# Pulmonary Hypertension in Chronic Neonatal Lung Disease: Mechanisms and Targets

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**Abstract**

Chronic neonatal lung disease is a common complication of preterm birth for which no effective preventive or rescue therapies currently exist. This condition has been and remains associated with serious pulmonary and neurological sequelae that have major lifelong health implications. Pulmonary hypertension is a common and important associated phenomenon, contributing to high mortality. Considerable gaps in knowledge exist, particularly with respect to pathogenesis, natural history, mechanisms contributing to right ventricular failure and the role, if any, of pulmonary vasodilators. Addressing these gaps will require careful prospective study of at-risk infants and improved understanding of pathophysiological mechanisms employing relevant animal models.

**Keywords**

Neonate • Prematurity • Extremely-low birth weight • Chronic lung disease • Bronchopulmonary dysplasia • Pulmonary hypertension • Nitric oxide • Rho-kinase

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## Bronchopulmonary Dysplasia

Positive pressure ventilation for the treatment of respiratory distress syndrome in prematurely-born infants was introduced into clinical practice in the mid-1960s [1]. Shortly thereafter, a chronic neonatal lung disease (CNLD), termed bronchopulmonary dysplasia (BPD), was first described by Northway and colleagues [2]. The affected infants were generally born preterm (the average postmenstrual age in Northway's cohort was 32 weeks) and all had severe respiratory failure, were ventilated with high $O_2$ concentrations and



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required high inflation pressures. The development of respiratory failure was accompanied by a classical sequence of radiological changes evolving from an initial "white-out", which cleared to reveal multiple cystic lesions. If infants survived this stage, a streaky pattern consistent with pulmonary fibrosis and/or distended lymphatics predominated. The mortality was very high at approximately 60% [2]. At autopsy there was evidence of both atelectasis and emphysematous changes, pulmonary fibrosis, marked proximal airway injury and vascular remodeling indicative of severe pulmonary hypertension (PHT) [2]. While the term BPD has been preserved over the intervening 50 years, the clinical, radiological and pathological features of CNLD have dramatically changed. The affected population is now much more immature at birth, the illness during the neonatal period is generally less severe, the classic sequence of radiological changes is no longer apparent, and proximal airway injury and fibrosis are no longer common pathological features.

## CNLD in the Present Era

Advances in neonatal care over the past 25 years have had a major impact on the survival of infants born ≤1000 g (known as extremely low birth weight (ELBW) infants, coinciding with ≤28 weeks' postmenstrual age at birth). CNLD now arises predominantly in ELBW infants with an overall incidence of around 50% [3, 4], leading to more than 10,000 new cases per year in the United States alone [5]. This incidence has not changed over the last 15 years, and in Canada has possibly even increased [4], despite advances in many aspects of neonatal care. CNLD has been and remains associated with serious pulmonary [6, 7] and neurological [8] sequelae that have major lifelong health implications.

Since the increasing use of antenatal corticosteroids and the advent of exogenous surfactant therapy in the early 1990s, the early respiratory course of ELBW infants has been generally characterized by minimal respiratory distress and little or no initial requirement for $O_2$ supplementation or ventilatory support. A subgroup will subsequently go on to have a progressive deterioration in respiratory function, requiring an increase in inspired $O_2$ and occasionally invasive or prolonged non-invasive respiratory support. The radiographic picture in these infants generally evolves from initial homogeneous hazy pulmonary opacities to a generalized coarse interstitial pattern [3]. The most widely used clinical definition of CNLD has been an $O_2$-dependency at 36 weeks' postmenstrual age. The use of this definition has limitations, in that it does not differentiate between mildly and severely affected infants. This has led to the development of a classification scheme which differentiates between three levels of severity (mild, moderate, severe) based on degree of supplemental $O_2$ requirement and need for respiratory support at 36 weeks' postmenstrual age [9]. A minority of ELBW infants will develop severe CNLD with significant and prolonged need for invasive ventilation and $O_2$ supplementation. Severe CNLD may be heralded during the first 2 weeks of life by a limited initial response to surfactant and/or deteriorations associated with development of air leaks, a hemodynamically significant patent ductus arteriosus and/or sepsis.

Although ELBW is the most common factor associated with development of CNLD, there is significant variability in the severity of lung disease amongst infants born at the same weight and gestational age, to which genetic factors are likely major contributors. Indeed, polymorphisms in tumor necrosis factor-α, toll-like receptor −10 and vascular endothelial growth factor are all suggested to play a role [10], as are single nucleotide polymorphisms in the fibroblast growth factor receptor-4 [11]. The barely understood complexity of a genetic contribution to CNLD is evident from a recent whole genome study in which there were alterations in expression of almost 10% of the genome [12].

## Pathological Features of CNLD

Five distinct stages of lung development have been defined: embryonic, pseudoglandular, canalicular, saccular and alveolar [13–16]. These stages are conserved among mammalian species

but with differing timing in relation to gestation, which has important implications for the relevance of experimental models recapitulating CNLD [17]. Preterm infants most as risk of developing CNLD are born during the transition between the late canalicular and early saccular phases, which are characterized by formation of primitive large distal airspaces, differentiation of Type I and Type II pneumocytes and expansion and thinning of the airway-capillary interface to an extent that is sufficient to support life. In humans, the alveolar stage, characterized by ingrowth of secondary crests into larger precursor saccules, commences in late gestation and continues well into childhood [18, 19]. The major pathological features of severe CNLD in the current era are an inhibition, or arrest, of alveolar formation, thickening of the interstitium and pulmonary inflammation [5, 20]. Hypoplastic, dysmorphic pulmonary microvasculature is also evident [21], resulting in reduced vascular surface area. Failure of alveolarization appears to last into adult life [22].

## Pulmonary Hypertension and CNLD

PHT is a common finding in patients with CNLD [23]. Available studies estimate the incidence of echocardiographic signs of PHT at between 17 and 43% of CNLD cases overall [24–27]. The incidence and severity generally increases in parallel with lung disease, being present in as many as 60% of severe CNLD [24]. Given the retrospective nature of the majority of published data, lack of long-term follow-up data, and the predominant reliance on echocardiography for diagnosis, the true incidence, severity and prevalence of PHT in formerly-premature infants is likely much greater than is currently appreciated. Other than the degree of prematurity, additional risk factors for PHT that are evident at birth include maternal pre-eclampsia, prolonged oligohydramnios and being born small for gestational age [28]. While genetic factors almost certainly contribute to the development of CNLD, no specific loci have yet been consistently associated with increased risk for PHT in this population.

As described above, the presence of evolving CNLD is usually evident within the first several weeks of life with respiratory deterioration (or lack of improvement) and persistent radiological abnormalities. Echocardiographic signs of PHT are frequently evident at this early stage and chronicity of PHT is generally established by 34–36 weeks' postmenstrual age [27]. Pathological contributors to PHT include sustained pulmonary vasoconstriction, exaggerated vasoreactivity (often precipitated by hypoxemic episodes), vascular hypoplasia and arterial wall remodeling due to smooth muscle hyperplasia and distal extension of smooth muscle into normally non-muscular arteries [29]. The extent to which the latter two structural features contribute to a "fixed" (i.e., non-reversible) form of chronic PHT is unknown. Since PHT is usually clinically silent, screening is recommended for all high-risk infants. It remains unclear whether the severity of PHT is simply a marker of CNLD severity or contributes to adverse outcomes in its own right. However, the diagnosis of PHT imposes a far greater burden of illness, resulting in lengthened hospital stay, prolongation of need for $O_2$ therapy, and a four-fold increase in mortality during the NICU stay [26]. Co-morbidities that worsen or inhibit recovery of lung function will also exacerbate PHT, including the persistence of left-to-right shunts that increase pulmonary blood flow (patent ductus arteriosus or large systemic-pulmonary collateral vessels) [30], airway abnormalities (subglottic stenosis, tracheomalacia, distal airway obstruction), gastro-esophageal reflux and factors contributing to poor growth, such as suboptimal nutrition and prolonged or repeated courses of corticosteroid therapy. The presence of pulmonary vein stenosis is an occasional finding in ex-preterm infants that heralds an extremely poor prognosis, especially in late-onset cases [31].

## Long-Term Outcome of PHT in CNLD

Retrospective data suggest that the majority of infants with CNLD-associated PHT will demonstrate gradual improvement in hemodynamic parameters during the first year of life, as lung

growth and function improves [30]. However, prospective long-term cohort data on these patients is lacking, and there is no knowledge regarding the (presumably high) potential for reappearance of PHT later in life [32]. For those patients with severe CNLD, progression of PHT is common, ultimately leading to right ventricular (RV) failure and early death, in most cases within 1 year of diagnosis [24]. Pulmonary hypertensive crises and cardiac arrest are also common [26], frequently precipitated by worsening hypercapnia and/or systemic hypotension in the settings of improperly-applied mechanical ventilation, anesthesia, sedation or intercurrent infection.

Long-term survival in progressive PHT is dependent upon an ability of the right ventricle to maintain adequate output in the face of increased pressure load, yet this aspect of disease has only recently been considered as a distinct therapeutic target [33, 34]. RV adaptation to increased pressure load evolves from a compensated (hypertrophied) state to a decompensated (dilated) state, in which a progressive decline in contractile function heralds imminent death [35]. The available evidence suggests that this evolution proceeds more rapidly in infants than in older children and adults [36]. Earlier dogma held that RV failure simply represented a mechanical response to increased pressure, which could be corrected by pulmonary vasodilators. Recent evidence has challenged this belief [33, 34, 37], indicating that discrete right ventricle-specific and/or pressure load-independent mechanisms may be responsible for RV contractile dysfunction. At this time, there is no specific knowledge on the pathogenesis of right heart failure in formerly premature infants.

## Current Therapies for Established CNLD and Associated PHT

The current mainstays of therapy for established CNLD with or without associated PHT include adequate nutrition, diuretics, prevention of infection, supplemental $O_2$ and correction of comorbidities (as described above) that may further contribute to lung injury. Optimizing nutrition in ELBW infants may impact the risk of CNLD [38], as under nutrition has been demonstrated in animal models to impair lung growth and to enhance lung injury [39]. Loop and thiazide diuretics are commonly used as therapy for CNLD [40]. Their use frequently leads to short-term improvements in oxygenation and requirement for respiratory support; however, there is no consensus on the dose, type or duration of diuretic therapy that is optimal or safe and no data suggesting any sustained or long-term benefits to their use [41].

Supplemental $O_2$, while necessary to avoid hypoxemia, can be directly cytotoxic to the lung due to increased reactive oxygen species (ROS) produced by mitochondria in direct proportion to the $PO_2$ to which the lung is exposed. Maturation of enzymatic antioxidants is gestation dependent [42, 43], and it has long been assumed that the ELBW infant is particularly at risk from ROS-mediated injury due to reduced antioxidant defenses [44]. That pulmonary toxicity due to supplemental $O_2$ occurs in ELBW infants has been demonstrated in trials comparing different target $O_2$ saturations in which the high target group ($SaO_2 \geq 96\%$) had more adverse pulmonary outcomes [45, 46], while low $O_2$ saturation targets ($SaO_2 < 90\%$) may lead to increased mortality [47]. These observations limit and render uncertain the $O_2$ saturation range that is available for safe clinical use. What is clear is that hyperoxia ($PaO_2 > 80$ mmHg) provides no further reduction to pulmonary vascular resistance (PVR) in excess of normoxia ($PaO_2$ 60–80 mmHg) [48] and may in fact further increase vasoreactivity [49].

PHT in CNLD is a dynamic phenomenon and its severity at any given time is strongly influenced by factors such as pH, $PaO_2$ and state of lung distension. The importance of these factors is generally underappreciated by clinicians. While pulmonary vasodilation with inhaled nitric oxide (iNO) has been used as rescue therapy for refractory hypoxemia in severe CNLD, often producing short-term improvements in oxygenation [50, 51], there is no data on long-term effects. Other therapies leading to improved outcomes in older children and adults with pulmonary arterial

hypertension [52] are unsupported by good quality clinical data in patients with CNLD and are suggested to be of doubtful value in this context [53]. Such therapies include Sildenafil (phosphodiesterase 5 inhibitor), Bosentan (endothelin receptor antagonist) or Epoprostenol (prostacyclin analogue), given either alone or in combination [54–58]. Sildenafil may have additional benefits on systolic function of the hypertrophied right ventricle [59, 60], aside from its vasodilator effects. Concerns regarding these agents relate to the potential for systemic hypotension, hepatotoxicity (Bosentan) and hypoxemia due to worsened ventilation-perfusion mismatch. Ideally, any consideration of long-term treatment with these agents should be accompanied by comprehensive evaluation of cardiopulmonary hemodynamics by cardiac catheterization, which allows for accurate determination of PHT severity, evaluation of acute vasodilator responsiveness and definitive exclusion of major collateral vessels, pulmonary vein stenosis and left heart disease as contributing factors [61].

## Prevention of CNLD and Associated PHT

The most effective way of preventing the development of CNLD would be to avoid prematurity. Given the current absence of any effective interventions targeting premature delivery, preventive therapy for CNLD and associated PHT must be directed at the contributing factors that lead to lung and cardiac injury, and their underlying mechanisms of action. Despite numerous trials of pharmacological treatments [62, 63], only three preventive agents have been convincingly demonstrated to reduce the incidence of CNLD: caffeine [64], Vitamin A [65] and early postnatal Dexamethasone [66]. The mechanism by which caffeine exerts its effects is unclear. Despite residual concerns surrounding the potential for increased mortality [67] or gut complications [necrotizing enterocolitis [68]] with caffeine, its prophylactic use is now common practice in North America, Europe and Australasia [69]. Vitamin A prophylaxis, which is modestly effective and shown to be safe [65, 70], has not been widely adopted. This likely relates to whether the small reduction in incidence of CNLD is seen to justify a prolonged course of intramuscular injections. Greater acceptance will likely depend on the results of ongoing trials assessing intravenous delivery. While certainly effective, early (within the first 7 days of life) postnatal Dexamethasone is not recommended due to high potential for adverse neurodevelopmental effects [66, 71]. Dexamethasone also reversed established PHT in adult rats [72], yet prolonged treatment of neonatal rats caused permanent lung hypoplasia, and augmented the severity of hypoxia-induced PHT when pups reached maturity [73]. Benefits of alternative strategies, such as systemic use of a less potent steroid, hydrocortisone, or inhaled steroids to reduce systemic side-effects remain unproven [63].

## Experimental Therapies

### Nitric Oxide (NO)

NO is a readily diffusible and highly-reactive free radical gas, first identified as the "endothelium-derived relaxing factor" in 1987. NO mediates smooth muscle relaxation via activation of soluble guanylate cyclase (sGC), leading to cyclic guanosine monophosphate (cGMP)-dependent calcium desensitization. NO-mediated signaling is critical to the rapid decrease in PVR following birth [74]. Properties of NO on the lung that are protective of experimental injury also include anti-inflammatory [75], antioxidant [76], anti-(smooth muscle) proliferative [77] and cytoprotective [78] effects. Abundant experimental evidence implicates deficient NO signaling as critical to the pathogenesis of CNLD and associated PHT [79–82]. Augmentation of NO signaling has also been shown to reverse sustained vasoconstriction, inhibit smooth muscle proliferation, and stimulate angiogenesis and alveolarization in experimental animals [83–88]. In neonates, iNO is employed as a short-acting pulmonary vasodilator, limiting hypoxemia by matching perfusion to ventilation and decreasing

right-to-left shunting. Unfortunately, despite promising preclinical studies [83, 85], iNO has proven ineffective as a preventive therapy for human CNLD [89–91]. Other than providing short-term improvement in oxygenation, iNO also does not appear to improve or slow the progression of CNLD-associated PHT [51, 92].

Assuming that the biological rationale for NO-based therapy in the prevention of neonatal lung and pulmonary vascular injury is sound, there are several possible explanations for the disappointing results of human studies despite strong supportive preclinical data. Firstly, that the beneficial effects of exogenous NO are counterbalanced by adverse ones and secondly, that inhalation of NO gas is a suboptimal means of providing NO to tissues in which endogenous production is deficient. Circulating and tissue-bound *S*-nitrosothiols (SNOs) contribute importantly to NO-cGMP signalling [93] and cause reversible post-translational regulation of protein function in a manner akin to phosphorylation. iNO has been shown inferior as a means of improving tissue NO function in experimental animals, when compared to SNO-based (ethyl nitrite) therapy [94]. In pilot human studies, inhaled ethyl nitrite improved oxygenation and hemodynamics in term infants with hypoxemic respiratory failure due to PHT [95]. No studies have been carried out to date in preterm infants. Another potential means to boost the potential benefits of exogenous NO may be as combination therapy with other agents known to improve lung growth and decrease lung injury, such as Vitamin A [96]. Treatment with Sildenafil, a PDE 5 inhibitor which, like NO, enhances cGMP signaling, attenuates both the PHT and the impairment of alveologenesis in experimental CNLD [84, 87]. Unfortunately, a recent pilot study of sildenafil for prevention of CNLD also proved disappointing [97].

## Strategies to Limit Adverse NO-Mediated Reactions

A biochemical barrier to effective NO-based therapy, that has yet to be overcome, relates to the high reactivity of NO (which is a free radical)—the dominant reaction depending upon the milieu in which NO is provided or generated (see Fig. 11.1). Physiological signaling of NO is regulated by reaction with heme proteins and reversible nitrosylation [98] of cysteine thiols, producing SNOs [93] either directly or following oxidation to nitrite anion. Under pathological conditions (e.g. oxidative stress, inflammation), NO will preferentially react with supra-physiological levels of $O_2$ or superoxide to produce reactive nitrogen species (RNS), nitrogen dioxide and peroxynitrite, respectively. Nitrogen dioxide is an important source of RNS in inflammatory states, where neutrophil-derived peroxidases and hydrogen peroxide are present in abundance [99]. These molecules cause nitration of tyrosine residues [100] that irreversibly inhibits protein function by forming 3-nitrotyrosine [101–104]. Nitration is linked to numerous disease states by triggering cellular responses ranging from pathological alterations in cell sig-

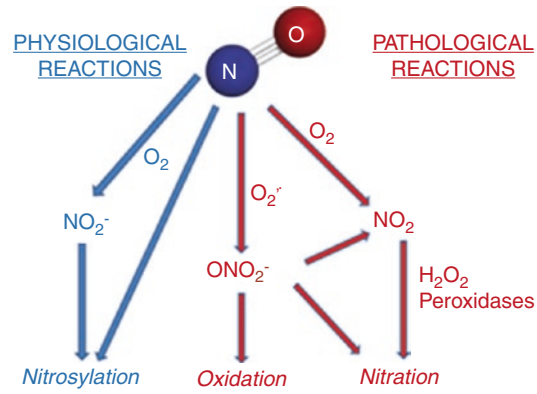


**Fig. 11.1** Reactions of nitric oxide (NO). Physiological effects of NO are mediated by *direct or indirect* (via oxidation to nitrite ($NO_2^-$)) *nitrosylation* reactions with divalent metal-based heme proteins, including soluble guanylate cyclase and hemoglobin, leading to alterations in cell signaling pathways and vascular function. Pathological reactions of NO are mediated by reaction with supraphysiological levels of molecular oxygen ($O_2$) to produce nitrogen dioxide ($NO_2$) or with superoxide ($O_2^{\bullet-}$) to produce peroxynitrite ($ONO_2^-$). Peroxynitrite is a potent oxidant and causes protein (tyrosine) *nitration*. This reaction may be direct, or via decomposition of $ONO_2^-$ to $NO_2$, which can mediate nitration reactions under inflammatory conditions in which hydrogen peroxide ($H_2O_2$) and heme peroxidases (such as myeloperoxidase) are present in abundance

naling to abnormal proliferation or cell death [105]. In human infants with CNLD, 3-nitrotyrosine levels from circulating and lung-derived proteins are a direct marker of disease severity [106, 107].

The reaction of NO with superoxide to form peroxynitrite occurs at the fastest rate constant known in biology ($1.9 \times 10^{10}$ $M^{-1}$ $s^{-1}$), such that the two molecules will always react when in proximity [108]. Peroxynitrite is both a nitrating agent [103, 109] and a potent oxidant [108, 110], leading to both tyrosine nitration [101–104] and cysteine oxidation [111]. Peroxynitrite also plays specific roles in the pathogenesis of experimental neonatal PHT, causing pulmonary vasoconstriction [112], vascular remodeling [113, 114] and RV dysfunction [115]. Inflammatory cell-derived RNS are also critical to hyperoxia-induced experimental CNLD [114]. Peroxynitrite decomposition catalysts that cause peroxynitrite to decompose to nitrate, rather than the toxic hydroxyl radical generated by spontaneous decomposition [108], have been employed with success by our group as preventive agents in experimental models of CNLD and PHT [113–115]. Unfortunately, currently available peroxynitrite decomposition catalysts are metalloporphyrin (iron or manganese-based) compounds which are potentially toxic, especially to the immature liver. This makes translation of the current generation of such compounds in ELBW infants highly unlikely.

## Antioxidant Therapies

Other than the direct cytotoxic effects of ROS on the lung discussed earlier, oxidative stress limits NO bioavailability (by steering NO toward production of RNS), limits the sensitivity of sGC to NO [116] and increases hydrolysis of cGMP via increased expression and activity of PDE 5 [117, 118]. Pharmacological therapy with broad spectrum antioxidants (e.g., Lazaroids or Tempol) is effective at limiting experimental chronic neonatal PHT [119]. However, our group has reported that a major adverse effect of effective antioxidant therapy in neonatal rats was inhibited lung cellular proliferation and decreased somatic growth [119], in keeping with a known critical role for low endogenous levels of ROS in normal growth and development [120]. A potentially safer alternative strategy is to supplement deficient antioxidant enzymes [such as superoxide dismutase (SOD)] or their co-factors. Unfortunately, clinical trials examining preventive effects of recombinant human SOD or selenium (co-factor for glutathione peroxidase) have not shown any impact on the incidence of CNLD [121, 122]. Similarly, preventive treatment with N-acetylcysteine, a glutathione precursor, was of no benefit [123]. The above highlights the challenges inherent in developing antioxidant therapies for the newborn that are both safe and effective [120]. The possibility must also be considered that, despite ample evidence of increased oxidative markers in the lungs of infants with evolving and established CNLD, increased ROS may not play a uniformly pathological role.

## Strategies to Improve Endogenous NO Function

Endogenous endothelial NO production by endothelial nitric oxide synthase (eNOS) requires an adequate supply of substrate, L-arginine, and arginine precursors, including L-citrulline. In the absence of sufficient substrate, "uncoupling" of eNOS results in a shift from NO to superoxide production. Up-regulation of arginases are an important cause of substrate deficiency directly contributing to inflammation and lung injury, which is preventable by hypercapnic acidosis in hypoxia-exposed neonatal rats [124] or by arginase-specific inhibitors in LPS-exposed Guinea pigs [125]. Supplementation of L-citrulline has also been shown to inhibit arginase and to prevent hyperoxia-induced lung injury in neonatal rats [88]. Tetrahydrobiopterin (BH4) is an important cofactor for eNOS to remain in a coupled state. Newborn mice haploinsufficient for GTP cyclohydrolase I, a rate-limiting enzyme in BH4 synthesis, spontaneously develop PHT [126]. eNOS function may be restored by treatment with L-sepiapterin, which serves as a substrate for BH4 synthesis [86].

## Alternative NO-Based Therapies

Nitrite was until recently considered a physiologically inert by-product of NO oxidation. It is now apparent that circulating nitrite is recycled in tissues to form NO, thereby acting as a stable endocrine pool for "NO-like" bioactivity that is complementary to endogenous NOS [127]. Systemic or inhaled inorganic nitrite possesses many theoretical advantages over other forms of NO-based therapy in that tachyphylaxis does not occur with chronic dosing, effects are of relatively rapid onset and last many hours and (sodium) nitrite is inexpensive and stable. Protective effects of sodium nitrite on adult experimental models of PHT have been reported [127–131] but no studies have been reported to date in neonatal animals.

## Rho-Kinase (ROCK) Inhibitors

Activation of the small GTPase, RhoA, by G-protein-coupled receptor ligands, and its downstream effector, ROCK [132–135], is a key pathway leading to sustained vasoconstriction and vascular remodeling in experimental chronic neonatal PHT [118, 136–140]. In neonatal rats with bleomycin-induced lung injury, ROCK is critical to inhibited pulmonary angiogenesis, possibly via up-regulation of anti-angiogenic thrombospondin (TSP)-1 [140]. ROCK mediates smooth muscle contraction by causing calcium sensitization [141, 142]. NO-mediated reversal of vasoconstriction is in part mediated through attenuating effects on RhoA expression [143] and activation [144–146], or through direct inhibitory effects on ROCK activity [147–149]. Hence, therapies which enhance cGMP signaling also suppress RhoA/ROCK activation [150, 151, 152].

The above insights have developed largely from experimental use of two kinase inhibitors, Y-27632 [153] and Fasudil (HA-1077) [154], which possess high specificity toward ROCK. Numerous animal studies [155] [136] and pilot reports using single doses or brief infusions of Fasudil in human adults [156, 157] and children [158] have confirmed an efficacy that is equal or superior to existing vasodilators. In addition to modulating vascular smooth muscle tone, evidence also indicates that ROCK regulates the expression of key mediators which modify smooth muscle phenotype, including actin polymerisation through LIM domain kinase-induced inhibition of cofilin [159] and changes in expression of mediators leading to increased proliferation and inhibited apoptosis of vascular smooth muscle, including platelet-derived growth factors [138, 160] and endothelin-1 [86, 113, 115, 137, 138, 161–166]. ROCK inhibitors are also effective when given by inhalation [157]. In addition, ROCK appears to play a role in cardiac failure with benefits of ROCK inhibition on the failing left ventricle being well-described [167–169]. Recent work in adult animals has also identified a direct role for ROCK in experimental RV hypertrophy and dysfunction [170]. Neonatal rat pups chronically exposed to hypoxia develop significant RV systolic dysfunction, secondary to afterload-independent mechanisms that involve up-regulated RV ROCK activity [171]. No studies employing ROCK inhibitors have been conducted to date in human neonates.

## Cell-Based Therapy

Endogenous endothelial and mesenchymal progenitor cells appear to play a role in normal lung development and in repair from lung injury [172–180]. Treatment with mesenchymal [181, 182] or endothelial [177] progenitor cells prevents experimental CNLD. These effects appear to be mediated by secreted factors [176, 181, 183], which as yet remain unidentified. A phase 1 study examining safety of intra-tracheal delivery of allogeneic umbilical cord blood-derived mesenchymal stem cells in preterm infants has been recently reported [184].

## Therapies Targeting Specific Growth Factors or Cytokines

Antagonizing growth factors or cytokines which are up-regulated during injury is another promising therapeutic approach. A candidate for which there is abundant supportive evidence is transforming growth factor (TGF)β1. Increased TGFβ is observed in lung tissue at autopsy of infants with CNLD [185] and bronchoalveolar lavage (BAL)

fluid of human preterm infants destined to develop CNLD [186]. Blockade of TGFβ1 signalling prevents vascular remodeling [187, 188] and inhibited alveolarization [189] in experimental animals. Interestingly, protective effects of attenuated ROCK signalling [190, 191] and peroxisome proliferator-activated receptor agonism [192–194] on the lung and heart may at least partially result from inhibition of TGFβ1 signalling. Another rational target is interleukin (IL)-1. Increased BAL and serum IL-1β is evident in infants developing CNLD [195, 196]. Transgenic mice over-expressing IL-1β develop a lung injury similar to CNLD, with lack of alveolar septation, and impaired vascular development of the lung, which may be mediated through effects on the retinoic acid pathway [197]. They also have inflammation mediated by the increased expression of neutrophil and macrophage chemokines [197]. Antagonism of IL-1β receptor signalling protects against hyperoxia-induced lung injury [198] and iNO-induced RV systolic dysfunction in neonatal rats [199].

## Conclusions

CNLD remains an important and unresolved health issue in infants born extremely preterm. If major therapeutic inroads are to be made in the future, it will need to be through novel pharmacologic interventions based on mechanistic insights derived from relevant animal models. Such studies ideally should: (1) examine for toxicity and dose-response in multiple models, (2) incorporate reversal of established disease as well as prevention as therapeutic strategies and (3) include evaluation of sex differences, functional effects (exercise capacity, airway and vascular reactivity) and longevity of effects into adult life.

With respect to understanding the determinants of PHT in CNLD, there are a number of barriers to improving upon our currently poor understanding of pathogenesis and natural history in human infants. Diagnosis of PHT and right heart dysfunction is problematic. Clinical signs are unreliable and catheterization is often not feasible until well after term corrected age, leading to a sole reliance on echocardiography. Performance of echocardiography can be challenging, especially for evaluation of the right heart due to the thin chest walls of premature infants and frequent presence of lung hyperinflation. Echocardiography-derived parameters indicating raised pulmonary arterial pressure, such as triscuspid regurgitant jet velocity, are not measurable in all patients, and when present have been shown to correlate poorly with pulmonary arterial pressure measured by catheter [61]. In addition, there are no agreed upon echocardiography-based definitions for diagnosis of PHT in neonates, and certainly none for evaluation of right heart function. Systematic study of echocardiographic parameters of RV function that are useful in newborns is required, incorporating new methodologies, including tissue Doppler and strain imaging, that have shown potential in older children and adults [200, 201]. Finally, lung or heart-lung transplantation, the only "curative" option for end-stage disease, is rarely feasible in CNLD-associated PHT, which contributes to a paucity of high quality human tissue available for study and a consequently much greater reliance on mechanistic and therapeutic insights from preclinical models [17].

While improved understanding of pathophysiological mechanisms will certainly facilitate the development of new therapies, challenges to clinical translation remain significant and include the inherent variability in phenotype and risk for CNLD and the development of associated PHT, the relatively low numbers of patients available for study and the uniquely high potential of this vulnerable population for off-target drug effects. Such considerations also have important implications for adoption of existing therapies employed in older children and adults.

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