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Can we Understand the Pathobiology of Bronchopulmonary Dysplasia??

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Fifty years after the original description of bronchopulmonary dysplasia (BPD) by Northway et al, BPD remains one of the most common complications of preterm birth encountered in the neonatal intensive care unit (NICU).^{1,2} Over those 50 years, substantial advances in the medical management of preterm birth have dramatically improved the survival of affected infants. At the same time, although BPD lung pathology has evolved in parallel with new patient management approaches, the incidence of BPD has not changed^{3–5}. Rather, the demographics of BPD have changed, resulting in a “left shift” in BPD epidemiology. Although the incidence of BPD has decreased in older (>28 weeks) preterm infants, due to marked increases in survival of extremely premature infants, the incidence of BPD has now substantially increased in the stratum of younger preterm infants (<28 weeks). The persistence of BPD as a neonatal intensive care problem underscores the need to better understand the pathobiology of BPD to: reduce incidence by implementing preventative strategies; limit long-term morbidity in survivors; and improve survival of the population with severe BPD that remain at high risk for mortality. To address the question: “can we understand the pathobiology of BPD?” we will discuss the current state-of-the-art, new directions for investigation that represent currently understudied areas of BPD pathobiology,

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and novel methodological approaches that will provide us with means to advance our knowledge about BPD pathobiology.

Understanding BPD pathobiology – where are we now?

In the original form of BPD described by Northway et al, the characteristic pathologic features of severe inflammation, marked fibrosis, and airway dysplasia and muscularization, appeared to result from severe lung injury superimposed on immature lungs.¹ In contrast, the “new” form of BPD, characterized by alveolar simplification, appears to result from a lesser magnitude of injury superimposed on highly immature lungs. The process of alveolarization is an intricate, highly orchestrated, developmental program involving multiple cell types within the lung⁶, and extensive investigation over the past few decades has identified numerous ante- and post-natal injuries capable of disrupting key developmental programs that are essential for alveolar and vascular growth. It is important to highlight that the current clinical definition of BPD, defined exclusively by oxygen use in affected infants, does not specifically reflect the complex and heterogeneous pathological events that together form the basis of BPD pathobiology. It is important to underscore that BPD is not a single entity, but rather a syndrome, where the relative contributions of perturbations to vascular, airway, and alveolo-capillary barrier function, as well as extra-pulmonary factors, vary from patient to patient. This represents one of the biggest challenges to our understanding of BPD pathobiology. In the discussion that follows, this will become more evident, where the extensive use of animal models does not permit the study of BPD *per se*, but rather, each animal model recapitulates a specific pathological event (e.g. alveolar simplification), that has been noted in BPD patients.

Injurious Stimuli that Disrupt Late Lung Development

Alveolarization is a complex developmental program requiring the temporal-specific activation of diverse signaling pathways, participation and coordination of multiple cell types interacting with the extracellular matrix, and influences of the lung microenvironment. Each component is necessary, but none are sufficient. As a result, the disruption of seemingly disparate molecular pathways by injurious stimuli (detailed below) have the ability to cause similar disruptions of alveolar and vascular growth.

Hyperoxia

In their original description, Northway et al noted that BPD appeared to “result from toxic effects of oxygen on the lung”¹, and later observed similar radiographic and histologic features in newborn guinea pigs exposed to 100% oxygen⁷. Subsequently, studies in multiple species confirmed the detrimental effects of hyperoxia on both the immature and the mature lung⁸, including characteristic acute pathologic changes such as epithelial and endothelial cell death, inflammation, pulmonary edema and hemorrhage^{9, 10}. More recent studies show that hyperoxia also compromises mitochondrial function^{11, 12}, and decreases lung resident¹³ and circulating progenitor cells¹⁴. The deleterious effects of hyperoxia result from both direct injury mediated by reactive oxidant species (ROS), and indirect injury from lung inflammation. Damage from ROS is mitigated by antioxidant enzymes, many of which are suppressed in experimental models of BPD¹⁵, and modulating antioxidant expression

impacts alveolarization, angiogenic gene expression, and vascular remodeling^{15–18}. The infiltration of inflammatory cells into the hyperoxia-exposed lung further potentiates injury by secreting cytokines, chemokines, and proteases^{19–22}. In many cases, selective targeting of these cells limits hyperoxia-induced lung inflammation and preserves alveolarization^{23–25}. However, subpopulations of inflammatory cells, such as alternatively activated monocytes, serve important immune regulatory functions, and deletion of these cells exaggerates hyperoxic lung injury²⁶. Taken together, these data highlight the need for a more granular understanding of the distinct populations of cells present in the lung at different stages of development, their function, and how they are modulated during disease to fully understand the pathobiology of BPD.

Cyclic Stretch and Mechanical Ventilation

Lung development *in utero* occurs in an environment of cyclic, negative pressure stretch induced by fetal breathing movements^{27, 28}, and pregnancy conditions that decrease amniotic fluid or impair fetal breathing are associated with lung hypoplasia^{29–34}. Physiologic stretch increases elastin remodeling during alveolarization³⁵, allows for mesenchymal thinning³⁶, regulates surfactant protein expression³⁷, and increases the expression of pro-angiogenic genes^{38, 39}. However, pathologic stretch is detrimental to late lung growth. Early preclinical studies combined injurious ventilation strategies with high levels of oxygen to model the severe injury observed in the original form of BPD described by Northway et al.⁴⁰ Modifications of these original models using less injurious ventilation strategies in more premature animals reproduce the alveolar hypoplasia and dysmorphic vascular development characteristic of the new BPD⁴¹. Cyclic over-distention of the lung activates inflammatory signaling in resident lung cells⁴², induces inflammatory cell recruitment^{43–47}, and directly affects numerous biologic pathways that are essential components of alveolarization, including elastin fiber assembly, angiogenesis, and metabolism^{48–52}. Applying lung protective or non-invasive methods of ventilatory support in premature animals decreases lung injury, lessens detrimental effects on alveolarization and elastin deposition⁵³, and limits neutrophilic infiltration⁵⁴. These and other studies served to motivate the adoption of lung protective ventilation, and early application of nasal continuous positive airway pressure in preterm infants^{55, 56}, in attempt to optimize lung expansion, avoid over-distention and limit lung injury. Further studies are needed to elucidate the separate pathways activated in physiologic and pathologic stretch, and to determine the optimal stretch necessary to promote normal lung growth.

Infection and Inflammation

Inflammation is a common pathway for many injuries that disrupt late lung development. Inflammatory cytokines are elevated in the tracheal aspirates of premature infants who later develop BPD^{57–61}, suggesting that early lung inflammation is an independent risk factor for disease. In animal studies, the administration of intrauterine endotoxin alters major developmental programs, and disrupts lung and vascular growth in preterm labs, rats, and mice^{62–67}. Chorioamnionitis increases the risk for preterm birth^{68–70}, however, whether it represents a significant risk factor for the development of BPD remains debated^{71–77}. In contrast, persistent perinatal lung inflammation and episodes of postnatal sepsis have consistently been identified as independent risk factors for the development of BPD^{78–81}. In

animal models, persistent bacterial colonization is associated with a greater impairment in lung growth, suggesting that differences in the maternal or fetal immune response may be a critical factor influencing the effect of inflammation on overall lung development⁸². Modulating the inflammatory response may represent an effective strategy to prevent BPD^{83–85}. However, it is important to recognize that these inflammatory pathways often have distinct and sometimes contrasting effects depending on the stage of lung development^{86–88}, and may promote important physiologic functions⁸⁹. Thus, continued efforts are needed to further our understanding of the detrimental and beneficial effects of ‘inflammatory’ pathways, and the influence of host factors on disease severity, to allow the development of effective, targeted therapies to treat or prevent BPD.

Nutrition and Growth Restriction

Intrauterine growth restriction (IUGR) appears to represent an additional injury that can disrupt late lung development and increase the risk for BPD^{90–96}, and also increase the risk for the development of pulmonary hypertension⁹⁷. Further, the postnatal growth restriction resulting from insufficient total calories that is commonly observed in premature infants^{98,99} may represent an additional risk factor¹⁰⁰, with evidence suggesting that adequate intake of protein¹⁰¹, fat¹⁰², and specific vitamins^{103–106} are particularly important. Data from animal models^{107–109} suggest that pre- and postnatal growth restriction alters antioxidant activity¹¹⁰, surfactant production¹¹¹, pulmonary endothelial function¹¹², and biologic pathways that regulate pulmonary vascular growth¹¹³. Despite these broad effects on key developmental pathways, recent clinical trials to optimize nutrition in preterm infants have demonstrated little or no impact on the incidence of BPD^{114–117}, with the exception of Vitamin A supplementation, which is associated with a small decrease in the risk of developing BPD¹¹⁸. Additional work is needed to fully characterize the function of individual nutritional components on essential developmental pathways, and identify the molecular pathways affected by growth restriction, in order to optimally translate these findings into efficacious therapeutic strategies.

Disruption of Essential Developmental Pathways

Impaired and Dysmorphic Pulmonary Vascular Growth

In addition to alveolar simplification, the pathobiology of the ‘new’ BPD includes abnormalities of pulmonary microvascular development. Pathologic examination of lung tissue from infants dying from the ‘new’ BPD¹¹⁹, or long-term ventilated preterm infants found evidence of either decreased and abnormally distributed pulmonary microvessels, or increased but simplified and immature capillaries¹²⁰. Taken together, these studies suggest that vascular abnormalities in BPD may be variable: with suppressed growth at early stages, and excessive, dysmorphic growth at later stages, perhaps representing a maladaptive compensatory response. Moreover, a subset of patients with BPD develop pulmonary hypertension, characterized by abnormal pulmonary vascular remodeling¹²¹ and tone^{122–124}. Although the mechanisms that specifically induce these pathologic changes remain to be fully defined, they are related, in part, to the detrimental effects of injuries on key regulators of pulmonary vascular growth and function, including the vascular endothelial growth factor (VEGF)^{50, 125–129}, hypoxia inducible factor (HIF)^{130, 131} and

nitric oxide (NO)^{66, 132, 133}. Directly blocking angiogenesis in animal models impairs alveolarization, while strategies to enhance angiogenesis^{134–136} preserve alveolarization¹³⁷, thus providing direct evidence that angiogenesis actively promotes distal lung growth¹³⁸. However, the cell- and matrix-derived signals that influence endothelial cell phenotype and allow for temporal-spatial control of angiogenesis, and how these signals are disrupted during BPD, remain to be fully defined.

Impaired Alveolar Epithelial Proliferation and Differentiation

In addition to profound effects on pulmonary vascular growth, both hyperoxia and mechanical stretch can directly affect alveolar epithelial cell (AEC) proliferation, survival, and fate. Although physiologic stretch promotes AEC differentiation^{37, 139, 140}, excessive stretch suppresses AEC proliferation⁴⁹. In vitro, hyperoxia induces apoptotic and necrotic AEC death^{141, 142}, and promotes type II AEC (AECII) differentiation to a type I AEC (AECI) phenotype¹⁴³. In vivo, hyperoxia induces rapid expansion of AECII in the neonatal lung, which are quickly depleted during recovery, resulting in fewer AECII in the adult lung, and potentially contributing to the emphysema observed in adult mice exposed to perinatal hyperoxia^{144, 145}. Of note, hyperoxia also re-induces telomerase activity in mature AECII, providing support for the notion that AECII may serve as population of resident progenitors after injury¹⁴⁶. However, further studies are needed to define the molecular mechanisms that control AEC proliferation and fate during development, to determine if injuries such as hyperoxia induce premature aging of the lung, and to potentially allow for these regenerative mechanisms to be re-invoked during injury.

Altered Extracellular Matrix Remodeling

The elastic theory of lung development places the extracellular matrix (ECM) of the developing lung in a pivotal regulatory position during alveolarization^{147, 148}. Dynamic changes in the expression of a large spectrum of ECM molecules, including collagens and elastin, matrix processing enzymes such as metalloproteinases¹⁴⁹ and neutrophil elastase^{150, 151}, and ECM cross-linking enzymes (lysyl oxidases, transglutaminases, and lysyl hydroxylases) occur during lung development. The expression of many of these molecules is dysregulated during aberrant lung development¹⁵². In general, the overall abundance of elastin is reduced, and elastin fibers in the developing septa are disorganized; although the general abundance of collagen and collagen cross-links is increased¹⁵³, and collagen fibers also take on an abnormal structure¹⁵⁴. How these changes directly result in disturbed lung development is not clear, and remains to be defined. How the ECM deposition is dysregulated in BPD is also a matter of much interest, and this idea is currently being extensively explored in the context of (myo)fibroblast subpopulations in the developing lung, where (myo)fibroblasts may be marked by platelet-derived growth factor receptor (PDGFR) α , or perilipin-2, or combinations thereof. Lineage tracing and other studies have highlighted the likely role of (myo)fibroblast subpopulation in normal and aberrant lung development^{155, 156}, and these studies await causal analysis, for example, by *in vivo* depletion studies. The dysregulation of signaling by the elastogenic growth factor, transforming growth factor (TGF)- β ¹⁵⁷; and the ability of TGF- β to regulate aberrant alveolarization and ECM production in a BPD animal model¹⁵⁸ underscores a key regulatory role for TGF- β in ECM production and remodeling in the developing lung. This TGF- β /

fibroblast/ECM axis is a clear area that warrants further study in the broader context of BPD pathobiology, where key players have been identified and validated as causal factors, but the pathogenic pathways still await discovery.

Understanding BPD pathobiology – future directions

Future studies to deepen our understanding of the pathobiology of BPD will continue to employ broad screens of human or animal model material, followed by targeted studies using interventional pharmacologic or genetic approaches to examine a causal role for a particular pathway or mediator. To this end, a large number of transcriptomic studies have been undertaken, addressing changes in mRNA and microRNA expression^{159–161}, and these data are only now being interrogated and explored. An integrated systems-biology approach will reveal new, or confirm suspected mechanisms that misdirect lung development in BPD patients, or in experimental animal models of BPD. These efforts are currently supported by the Lung Gene Expression Analysis (LGEA) web portal, as part of the LungMAP consortium, which facilitates the analysis, display, and interpretation of gene expression patterns obtained from single cells, sorted cell populations and whole lung tissues¹⁶². Early twin studies suggested that genetic factors contribute substantially to the risk of BPD^{163, 164}. Subsequently, however, genome wide association studies were unable to identify single nucleotide polymorphisms significantly associated with an increased risk of BPD^{165, 166}. Additional screens to detect epigenetic marks¹⁶⁷ and changes in long non-coding RNA¹⁶⁸, have recently been performed, and others such as proteomic and metabolomic screens, have not yet been undertaken. Although these advances in high throughput, ‘omic’ technologies can yield vast amounts of data, heterogeneity in the diseased population and the presence of multiple confounding factors can limit the power to differentiate true signals from background noise. Moving forward, the integration of data sets from different types of ‘omics’ in a multi-omic approach has great potential to more comprehensively understand complex disease etiology¹⁶⁹. Similarly, computational biology approaches to allow multicohort analysis of publically available gene expression data have been used successfully to identify robust gene sets that can accurately distinguish patients with similar yet distinct diseases, raising the possibility that similar approaches could be used to identify a “gene signature” for BPD¹⁷⁰. Central to all these studies is the source of the material under study. Autopsy material from BPD patients is very limited, and originates from the “old BPD” phenotype rather than the “new BPD” prevalent today. There is a pressing need to coordinate efforts to acquire pathologic samples of lung and other tissues from BPD non-survivors, and to more readily acquire and distribute available material from instrumented BPD patients, such as cells from endotracheal aspirates.

There is also much scope for the refinement of animal models of BPD¹⁷¹. Currently, there is little standardization across studies, regarding both the animals, and the injurious stimuli employed. BPD may be modeled in mice¹⁷², rats¹⁷³, rabbits¹⁷⁴, lambs¹⁷⁵, pigs^{176, 177}, and non-human primates¹⁷⁸, and these models have proved highly valuable in studies on pathobiology¹⁷⁹ and therapy development¹⁸⁰. Recent reports have documented no less than 40 different hyperoxia exposure protocols used in a two-year period – in mice alone – to study BPD^{181, 182}. The recognition that the lung response to injuries such as hyperoxia is highly dependent on mouse strain, has stimulated efforts to standardize experimental models

of hyperoxia, by directly comparing either strain or specific injury protocols, in an effort to make studies performed in different laboratories directly comparable. At the same time, it is important to recognize that the use of diverse models has provided much fruitful information on BPD pathobiology. In addition, there is a pressing need for translationally relevant models which combine a background of infection/inflammation, with oxygen injury and/or ventilation. The utility of transgenic mice to address pathogenic pathways at a molecular level has driven much research in the direction of rodents and other small-animal models. However, term rats and mice have lungs that are not only structurally different from those of humans, but also properly adapted for efficient gas exchange at birth, and do not represent poorly-adapted, preterm lungs characteristic of infants at risk for BPD. The disengagement from the preterm lamb and non-human primate models represents a step back from progress towards understanding BPD pathobiology, although the costs and ethical issues related to such models are fully appreciated.

There is also much scope for improvement in how experimental BPD and patient tissue is used and analyzed. Studies on transcriptomic changes during normal and aberrant lung alveolarization have generally relied on whole-organ studies, and later, with increased appreciation of the compartmentalization of developmental pathways acting in an orchestrated manner, investigators have focused on developmentally-relevant pathways in discrete lung compartments and cells. Although this approach has resulted in studies in cultured cells and laser-capture micro-dissected tissue, *ex vivo* single-cell transcriptomic analyses have not yet been undertaken to address BPD pathobiology¹⁸³. Moreover, although the isolation and use of primary cells represents a significant advance over commercially available cell lines, even short term culturing of cells significantly alters gene expression, highlighting the importance of taking advantage of innovative single-cell analyses on freshly dispersed cells¹⁸⁴.

There has also been much refinement in the methods used to quantify changes in the lung architecture under pathological conditions, including the use of design-based stereology to analyze thickening of the alveolar septal walls and the number of alveoli in the lung¹⁸⁵. Specific recent advances include methodology to assess the number¹⁸⁶ and formation¹⁸⁷ of alveolar capillaries in the lung, and assembling serial histology sections to create digital three-dimensional reconstructions of the alveolo-capillary network^{188, 189}, to permit visualization of perturbations that occur during aberrant lung development. It remains desirable to also study these changes by radiological imaging, such as that described to study alveolarization in human subjects with hyperpolarized gas in ³He magnetic resonance imaging¹⁹⁰; however, this approach has not yet been applied to experimental animal models. The current clinical definition of BPD relies exclusively on the need for – and degree of – oxygen supplementation in affected neonates; however, lung structure plays no role in this definition. In contrast, no studies on respiratory function or lung mechanics¹⁹¹, which include parameters central to current diagnostic criteria for BPD, are currently performed in animal models of BPD, largely due to technical challenges associated with adapting available technology to very small animals. Studies on the relationship of lung structure to function in experimental animals are essential, similar to a recent study that examined this relationship in oxygen-injured developing lungs¹⁹². Further studies addressing how perturbations to lung architecture translate to compromised gas exchange

and respiratory mechanics in experimental animals are essential, if our understanding of BPD pathobiology is to develop further. Thus, the application of forced oscillation methods or whole-body plethysmography is highly desirable in future studies in animal models of BPD.

Beyond animal models, new *in vitro* approaches have also shown promise in furthering our understanding of BPD pathobiology. Notable recent developments include the use of co-culture models of the alveolo-capillary barrier¹⁹³, and the generation of organoids which undergo a process comparable with that of alveolarization^{194–196}. Organoids in particular are an attractive opportunity to study lung development *in vitro*, because the cell-populations combined in organoids can be tailored to the biological questions posed, and may allow for genetic or pharmacological interventions to be applied *in vitro* that are not possible *in vivo* secondary to toxicity or lethality. Similarly, the use of decellularized lungs permits the unique opportunity to define the ECM-derived signals that direct lung cell fate during development, and how these signals are disrupted during injury¹⁹⁷. Along these lines, the initial flurry of interest in the application of precision-cut lung sections to study lung alveolarization¹⁹⁸ has not yet found widespread application. Both organoids and lung sections present a wonderful opportunity for advanced molecular analyses to understand cell-cell and cell-matrix interactions and developmental pathways pertinent to BPD pathobiology. Such approaches include new methods for spatially resolved “omics”¹⁹⁹, such as that described for human and mouse brains²⁰⁰ where quantitative single-cell transcriptomic and proteomic^{201–203} analyses are performed *in situ* on tissue sections, to retain single-cell data while preserving positional information. These suggestions for future directions are summarized in Table I (for methodology) and Table II (for research priorities).

Summary

Since the original description of BPD, the past 50 years have seen tremendous efforts to characterize and understand the pathobiology of BPD, using both human BPD patient material and experimental animal models of arrested alveolarization that span the gamut from mouse to non-human primates. All of these approaches have generated a very solid foundation of knowledge for future studies, which must include a better appreciation of the nature of the pathological processes at play, and the validation of causal roles for pathogenic pathways that have been demonstrated to accompany disordered lung development associated with BPD. Massive strides continue to be made in the development of technology to study these biological processes. Indeed, it is now possible to relatively easily and inexpensively obtain the proteome or the transcriptome of a single cell, *in situ*. It is also possible to tag multiple cell-types, track the movement of these cells simultaneously, and observe their phenotypic transitions in a temporal and spatial context, in a living organism. Furthermore, the rapid evolution of computational and systems biology now allows for the meaningful analysis of increasingly complex datasets. Despite widespread application in the neurobiology and molecular oncology fields, few of these exciting methodologies have been applied to questions of aberrant lung development. It is now time to do exactly that. In doing so, the next 50 years of BPD research no doubt hold many exciting observations and discoveries in store for us.

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ABBREVIATIONS

AEC	alveolar epithelial cell
AECI	type I alveolar epithelial cell
AECII	type II alveolar epithelial cell
BPD	bronchopulmonary dysplasia
HIF	hypoxia-inducible factor
IUGR	intrauterine growth restriction
LGEA	Lung Gene Expression Analysis
NF	nuclear factor
NICU	neonatal intensive care unit
NO	nitric oxide
PDGFR	platelet-derived growth factor receptor
ROS	reactive oxygen species
TGF	transforming growth factor
VEGF	vascular endothelial growth factor

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Table I

Methodologies that will impact our understanding of the pathobiology of bronchopulmonary dysplasia that are yet to be applied to the study of aberrant lung alveolarization.

1.	Methodological approach and projected contribution	Reference
	Use of spatially-resolved “omics” to provide information about gene and protein expression while retaining crucial positional information during aberrant lung alveolarization	
	• Mass cytometry	201
	• Imaging mass spectrometry	203
	• Single-molecule fluorescence <i>in situ</i> hybridization	202
	• Single-cell transcriptomics <i>in situ</i>	200
	2. Use of single-cell RNA-seq to identify and characterize cell subpopulations with different phenotypes, and to delineate how individual cell subpopulations evolve during aberrant lung alveolarization.	183
	3. Use of primary cells with minimal to no passaging to reduce artifacts that develop in cell culture.	184
	4. Use of comprehensive multicohort analysis to establish a “gene signature” for BPD	170
	5. Use of design-based stereology approaches for the analysis of lung structure in experimental animals to precisely define structural changes that accompany – or result from – aberrant lung development	185
	6. Development of refined experimental animal models that recapitulate pathological hallmarks of currently prevalent forms of BPD. These include the development of “two-hit” small animal models, and re-establishment of true preterm large animal models.	171, 180
	7. Use of <i>ex vivo</i> and <i>in vitro</i> models of alveolarization	
	• Decellularized lung scaffolds	197
	• Organoids	196
	• Precision-cut lung slices	198
	• Co-cultures	193
	8. Use of computational approaches to generate complex three-dimensional renditions of the structure of the alveolus, from digitized libraries of serial lung tissue sections.	188, 189
	9. Development of methodology to assess respiratory mechanics to correlate changes in lung structure in experimental animals with changes in lung function.	191, 192
	10. Development of radiological approaches to image aberrant lung growth at high resolution with serial (longitudinal) analyses of the same experimental animals and human subjects.	187, 190
	11. Establishment of coordinated efforts to biobank biological material from BPD patients from multiple centers and make material available to investigators.	none

Table II

Research priorities to further our understanding of the pathobiology of bronchopulmonary dysplasia.

Research priority	
Key pathobiology questions to be addressed in experimental animal models of BPD	
1.	To delineate the complex roles of inflammation and inflammatory mediators in normal and aberrant lung alveolarization.
2.	To clarify the utility of stem-cell approaches to correct aberrant lung alveolarization.
3.	To clarify how the extracellular matrix (ECM) and ECM-cell interactions contribute to arrested lung alveolarization.
4.	To understand how specific microenvironments within the lung alter cell phenotype to promote or impair alveolarization.
5.	To include extra-pulmonary organs and systems alongside that of the lungs in studies on BPD pathobiology; particularly when extra-pulmonary organ dysfunction, as a consequence of pulmonary injury, may feedback to impact the progress of lung alveolarization.
6.	To determine the source (lineage) of cell progenitors, both for <i>de novo</i> lung development and for repair after injury, and define the molecular pathways that enhance or impair progenitor cell function.
7.	To encourage the inclusion of studies that validate causality alongside descriptive studies that identify candidate mediators of pathology.
Structural microscopic analyses to be undertaken on BPD patient material	
8.	To clarify the pathological characteristics of the changes to the airways, capillaries, conducting vessels, and the microstructure of the alveolo-capillary barrier of prevalent forms of BPD today, in order to facilitate development and refinement of experimental animal models that correctly recapitulate pathology.
Genetic analyses to be undertaken in BPD patient cohorts	
9.	To assess whether computational biology approaches allow for the multicohort analysis of gene expression data sets obtained from premature infants to identify a molecular signature of BPD.
10.	To delineate the genetic contributions to BPD in human populations. These studies would include identifying genetic modifiers of response to injury, as well as susceptibility, and pharmacogenetic responses to drugs.
Key pathobiology considerations in both clinical and experimental BPD	
11.	An assessment of the prenatal, postnatal, and intergenerational responses to candidate pathogenic triggers or mediators, including toxins, infection, stress, smoke, and nutrition.
12.	An assessment of the impact of maternal-fetal interactions on post-natal lung growth, including the roles of the placenta, maternal illness, and intrauterine growth restriction.