vascular remodelling in pulmonary arterial hypertension. *Cardiovasc Res* 2014;**103**(4):573–584.
50. Meloche J, Potus F, Vaillancourt M, Bourgeois A, Johnson I, Deschamps L, Chabot S, Ruffenach G, Henry S, Breuils-Bonnet S, Tremblay E, Nadeau V, Lambert C, Paradis R, Provencher S, Bonnet S. Bromodomain-containing protein 4: the epigenetic origin of pulmonary arterial hypertension. *Circ Res* 2015 (Aug 28);**117**:525–535.
51. Dickinson MG, Bartelds B, Molema G, Borgdorff MA, Boersma B, Takens J, Weij M, Wichers P, Sietsma H, Berger RM. Egr-1 expression during neointimal development in flow-associated pulmonary hypertension. *Am J Pathol* 2011;**179**:2199–2209.
52. Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, Trembath RC, Jennings S, Barker L, Nicklin P, Walker C, Budd DC, Pepke-Zaba J, Morrell NW. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* 2010;**122**:920–927.
53. Selimovic N, Bergh CH, Andersson B, Sakiniene E, Carlsten H, Rundqvist B. Growth factors and interleukin-6 across the lung circulation in pulmonary hypertension. *Eur Respir J* 2009 Sep;**34**:662–668.
54. Price LC, Wort SJ, Perros F, Dorfmuller P, Huertas A, Montani D, Cohen-Kaminsky S, Humbert M. Inflammation in pulmonary arterial hypertension. *Chest* 2012;**141**:210–221.
55. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res* 2014;**115**:165–175.
56. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. *Circ Res* 2009;**104**:236–244, 28p following 244.
57. Crosby AA. Praziquantel reverses pulmonary hypertension and vascular remodeling in murine schistosomiasis. *Am J Respir Crit Care Med* 2011;**184**:467–473.
58. Bartelds B, van Loon RL, Mohaupt S, Wijnberg H, Dickinson MG, Boersma B, Takens J, van Albada M, Berger RM. Mast cell inhibition improves pulmonary vascular remodeling in pulmonary hypertension. *Chest* 2012;**141**:651–660.
59. Upton PDPD. The transforming growth factor-b-bone morphogenetic protein type signalling pathway in pulmonary vascular homeostasis and disease. *Exp Physiol* 2013;**98**:1262–1266.
60. Lane K, Machado R, Pauciulo M, Thomson J, Phillips J, Loyd J, Nichols W, Trembath R. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000;**26**:81–84.
61. Machado RDRD. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 2006;**27**:121–132.
62. Atkinson Carl C. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* 2002;**105**:1672–1678.
63. Graham Brian BBB. Transforming growth factor-b signaling promotes pulmonary hypertension caused by Schistosoma mansoni. *Circulation* 2013;**128**:1354–1364.
64. Gilbane Adrian JAJ. Impaired bone morphogenetic protein receptor II signaling in a transforming growth factor-b-dependent mouse model of pulmonary hypertension and in systemic sclerosis. *Am J Respir Crit Care Med* 2015;**191**:665–677.
65. Gao Bao-Hui BH. Expression and pathological implication of transforming growth factor-beta1 mRNA and endothelin-1 mRNA in intraacinar pulmonary arterioles of congenital heart disease accompanied with pulmonary hypertension. *Zhonghua Bing Li Xue Za Zhi* 2005;**34**:159–162.
66. Dalvi Pranjali P. Downregulation of bone morphogenetic protein receptor axis during HIV-1 and cocaine-mediated pulmonary smooth muscle hyperplasia: implications for HIV-related pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2013;**33**:2585–2595.
67. Trembath RCRC. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001;**345**:325–334.
68. Ranchoux B, Antigny F, Rucker-Martin C, Hautefort A, Pechoux C, Bogaard HJ, Dorfmuller P, Remy S, Lecerf F, Plante S, Chat S, Fadel E, Houssaini A, Anegon I, Adnot S, Simonneau G, Humbert M, Cohen-Kaminsky S, Perros F. Endothelial-to-mesenchymal transition in pulmonary hypertension. *Circulation* 2015;**131**:1006–1018
69. Spiekerkoetter EE. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013;**123**:3600–3613.
70. Long Lu L. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med* 2015;**21**:777–785.
71. Nasim Md Talat MT. BMPR-II deficiency elicits pro-proliferative and anti-apoptotic responses through the activation of TGFb-TAK1-MAPK pathways in PAH. *Hum Mol Genet* 2012-6-1;**21**:2548–2558.
72. Nickel Nils N. GDF-15 is abundantly expressed in plexiform lesions in patients with pulmonary arterial hypertension and affects proliferation and apoptosis of pulmonary endothelial cells. *Resp Res* 2011;**12**:62.
73. Davies Rachel JRJ. BMP type II receptor deficiency confers resistance to growth inhibition by TGF-b in pulmonary artery smooth muscle cells: role of proinflammatory cytokines. *Amer J Physiol Lung Cell Mol Physiol* 2012(15 Mar);**302**:604–615.
74. Soon Elaine E. BMPR-II deficiency promotes pulmonary hypertension via increased inflammatory cytokine production. *Am J Respir Crit Care Med* 2015;**192**:859–872.
75. Spiekerkoetter E, Sung YK, Sudheendra D, Bill M, Aldred MA, van de Veerdonk MC, Vonk Noordegraaf A, Long-Boyle J, Dash R, Yang PC, Lawrie A, Swift AJ, Rabinovitch M, Zamanian RT. Low-dose FK506 (Tacrolimus) in end-stage pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2015;**192**:254–257.
76. Nickel NP, Spiekerkoetter E, Gu M, Li CG, Li H, Kaschwich M, Diebold I, Hennigs JK, Kim KY, Miyagawa K, Wang L, Cao A, Sa S, Jiang X, Stockstill RW, Nicolls MR, Zamanian RT, Bland RD, Rabinovitch M. Elafin Reverses pulmonary hypertension via caveolin-1-dependent bone morphogenetic protein signaling. *Am J Respir Crit Care Med* 2015;**191**:1273–1286.