The University of Vermont Larner College of Medicine, in collaboration with the National Heart, Lung, and Blood Institute (NHLBI), the Alpha-1 Foundation, the American Thoracic Society, the Cystic Fibrosis Foundation, the European Respiratory Society, the International Society for Cell & Gene Therapy, and the Pulmonary Fibrosis Foundation, convened a workshop titled “Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Diseases” from July 24 through 27, 2017, at the University of Vermont, Burlington, Vermont. The conference objectives were to review and discuss current understanding of the following topics: 1) stem and progenitor cell biology and the role that they play in endogenous repair or as cell therapies after lung injury, 2) the emerging role of extracellular vesicles as potential therapies, 3) ex vivo bioengineering of lung and airway tissue, and 4) progress in induced pluripotent stem cell protocols for deriving lung cell types and applications in disease modeling. All of these topics are research areas in which significant and exciting progress has been made over the past few years. In addition, issues surrounding the ethics and regulation of cell therapies worldwide were discussed, with a special emphasis on combating the growing problem of unproven cell interventions being administered to patients with lung diseases. Finally, future research directions were discussed, and opportunities for both basic and translational research were identified.

Keywords: cell therapy; bioengineering; endogenous lung progenitor cells; induced pluripotent stem cells; extracellular vesicles

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Overview

The Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Diseases 2017 conference was the seventh in a series of biennial conferences focused on the rapidly progressing fields of stem cells, cell therapies, and ex vivo bioengineering in lung biology and disease. Since the last conference in 2015, there have been a number of exciting developments that include but are not limited to the following:

1. Increased understanding of the identity and functional roles of endogenous progenitor cells of the lung epithelium and their stem cell niche;
2. Progress in understanding the steps necessary to have induced pluripotent stem cells (iPSCs) differentiate into functional airway–like and alveolar epithelium–like cells;
3. Increased delineation of the potential roles of mesenchymal stromal cells (MSCs) and endothelial progenitor cells (EPCs) as cell therapy agents for a widening range of lung diseases;
4. A steadily increasing number of clinical trials, particularly of MSCs, in a widening range of lung diseases;
5. Disquieting growth of unproven cell-based interventions and the global regulatory frameworks surrounding cell therapy;
6. Emerging techniques to evaluate promising therapies preclinically with the goal of improving translation toward clinical trials; and
7. Progress in bioengineering techniques, including further development of decellularized whole lungs as scaffolds for ex vivo lung bioengineering and as research tools.

Despite significant progress in each of these areas, many questions remain that need to be explored in the coming years. Extensive discussion of each topic area during the conference resulted in updated overall recommendations for how best to move each area forward, summarized in Table 1.

A full agenda, including all moderators, speakers, and facilitators, is provided as a data supplement.

Introduction

This conference provided follow-up of the six previous biennial conferences held at the University of Vermont since 2005 (1–6). The conference was initiated in 2005 by Drs. Daniel J. Weiss (University of Vermont) and Darwin Prockop (Texas A&M University) and saw continued leadership and growth in the conference with Dr. Weiss serving as conference chair until 2015. Dr. Darcy E. Wagner (Lund University, Sweden) became the conference chair for 2017, with Drs. Amy L. Ryan (Firth) (University of Southern California) and Laertis Ikonomou (Boston University) serving as co–vice chairs.

Investigation of stem cells, cell therapies, and ex vivo bioengineering in lung biology and disease have made rapid progress since 2015. Exciting advances continue in the use of iPSCs, with the most recent data showing derivation of cells with more convincing phenotypic and, in some cases, functional characteristics of both airway and alveolar epithelial cells. Significant progress also continues to be made in investigations of endogenous stem and progenitor cells resident in adult lungs. Advances in lineage-tracing approaches, novel uses of lung injury models, development of lung organoid cultures, and other techniques continue to provide important insights into the identity and lineage expansion properties of previously identified putative endogenous stem and progenitor populations and demonstrate an increasingly complex network of cellular repair after injury. Despite this progress, ongoing challenges remain with respect to 1) better defining, accessing, and manipulating the appropriate niches; 2) developing more refined lineage tracing systems; and 3) evolving techniques to define, characterize, and explore potential therapeutic and/or pathological properties of endogenous lung progenitor cells. A challenging limitation of current studies is that the identity of endogenous progenitor cells primarily comes from studies using mouse models, with ongoing controversy concerning the identity and characterization of human progenitor cells. Data from several laboratories are moving researchers closer to better defining the physiological and pathophysiological roles of upper airway basal epithelial cells in homeostasis and in development of chronic obstructive pulmonary disease (COPD), lung cancers, and other lung diseases (7–9).

Guidelines have been published describing specific nomenclature of stem and progenitor cells in the lung, including in previous conference reports (1–6). However, as knowledge evolves, it remains necessary to continually refine and expand these criteria to provide a more precise definition of progenitor and stem cell populations within the lung. Continued attention must be paid to evolving concepts of different capacities to mediate regeneration in cells originating from different tissues, notably MSCs and EPCs, that might be used in lung disease therapies. Phenotypic and functional attributes of cells are context dependent, and thus phenotyping cells needs to take this into consideration. Cells previously considered to be differentiated airway or alveolar epithelial cells can proliferate and differentiate into other lung epithelial cell types under varying circumstances, and, as such, paradigms of lung cell behavior are continuously evolving.

Continued preclinical studies of immunomodulation and paracrine effects of adult MSCs derived from bone marrow, adipose, placental, and other tissues are providing evidence of safety and efficacy in ameliorating injury and inflammation in preclinical animal models of acute lung injury, asthma, bronchopulmonary dysplasia, COPD, sepsis, ventilator-induced lung injury, and other lung diseases (10, 11). In parallel, investigations with other cell
Table 1. Overall Conference Summary Recommendations and Focus Areas

**Basic science: endogenous stem and progenitor cell biology**
- Continue to encourage new research to elucidate molecular programs for development of lung cell phenotypes. Incorporate technological advances, including single-cell sorting and analyses (e.g., single-cell RNA-seq, single-cell ATAC-seq, single-cell proteomics) and gene editing (e.g., CRISPR/Cas9).
- Continue to refine the nomenclature used in study of endogenous lung stem and progenitor cells.
- Comparatively identify and study endogenous stem/progenitor cell populations between different lung compartments and between species.
- Identify additional cell surface markers that characterize lung cell populations for use in visualization and sorting techniques.
- Increase focus on study of endogenous pulmonary vascular and interstitial progenitor populations.
- Continue to develop robust and consistent methodologies for the study of endogenous lung stem and progenitor cell populations. This includes exploration of different lung injury models that provide individually novel and grouped complementary data.
- Develop more sophisticated tools to identify, mimic, and study ex vivo the relevant microenvironments ( niches) for study of endogenous lung progenitor/stem cells.
- Continue to develop functional outcome assessments for endogenous progenitor/stem cells.
- Elucidate how endogenous lung stem/progenitor cells are regulated in normal development and in diseases, with a focus on human lung tissue.
- Identify and characterize putative lung tumor-initiating cells and regulatory mechanisms guiding their behavior.
- Devise better definitions of “lung in a dish” studies. Is expression of a few phenotypic genes enough? What functional assays are currently available, and how can these be expanded?

**Basic science: cell therapy and iPS/ESC differentiation**
- Continue to elicitate mechanisms of potential recruitment, mobilization, and homing of circulating or therapeutically administered cells to lung epithelial, interstitial, and pulmonary vascular compartments for purposes of either engraftment or immunomodulation.
- For studies evaluating putative engraftment of any type of cell, including endogenous lung progenitor cells and/or iPSC-derived lung cells, as either lung epithelial, interstitial, or pulmonary vascular cells, advanced histological imaging techniques (e.g., confocal microscopy, deconvolution microscopy, EM, laser capture dissection) must be used to avoid being misled by inadequate photomicroscopy and immunohistochemical approaches. Imaging techniques must be used in combination with appropriate statistical and other quantitative analyses of functional cell engraftment to allow an unbiased assessment of engraftment efficiency.
- Continue to elucidate mechanisms by which ESCs and iPSCs develop into lung cells/tissue.
- Comparative assessments of different ESC and iPSC differentiation protocols. Should protocols be standardized?
- Continue to develop disease-specific populations of ESC-/iPSC-derived cells, such as for cystic fibrosis and alpha-1 antitrypsin deficiency as well as other lung diseases.
- Expand use of these cell populations for drug screening and as tools for probing basic disease-specific molecular and cellular pathophysiology.
- Further understanding of how ESC-/iPSC-derived lung lineages will behave in the diseased microenvironment in vivo.
- Develop fluorescent reporter–independent flow-sorting strategies for purification of ESC-/iPSC-derived lung lineages to reduce potential tumorigenicity of transplanted populations.
- Strong focus must be placed on understanding immunomodulatory and other mechanisms of cell therapy approaches in different specific preclinical lung disease models.

**Basic science: bioengineering**
- Continue to develop approaches for ex vivo engineered trachea and large airways for clinical use in both pediatric and adult patients. Increase focus on producing biologically epithelialized and otherwise functional scaffolds. Increase studies on the underlying biology of engineered tracheal scaffolds.
- Continue to explore lung tissue bioengineering approaches such as artificial matrices, three-dimensional (3D) culture systems (e.g., extracellular matrix environments for organoid culture), 3D bioprinting, and other novel approaches for generating lung ex vivo and in vivo from stem cells, including systems that facilitate vascular development.
- Develop standards for potential clinical use of ex vivo engineered trachea and lung.
- Work to define consensus endpoints for the functional assessment and validation of engineered lung tissue.
- What is the optimal environment for growing and/or maintaining lungs ex vivo? Develop advanced bioreactor systems for doing this. Current ex vivo lung perfusion systems can keep tissue viable only in the range of a few hours.
- Conduct studies on perfusate compositions and how they may support multiple cell types.
- Evaluate effect of environmental influences, including oxygen tension, and mechanical forces, including stretch and compression pressure, on development of lung tissue from stem and progenitor cells.
- Incorporate studies of pulmonary nervous and lymphatic structure and function in ex vivo lung bioengineering.

**Basic science: overall**
- Improved preclinical models of lung diseases are necessary.
- Influence of carbohydrates and lipids in lung regeneration.
- Influence of the microbiome and its metabolites in lung regeneration.
- Effects of sex and aging on stem/progenitor cell biology and therapies.
- Disseminate information about and encourage use of existing core services, facilities, and web links.
- Increase tissue sharing of human tissue (adult and fetal). In order to accomplish this, new funding initiatives that support research and infrastructure for storage and shipment would be beneficial.
- Actively foster interinstitutional, multidisciplinary research collaborations and consortiums as well as clinical/basic partnerships. Include a program of education on lung diseases and stem cell biology. A partial list includes the NHLBI PACT, NCATS stem cell facilities, GMP vector cores, small animal mechanics, and CT scanner facilities at several pulmonary centers.

(Continued)
Table 1. (Continued)

Translational/clinical
- Support high-quality translational studies focused on cell-based therapy for human lung diseases. Preclinical models will provide proof of concept; however, these must be relevant to the corresponding human lung disease. Disease-specific models, including large animal models when feasible, should be used and/or developed for lung diseases.
- Basic/translational/preclinical studies should include rigorous comparisons of different cell preparations with respect to both outcome and toxicological/safety endpoints. For example, it remains unclear which MSC or EPC preparation (species and tissue source, laboratory source, processing, route of administration, dosing, vehicle, etc.) is optimal for clinical trials in different lung diseases.
- Incorporate rigorous techniques to unambiguously identify outcome measures in cell therapy studies. Preclinical models require clinically relevant functional outcome measures (e.g., pulmonary physiology/mechanics, electrophysiology, and other techniques).
- Continue to expand well-designed and appropriately regulated clinical investigations of cell-based therapies for pulmonary diseases and critical illnesses. This includes full consideration of ethical issues involved, particularly which patients should initially be studied.
- Develop uniform criteria for outcome measures and clinical assessments in cell therapy trials and in patients who receive engineered tracheal implantations or lung implementations when applicable.
- Provide increased clinical support for cell therapy trials in lung diseases. This includes infrastructure, use of NIH resources such as the PACT program and the NCATS/SCTL (IPSC; https://ncats.nih.gov/stemcell), coordination among multiple centers, and use of registry approaches to coordinate smaller clinical investigations.
- Clinical trials must include evaluations of potential mechanisms, and this should include mechanistic studies as well as assessments of functional and safety outcomes. Trials should include, whenever feasible, collection of biological materials such as lung tissue, BAL fluid, and blood for investigation of mechanisms as well as for toxicology and other safety endpoints. Correlations between in vitro potency and in vivo actions of the cells being used should be incorporated whenever possible.
- Creation of an international registry to encompass clinical and biological outcomes from all cell therapy–based and ex vivo trachea and lung bioengineering trials.
- Partner with existing networks, such as PETAL (NHLBI) Clinical Trials Network for the Prevention and Early Treatment of Acute Lung Injury or American Lung Association Airways Clinical Research Centers, nonprofit respiratory disease foundations, and/or industry as appropriate to maximize the scientific and clinical aspects of clinical investigations.
- Integrate with other ongoing or planned clinical trials in other disciplines in which relevant pulmonary information may be obtained. For example, inclusion of pulmonary function testing in trials of MSC in graft-versus-host disease will provide novel and invaluable information about potential MSC effects on development and the clinical course of bronchiolitis obliterans.
- Work with industry to have access to information from relevant clinical trials.
- All relevant investigators should take a strong stand against marketing of unproven stem cell-based interventions and be familiar with the resources available to patients, caregivers, and all involved healthcare professionals on the websites of respiratory disease and patient advocacy groups as well as those of the leading stem cell societies (International Society for Cell & Gene Therapy and the International Society for Stem Cell Research).

Definition of abbreviations: ATAC-seq = assay for transposase-accessible chromatin using sequencing; CRISPR = clustered regularly interspaced short palindromic repeats; CT = computed tomography; GMP = good manufacturing practice; EPC = endothelial progenitor cells; ESC = embryonic stem cells; IPSC = induced pluripotent stem cells; MSC = mesenchymal stromal cells; NCATS = National Center for Advancing Translational Sciences; iPSC = induced pluripotent stem cells; MHC = major histocompatibility complex; MSKCC = Memorial Sloan Kettering Cancer Center; NBA = National Board of Medical Examiners; NC = National Cancer Institute; OB = obliterating bronchiolitis; PACT = Production Assistance for Cellular Therapies; PETAL = National Heart, Lung, and Blood Institute; NIH = National Institutes of Health; RNA-seq = RNA sequencing; SCTL = Stem Cell Translation Laboratory.

populations, including bone marrow mononuclear cells and EPCs, have shown that these cells ameliorate injury in preclinical models of lung diseases (12, 13). A growing number of clinical investigations are either in progress or planned in a range of pulmonary diseases, including acute respiratory distress syndrome (ARDS), sepsis, bronchopulmonary dysplasia, and idiopathic pulmonary fibrosis, and continuing trials in COPD listed at www.clinicaltrials.gov are taking place around the world (14). Notably, a recently completed, phase II, multicenter, double-blind, placebo-controlled trial of bone marrow–derived, non–human leukocyte antigen–matched allogeneic MSCs for patients with severe ARDS demonstrated safety but no efficacy (15). Although there are a number of potential reasons for this, including dosing and timing of administration, post hoc analyses demonstrated that a substantial number of the MSCs were nonviable at the time of administration. This raises additional considerations for effective implementation of cell therapy–based clinical investigations in lung diseases and critical illnesses (16).

It should be noted that some of the trials listed at www.clinicaltrials.gov are pay-to-participate studies that therefore do not conform to widely accepted ethical and scientific criteria for clinical trials (17). This is a growing problem, both in the United States and globally. An open discussion comprehensively addressing such issues was conducted for the first time at the 2017 conference. This is a significant issue that will require continued and concerted leadership from a coalition of respiratory disease societies and patient advocacy groups to provide appropriate patient, family, and caregiver information on the current state of cell-based therapies for lung diseases, and this is discussed further in the workshop report.

Significant advances continue to be made in novel areas of investigation, in particular those exploring the use of three-dimensional (3D) culture systems and bioengineering approaches to generate functional lung tissue and airways ex vivo. Each of these areas is reviewed in the next sections, followed by recommendations for ongoing and future studies, listed in Table 1. The consensus of conference participants was strongly positive and was supportive of continued investigations into each of these areas.
and production of the conference report is included in the data supplement.

Session 1: Extracellular Vesicles in Lung Regenerative Medicine
MSCs elicit benefits in wound healing, inflammation, hypertension, cardiovascular disease, brain injury, and cancer (18). Recently, MSCs were shown to exert their effects in a paracrine manner, raising the possibility that these effects are mediated by extracellular vesicles (EVs) (19, 20). The opening session focused on the use of MSCs and MSC-derived EVs (MSC-EVs) as therapeutic agents. It is currently unclear whether the mechanism of action of MSCs is comparable to that of MSC-EVs in terms of their potency for therapeutic use. MSC-EVs have been administered successfully to a patient with steroid-refractory acute graft-versus-host disease and have shown efficacy in several animal models of different diseases (e.g., acute kidney injury, acute myocardial infarction, acute liver injury, hepatic failure, stroke, traumatic brain injury) (21, 22). Despite the fact that only 25% of the MSC-derived exosomes were active, they were able to reduce infarct size in a mouse model of acute myocardial ischemia/reperfusion injury (23). Comparable studies evaluating the percentage of active exosomes in lung injury models have not yet been performed, and this will be important as more studies explore the potential of MSC-EVs.

Both MSCs and MSC-EVs are heterogeneous populations, and further studies are needed to understand the implications of this heterogeneity. One promising area of research pioneered by Dr. Donald Phinney’s group has mechanistically linked MSC stem/progenitor and effector functions to TWIST1 (Twist-related protein 1), the expression of which can be specified in populations via a process that can be modeled hierarchically. Dr. Phinney’s group has used this approach to develop the Clinical Indications Prediction scale, which demonstrates that high TWIST1 indicates proangiogenic effects, whereas low TWIST1 indicates anti-inflammatory effects (24). The Clinical Indications Prediction scale has been validated by using MSCs in a bleomycin-induced lung injury model in which low TWIST1 MSCs were shown to be protective against inflammation. Additional factors, such as colony forming units-fibroblast assay activity and FGFR2IIc (fibroblast growth factor 2 receptor IIIc) expression, may also be useful metrics to evaluate MSC potency and fitness (25, 26).

Overall, however, the mechanism of action of MSCs remains incompletely understood. MSCs are known to secrete both exosomes (50–100 nm) and microvesicles (100–1,000 nm), and both of these have been proposed as the primary MSC effectors (27).

Dame Julia Polak Bioengineering Sessions
The bioengineering sessions of the conference have been renamed in memory of Dame Dr. Julia Margaret Polak. Dame Julia Polak was one of the longest-surviving nurses in the United Kingdom and was a prestigious investigator who had an incredible influence on the field of tissue engineering. She was one of the early pioneers for lung tissue engineering.

Session 2: Ex Vivo Bioengineering of Lung and Airway Tissue
Detection of extracellular matrix (ECM) proteins has remained elusive because of difficulties in detecting these proteins, which have low solubility. However, new mass spectrometry–based proteomic methods based on sequential detergent extraction techniques or decellularization of tissue have been shown to enrich for ECM detection and could be used to help improve understanding of how ECM can drive signaling in lung repair and disease (28). The development of these novel proteomic methods can be used to better understand the molecular identification and plasticity of the ECM niche in varied models of lung regeneration.

The ECM is one of the major constituents in whole-organ decellularized scaffolds that are being explored for ex vivo engineering of lung tissue. Although a number of key hurdles have been overcome (e.g., protocols to decellularize human and large animal scaffolds, expansion of cells for re-endothelialization and re-epithelialization of whole human and large animal constructs) (29), a number of challenges remain. Recent work by Dr. Harald Ott’s group in a short-term porcine model of transplantation has identified a number of key hurdles, including achievement of higher-level cellular function such as surfactant expression. Going forward, the goal remains achieving full organ recellularization and, further, patient-specific regeneration of functional lung grafts for clinical transplant (30).

Because there are a number of challenges to address before de- and recellularized lungs can be used in the clinic, technologies that can help accelerate progress and identify promising developments are needed. Alternative platforms and technologies, such as the development of ECM hydrogels derived from decellularized tissues, could be used to evaluate important cell responses after recellularization under defined and controllable conditions. Furthermore, the use of noninvasive techniques to monitor recellularization, such as bioluminescence, could be used to monitor recellularization and graft maturation in real time (31).

One of the major challenges faced in whole-organ lung bioengineering efforts is the generation of functional pulmonary vasculature in decellularized lung scaffolds. Dr. Takaya Suzuki of the University of Toronto showed preliminary work on partially reprogrammed pulmonary endothelial cells and demonstrated partial coverage of lung scaffolds after repopulation in ex vivo culture (T. Suzuki, unpublished data). This work highlighted the utility of novel iPSC-derived and endogenous progenitor cell-derived populations for lung tissue regeneration.

Although natural biomaterials such as decellularized lung ECM have the benefit of containing organ-specific signaling molecules and can provide architecture that closely mimics the native structure, synthetic materials that can be more readily controlled and manufactured on demand are emerging as a potential alternative. New advanced methods for creating tissue-specific materials for use in airway regeneration studies are emerging that can be used with novel biomaterials which can be further biochemically and biomechanically modified to direct cell behavior (32). This will be an important area of future investigation.

Session 3: Bioengineering Next-Generation Devices for Lung Diseases
In addition to efforts to bioengineer new lung tissue for transplant, new research
efforts that integrate engineering with lung biology are emerging as a promising option for further understanding lung biology and disease. One of the leading technologies in this area is "lung on a chip" (33, 34). This platform can be used for high-throughput drug testing and is a complementary tool for bioengineering and lung disease research. Several individual stimuli have been identified as important for understanding of the cells with respect to their environment: cyclic stretch, shear stress, gas partial pressures, and stiffness and topography of the matrix that cells are attached to or embedded in (35). All of these can be studied in a systematic individual or combinatorial fashion with the advent of new in vitro devices, which cover both the micro and macro scales (33, 36).

Advances in manufacturing, coupled with understanding of how cells interact with biomaterials, present new opportunities for improving existing technologies such as in extracorporeal membrane oxygenation (ECMO) devices. One of the major problems in ECMO devices is formation of thrombi on the hollow-fiber membranes. Overcoming the problem of blood clotting inside those fibers would drastically improve the applicability of ECMO and potentially make it a valid alternative for lung transplant. Recent work has shown that albumin/heparin and titanium dioxide coatings can promote an endothelial cell layer resistant to high physiological shear stresses with no change in procoagulative, proinflammatory, and reactive oxygen species genes and no accumulation of reactive oxygen species within 48 hours (37). These coatings are a promising step toward the feasibility of generating bioartificial lungs.

In addition to combining advances in manufacturing and material development, new imaging and computational approaches combining 2D and 3D imaging have opened new doors to understanding alveolar development. In one study, it was found that alveoli form by buckling off from a thinning luminal epithelium into the mesenchyme. First, rings of elastin are formed, and these later become stabilized by rings of collagen and elastin fibers surrounding each unit, leaving space for the capillary network, lymphatics, nerves, and conducting airways (38). Development of new imaging modalities and image-processing techniques will help advance understanding of lung development and regeneration.

One such example was illustrated by John Sembrat (University of Pittsburgh), who showed his work on reseeding decellularized mouse lungs with GFP-expressing bone marrow MSCs (GFP-B-MSCs) and heterotopic implantation into the dorsum of wild-type mice (J. Sembrat, unpublished data). Two-photon microscopy confirmed the existence of vasculature in the transplanted constructs containing GFP-B-MSCs after 1 month. Some cells inside the lung constructs lacked GFP, indicating that they originated from the transplant recipient. Some of the GFP-negative cells inside the transplanted constructs were CD45 positive, indicating their hematopoietic origin, or CD31 positive (endothelial cell marker). Therefore, this work indicates that B-MSCs can induce endogenous cell recruitment after scaffold implantation.

Session 4: Endogenous Progenitor Cells

How lungs repair after injury is an area of intense investigation. The lung is comprised of different regional stem/progenitor populations that expand and differentiate into mature adult cell types after injury. It is increasingly recognized that these cells are deranged in chronic lung diseases and may be targets for new therapies. However, most of the understanding of these cells and their behavior is derived from animal models using lineage tracing. Differences are known to exist between mouse and human progenitor populations during development, in addition to the known anatomic differences (39, 40); thus, translation of knowledge from murine studies to human lung biology and eventual therapies is a priority area for future research.

One area in particular that has received increased attention is the development of gene and cell therapy attempts for cystic fibrosis (CF). Although there is a strong body of literature informing methods of isolation, culture, and in vitro characterization of human airway basal cells, techniques for using gene-corrected somatic stem and progenitor cells in cell therapy are needed. Recent work by Dr. Scott Randell’s group at the University of North Carolina has aimed to optimize and characterize different in vitro expansion methods to safely expand cell numbers to clinically relevant numbers. Furthermore, they have established a series of in vitro and preclinical in vivo assays to assess potential recovery of cystic fibrosis transmembrane conductance regulator (CFTR) function with human bronchial epithelial cell populations. They determined that 10% of cultured cells need to have functional CFTR activity to recover function in an Ussing chamber assay, but difficulties remain in achieving a sufficient number of engrafted cells in vivo to recover function. Polidocanol has been used by Dr. Randell’s group and others to regionally strip resident airway cells and provide access to the basement membrane for cellular engraftment (41, 42). This is a major step toward improving understanding of how to encourage cell engraftment of exogenously gene-corrected airway cells for patients with CF.

Despite advances in improving cellular delivery to damaged or diseased lung tissue, one important consideration is the niche to which they are being delivered and how this might affect cellular behavior. Currently, much more is known about mechanical signaling in mammary epithelial progenitor cells than about mechanical signaling in lung epithelial progenitor cells. For example, ECM and tissue stiffness have been shown to drive cell behavior in breast epithelial cells (43). Work done at Dr. Valerie Weaver’s laboratory has previously shown that tissue stiffness drives epithelial–mesenchymal-like transition in breast epithelial cells in breast cancer through β1-integrin signaling, leading to worse outcomes. A number of tools exist to explore mechanical signaling that could be applied to further understanding of the role of mechanical signaling in lung epithelial progenitor cells.

In addition to matrix and stiffness considerations, how cells in the stem cell niche drive proliferation and differentiation of distal endogenous epithelial progenitor cells in the lung after injury is an important consideration for designing new therapies (44, 45). Region-specific mesenchymal and endothelial populations help determine distal epithelial progenitor cell fate after injury in murine models. Similarly, recent work from Dr. Emma Rawlins’s group has identified multipotent progenitors in the developing distal branching tips of the murine and human lung (39). A Sox9+ progenitor cell population was self-
renewable and could give rise to both bronchiolar and alveolar cells in organoid culture using differential combinations of multiple signaling molecules. These adult human cells could efficiently engraft in a murine xenotransplantation model after bleomycin injury. Thus, cell-intrinsic behavior of epithelial progenitors is not solely responsible for driving regeneration; instead, these cells integrate cues from neighboring niche cells to regenerate. Further understanding of how these stromal cells support and direct regeneration in both murine and human models, as well as the cues that the epithelial cells provide to these stromal cells, will be an important area of future focus.

In addition to region specificity and heterogeneity of epithelial cells, recent work from Dr. Russel Braeuer at the University of Michigan aimed to characterize the heterogeneity of the mesenchymal populations of the lung using genetically labeled mice (collagen type I and the lung embryonic mesenchymal transcription factor Foxf1) (R. Braeuer, unpublished data). They found that Foxf1 was present only in a subpopulation of collagen-positive cells in the adult lung with high Sca-1 (stem cell antigen 1) expression as well as CD90 and PDGF-α (platelet-derived growth factor-α) positivity. Further understanding these lineages in the adult human lung and how these populations are deranged in disease will be important areas for future research.

With regard to cell therapy, understanding the cell source is critical for designing effective therapies. Merline Kocheekkaran Benny of Brown University has characterized preterm versus term umbilical cord–derived MSCs (hUC-MSCs) for neonatal lung injury (M. Kocheekkaran Benny, unpublished data). Term and preterm hUC-MSCs had similar multilineage differentiation capacity, in vitro wound-healing abilities, and cytokine secretion profiles in response to LPS-induced stress response. Thus, preterm hUC-MSCs may have a role in the management of neonatal injuries.

Session 5: iPSC and ESC Biology and Application in Lung Regenerative Medicine

Application of pluripotent stem cells to lung regenerative medicine has witnessed considerable advances since the 2015 meeting. Advances in iPSC biology for lung regeneration highlighted the work of four young investigators who have made progress in improving directed differentiation protocols, understanding the role of the niche in fate decisions and disease modeling.

Wnt signaling was recently shown to play a critical role in both lung progenitor specification and proximodistal patterning of iPSCs (46). Temporal changes in Wnt signaling were found to be required for NKX2-1 (NK2 homeobox 1) induction, as assessed using an NKX2-1<sup>GFP</sup> iPSC reporter line. Subsequent withdrawal of Wnt signaling led to rapid upregulation of a proximal lung gene expression program. These cells formed 3D “bronchospheres” and air–liquid interface cultures composed of secretory, goblet, and basal cells with functional CFTR channels. Surprisingly, the Wnt-dependent patterning response was found to be intrinsic to the lung epithelium and not dependent on signaling from nonlung cell types. This is in contrast to previous studies during mouse lung development that have described how endodermal–mesenchymal interactions are vital to instructing lung morphogenesis (reviewed in References 47 and 48). It will be interesting to follow progress in this area as researchers strive to address some of the limitations that remain to be answered in this field, including the following:

1. How similar are the iPSC-derived epithelial cells to mature adult airway epithelium?
2. What factors contribute to the variability in the organoid swelling?
3. Does the epithelium function and respond differently when mesenchymal niche cells are present?

In addition to using iPSCs to understand lung development and their potential use in cell therapies or bioengineering, there has been an increase in the use of iPSCs for disease modeling and drug testing. Genetically engineered iPSCs show promise for personalized disease modeling, and one such example is the development of a tdTomato fluorescence reporter under the control of the CFTR locus using transcription activator–like effector nucleic–based genome engineering (49). These cells can then be used in high-throughput screening approaches to evaluate CFTR function, focusing on the complexity of mutant CFTR maturation and how genetic modifiers likely have a key role in CFTR processing and function. The AAVS1 (adeno-associated virus integration site 1) “safe harbor” locus was used to introduce a halide-sensitive yellow fluorescent protein to monitor CFTR function. Directed differentiation toward intestinal/biliary epithelium was then performed using mutant and CFTR gene-corrected reporter iPSCs. Thus, cells expressing both yellow fluorescent protein and tdTomato, which actively responded to CFTR stimulation by the addition of forskolin, were generated. These data complement studies by Hans Clever’s laboratory, which pioneered the use of organoid systems for evaluating CFTR function (50, 51). It will be interesting to see if intrinsic differences exist in CFTR responses between intestinal and airway epithelia.

iPSCs may be particularly useful for in vitro studies to investigate disease-modifying mutations in patients with CF with extreme phenotypes. Dr. Hongmei Li of Boston Children’s Hospital showed preliminary work that highlights the power of using gene manipulation in evaluating genotype–phenotype relationships using patient-specific iPSCs. Previously, the epithelial sodium channel (ENaC) was identified as a genetic modifier in the long-term nonprogressive phenotype associated with the F508del phenotype in patients with CF (52). Five patients with long-term nonprogressive CF were evaluated in the study. Whole-exome sequencing identified extremely rare or never reported variants in the SCN1D and SCN1B (amiloride-sensitive sodium channel subunits delta and beta, respectively) genes of ENaC in four of the five patients. Interestingly, one of the four mutations had a functional consequence on ENaC; yet, all five patients had no decline in lung function. Their data suggest a potential role of δ-ENaC as a disease modifier in CF, further supporting the plausibility of ENaC as a therapeutic target to ameliorate disease. It will be exciting to follow progress in this work and see if ENaC is important to CF disease.

As previously discussed, pulmonary endothelial cells play a role in directing regeneration of distal endogenous airway and alveolar progenitor cells. Therefore, the ability of human iPSC-derived endothelial cells (iECS) to influence fate...
decisions of lung progenitor cells is of particular interest because efficient generation of distal lung epithelium from iPSC remains challenging. Dr. Miriel Ho of Ottawa Hospital Research Institute proposed that efficient generation of functional distal types I and II alveolar epithelial cells might require endothelial cells because of their intimate relationship in the alveolar–capillary barrier. In support of this, she showed preliminary data on deriving iECs using a combination of BIO (6-bromoindirubin-3′-oxime) for GSK3β (glycogen synthase kinase-3β) inhibition, and VEGF (vascular endothelial growth factor) followed by immunomagnetic selection of VE-cadherin+ (vascular endothelial cadherin–positive) iECs (M. Ho, unpublished data). These cells were at least 80% pure for endothelial cell markers CD34, PECAM-1 (platelet endothelial cell adhesion molecule 1), and VEGFR2 (vascular endothelial growth factor receptor 2) and formed a cobblestone monolayer in culture. When iPSC-derived NKX2-1+ and SOX9 (sex-determining region Y-box 9)-expressing lung progenitors were treated with conditioned media from iEC cultures, distal lung markers AQPS5 (aquaporin 5) and SFTPC (surfactant protein C) increased with a concomitant decrease in proximal epithelial markers (e.g., FOXJ1 and MUC5AC). This suggests an instructive role for paracrine signaling from endothelial cells in the differentiation of distal lung epithelium from iPSCs.

Session 6: Commercialize Your Research for Industry Sponsorship
Small Business Innovation Research/Small Business Technology Transfer Collaboration
Details of this session are provided in the data supplement.

Session 7: Regulation of Stem Cell and Regenerative Medicine Treatments
The marketing of unproven “stem cell”–based interventions (SCBIs) for various diseases, including lung diseases, has become a global public health problem and has proved refractory to various regulatory efforts (53, 54). The recent statement from U.S. Food and Drug Administration (FDA) Commissioner Scott Gottlieb regarding increasing FDA enforcement actions highlights the extent of the problem even in countries with well-developed cell therapy regulatory frameworks (55). For the first time, an entire session dedicated to regulation of regenerative medicine products, co-organized by the International Society for Cell & Gene Therapy (ISCT), was included at the conference. This session was a direct result of discussions that had occurred at the 2015 Vermont Stem Cell Conference on the importance and possible ways of raising awareness of this issue among all stakeholders—patients, caregivers, clinicians, scientific societies, and regulatory agencies (6).

There has been an alarming increase in the number of U.S. clinics that market unproven SCBIs. Contrary to the familiar narrative of “stem cell” clinics thriving in countries with inadequate or lax regulatory oversight, hundreds of businesses offering direct-to-consumer SCBIs are active in the U.S. market, with almost half of these clinics operating in three states (California, Florida, and Texas) (56, 57). These businesses increasingly target patients with chronic lung diseases, such as COPD, using a wide range of marketing ploys such as exaggeration of clinical benefits, minimization of potential risks, and uncontrolled patient testimonials. The U.S. federal www.clinicaltrials.gov database has become a marketing device for “pay-to-participate” cell-based studies that have not been cleared by the FDA (58). Dr. Leigh Turner of the University of Minnesota called for more effective oversight by both federal agencies (FDA, Federal Trade Commission) and state medical boards.

The ISCT Presidential Task Force, chaired by Dr. Massimo Dominici, has been actively working on the problem associated with the use of unproven cellular therapies. The ISCT has a number of ongoing activities in an effort to combat this problem, including the publication of a reference guide and several articles covering a variety of issues arising from the use of unproven and unregulated SCBIs (59–61). Dr. Dominici stressed the importance of international cooperation between professional societies, regulators, and patient foundations to combat the rise of unproven SCBIs.

The dangers associated with the administration of autologous experimental cell products with little oversight have been documented recently by Dr. Ajay Kuriyan and his group at the University of Rochester (62). Three patients who received simultaneous, bilateral, intravitrreal injections of autologous adipose tissue–derived “stem cells” in a business offering unproven SCBIs experienced blinding as a complication. Dr. Kuriyan highlighted the flawed informed consent process in such businesses and the difficulty of collecting clinical data related to complications from unproven “stem cell” administration due, in large part, to nondisclosure agreements signed by patients.

Regulatory oversight, such as premarket demonstration of safety and efficacy, for medical products remains challenging. There are sustained efforts for health product deregulation, including regenerative medicine products, and this is further driven by ideological underpinnings and political and economic factors. Douglas Sipp of the RIKEN Center for Developmental Biology drew parallels between conditional approval pathways recently introduced in Japan and the United States (21st Century Cures Act, Public Law 114-255) and discussed the potential implications of such pathways, such as lower safety and efficacy standards, potential risks to patients, and increasing financial burden to national healthcare systems (63, 64).

In summary, the speakers addressed the complex regulatory, bioethical, financial, and societal issues associated with the emergence of unregulated SCBIs. The lively discussion and engaging comments by both the audience and the speakers made it clear that there is a need for this type of session, and future conferences will engage participation of other leading stem cell organizations such as the International Society for Stem Cell Research in this area.

Session 8: Careers in Stem Cells, Cell Therapies, and Lung Bioengineering
At two previous editions of this conference, this session has provided useful information on academic career support and early career development (5, 6). This session aimed to inform attendees about various funding mechanisms and programs within the National Institutes of Health (NIH) available to researchers across all levels. Details of this session and further resources are provided in the data supplement.

Session 9: Cell Therapies (MSCs, EPCs, and ASCs) in Lung Disease
The final scientific session covered advances in clinical and preclinical work using stem
cell therapy in lung disease. Preclinical studies from Dr. Michael Matthay’s group at the University of California, San Francisco, and others have shown the promise of using MSCs in ARDS. This led to a phase I dose escalation clinical trial of 1 million, 5 million, or 10 million MSCs per kilogram delivered intravenously, which demonstrated no safety issues (65). The phase IIa trial was recently completed and published (15). This was a National Heart, Lung, and Blood Institute (NHLBI)-supported, double-blind, placebo-controlled trial of MSC administration for moderate to severe ARDS in 60 patients conducted at five U.S. university centers. There were no issues regarding safety in the phase IIa trial, and although there were encouraging trends in the secondary efficacy endpoints, no efficacy was observed (15). Dose and timing of administration, as well as post hoc analyses, demonstrated that a substantial number of the MSCs were nonviable at the time of administration and raise ongoing questions for effective implementation of cell therapy–based clinical investigations in lung diseases and critical illnesses (16).

Previous studies have shown that preclinical studies using autologous cells from patients with chronic disease were less effective than those from healthy individuals. Therefore, Dr. David Courtman of the Ottawa Hospital Research Institute has been working on developing a manufacturing pipeline to develop autologous cells in isolated, controlled units that can also record environmental parameters affecting the final therapeutic cell product. This is geared toward optimizing the preclinical and clinical paths to the first-in-human clinical trial for pulmonary hypertension using gene-enhanced autologous EPCs. Their group has recently begun a phase II trial for pulmonary hypertension (SAPPHIRE [Study of Angiogenic Cell Therapy for Progressive Pulmonary Hypertension: Intervention with Repeat Dosing of eNOS-enhanced EPCs]; www.clinicaltrials.gov identifier NCT03001414). This pipeline, based on good manufacturing practice principles, could be applied more broadly for other cell therapies in the future.

As highlighted earlier in this report, the cells themselves may not be necessary, and secreted products from cells might be sufficient in some diseases. EVs from MSCs were recently shown to be capable of modulating human macrophages (66). Human MSCs were able to promote an unconventional M2-like phenotype (i.e., increased phagocytic activity) when cultured in the presence of LPS BAL fluid derived from patients with ARDS. These effects were mediated through mitochondrial transfer from the MSC-derived EVs and a subsequent enhancement of macrophage oxidative stress. Further studies are needed to understand the potential efficacy of EVs in acute and chronic lung diseases.

There have also been recent advances toward regulating and directing cell behavior of MSCs through engineering approaches. Improved targeting of MSCs to the lungs can be achieved using endocytosed superparamagnetic iron oxide nanoparticles (67). MSCs that had been magnetized, delivered intravenously, and directed to the lungs using extracorporeal magnets on the thorax of animals that had received silicosis had a reduced fibrotic burden compared with MSCs that had been magnetized but not directed to the thorax with extracorporeal magnets (L. Silva, unpublished data). In addition to directing MSCs to the lung, new advances in engineering MSC delivery vehicles might help to regulate their effects. Dr. Jae-Won Shin showed his group’s preliminary data on the effect of substrate stiffness on MSC cytokine production. MSCs that were cultured on a softer alginate matrix had enhanced matrix metalloproteinase as well as TSG6 (TNF-stimulated gene 6) production compared with those cultured in the stiff alginate matrix upon activation with TNF-α (J. W. Shin, unpublished data). MSCs were thus encapsulated in soft alginate hydrogel spheres and injected into mice that had received bleomycin 1 week prior. These MSCs were able to reduce collagen burden more than unencapsulated MSCs. Both of the latter two approaches indicate an emerging theme of incorporating bioengineering- and biomaterial-based approaches to improve MSC-based cell delivery.

### Setting Priorities and Recommendations to the NIH and Other Organizations Regarding Future Research Opportunities

The broad field of lung regenerative medicine continues to evolve at an accelerating pace. The NIH, nonprofit respiratory disease foundations, and other sources of scientific and funding support remain positive, and this will be important for continued development. A series of scientific and funding recommendations coming out of discussions at the conference and postconference surveys are presented in Table 1.

There is an increasing consensus and actions geared toward removing the growing problem of marketing of unproven SCBs. Conference participants were encouraged by the recent actions taken by the FDA and other organizations. The American Thoracic Society (ATS) Respiratory Cell and Molecular Biology Stem Cell Working Group will remain a prominent focal point in these actions, and the continued long-term goal is to have a functional coalition of respiratory disease societies, professional organizations, and patient advocacy groups working in concert to best educate patients and caregivers to diminish and ultimately remove this problem.

### Conclusions

A continuing accumulation of data in animal models, novel ex vivo models using human tissue and cells, and clinical trials suggests that cell-based therapies and bioengineering approaches may be potential therapeutic strategies for lung repair after injury or in end-stage lung disease. Further insight into the role of endogenous lung progenitor cells, the stem cell niche in which they reside, and how the niche cells and progenitor cells orchestrate development and regeneration will provide an opportunity for novel therapeutic strategies. Significant progress has been made in the development of protocols and technologies for generating iPSC-derived lung cells from patients with genetic disorders, such as CF. Remarkable progress has been made in each of these areas since the last conference 2 years ago. We hope that the workshop recommendations (Table 1) will spark new research that will provide further understanding of mechanisms of lung injury repair and a sound scientific basis for therapeutic use of stem cells and cell therapies, in concert with bioengineering strategies, in lung diseases.
This workshop report was prepared by an ad hoc subcommittee of the ATS Assembly on Respiratory Cell and Molecular Biology Stem Cell Working Group.

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