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An Official American Thoracic Society Workshop Report 2015
Stem Cells and Cell Therapies in Lung Biology and Diseases

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Abstract

The University of Vermont College of Medicine, in collaboration with the NHLBI, Alpha-1 Foundation, American Thoracic Society, Cystic Fibrosis Foundation, European Respiratory Society, International Society for Cellular Therapy, and the Pulmonary Fibrosis Foundation, convened a workshop, "Stem Cells and Cell Therapies in Lung Biology and Lung Diseases," held July 27 to 30, 2015, at the University of Vermont. The conference objectives were to review the current understanding of the role of stem and progenitor cells in lung repair after injury and to review the current status of cell therapy and *ex vivo* bioengineering approaches for lung diseases. These are all rapidly expanding areas of study that both provide further insight into and challenge traditional views of mechanisms of lung repair after injury and pathogenesis of several lung diseases. The goals of the conference were to summarize the current state of the field, discuss and debate current controversies, and identify future research directions and opportunities for both basic and translational research in cell-based therapies for lung diseases. This 10th anniversary conference was a follow up to five previous biennial conferences held at the University of Vermont in 2005, 2007, 2009, 2011, and 2013. Each of those conferences, also sponsored by the National Institutes of Health, American Thoracic Society, and respiratory disease foundations, has been important in helping guide research and funding priorities. The major conference recommendations are summarized at the end of the report and highlight both the significant progress and major challenges in these rapidly progressing fields.

Keywords: bioengineering; cell therapy; endogenous lung progenitor cells; lung; stem cells

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The conference was held at the University of Vermont, Burlington, July 27–30, 2015.

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Overview

This was the sixth 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 diseases. Since the last conference there have been a number of exciting developments that include but are not limited to: (*1*) increased understanding of the identity and functional roles of endogenous progenitor cells of the lung epithelium; (*2*) progress in understanding the steps necessary to have induced pluripotent stem cells differentiate into both airway and alveolar epithelial cells; (*3*) increased delineation of the potential

roles of mesenchymal stromal cells and endothelial progenitor cells as cell therapy agents for a widening range of lung diseases; (*4*) a steadily increasing number of clinical trials, particularly of mesenchymal stromal cells, in a widening range of lung diseases; (*5*) identification of additional cell populations that may have a role in treatment of lung diseases; (*6*) progress and controversy in *ex vivo* tracheal bioengineering; and (*7*) progress in development of decellularized whole lungs as scaffolds for *ex vivo* lung bioengineering and as research tools. Conversely, there has been growth in use of unproven cell-based therapies for lung diseases (i.e., stem cell medical tourism), an area of increasing concern.

However, there remain many questions in each of these areas. Extensive discussion of each topic area during the conference resulted in an updated series of recommendations on nomenclature, summarized in Table 1, and updated overall recommendations for how to best move each area ahead, summarized in Table 2.

Introduction

This conference was a follow up to five previous biennial conferences held at the University of Vermont in 2005, 2007, 2009, 2011, and 2013 (1–6). Since the last conference in 2013, investigations of stem cells, cell therapies, and *ex vivo* bioengineering in lung biology and diseases have continued to rapidly progress. Exciting advances continue in studies of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), with recent data demonstrating more convincing evidence of derivation of cells with phenotypic and in some cases functional characteristics of both airway and alveolar epithelial cells. Significant progress also continues to be made in investigations of local (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 understanding of 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. However, ongoing challenges remain with respect to better defining, accessing, and manipulating the appropriate niches and to continue to devise more refined lineage tracing and other techniques to define, characterize, and explore potential therapeutic and/or pathologic properties of endogenous lung progenitor cells. This includes studies of lung cancer stem cells, an area of increasing focus and high interest that remains incompletely understood. Another challenge has been that most previous studies of endogenous progenitor cells used mouse models, with ongoing controversy concerning the identity and characterization of human progenitor cells. However, increasing data from several laboratories are better defining the physiologic and pathophysiologic roles of upper airway basal epithelial cells in homeostasis and in development of chronic obstructive pulmonary disease (COPD), lung cancers, and other lung diseases.

Previous conferences and other sources have suggested guidelines for the nomenclature of stem and progenitor cells in the lung. However, precise definitions and characterizations of specific cell populations, as well as mesenchymal stem (stromal) cells and endothelial progenitor cells, remain in evolution. This reflects in many respects increasing appreciation that the phenotypic and functional attributes of cells are context dependent. 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 in evolution. Although less so than in previous years, the terms "stem cell" and "progenitor cell" are still used with varying degrees of clarity and precision by different investigators and in recent publications. This continues to complicate comparison of different investigative approaches and fuller understanding of the role of endogenous lung progenitor cells both in normal homeostasis and in response to different types of lung injuries. A suggested glossary of relevant working definitions applicable to lung, originally presented in the report of the 2007 conference, has been updated in consultation with thought leaders in the relevant fields and is depicted in Table 1. This glossary does not necessarily reflect an overall consensus for the definition of each term and will undergo continuing evolution as overall understanding of the cell types and mechanisms involved in lung repair continue to be elucidated. Nonetheless, it remains a useful framework for further discussion and to guide future experiments.

Continued preclinical studies of immunomodulation and paracrine effects of adult mesenchymal stromal (stem) cells (MSCs) derived from bone marrow, adipose, placental, and other tissues continue to provide evidence of safety and efficacy in ameliorating injury and inflammation in animal models of acute lung injury, asthma, bronchopulmonary dysplasia, COPD, sepsis, ventilator-induced lung injury, and other lung diseases. In parallel, a steadily increasing number of investigations with other cell populations, including bone marrow mononuclear cells and amniotic fluid (AF)-derived cells, demonstrate efficacy in ameliorating injury in preclinical models of lung diseases. In parallel, more sophisticated understanding of the mechanisms by which each of these different cells can act has provided growing insight into their potential applicability for clinical lung diseases. There are subsequently a growing number of clinical investigations either in progress or planned in a range of pulmonary diseases, including acute respiratory distress syndrome (ARDS), sepsis, bronchopulmonary dysplasia (BPD), and idiopathic pulmonary fibrosis as well as continuing trials in COPD listed on ClinicalTrials.gov that are taking place in the United States, Canada, Brazil, Europe, and Australasia. Notably, a phase I multicenter dose-escalation trial of bone marrow–derived non–HLA-matched allogeneic MSCs for patients with severe ARDS demonstrated safety without evidence of infusional toxicities. The phase II trial is underway, and results are anticipated in the spring of 2016.

Significant advances also continue to be made in novel areas of investigation, particularly increasing exploration of three-dimensional (3D) culture systems and bioengineering approaches to generate functional lung tissue *ex vivo*. In parallel, *ex vivo* bioengineered trachea and upper airways have been used clinically with varying degrees of success and have generated significant controversies about the approaches used. A significant challenge will be to develop a fuller

Table 1. Glossary and definition of terminology

Potency: Sum of developmental or differentiation capacity of a single cell in its normal environment *in vivo* in the embryo or adult tissue. A change in potency may occur by dedifferentiation or reprogramming, after transplantation to another site or in response to local inflammation or injury. Demonstrating this change in potency requires lineage tracing the fate of single cells.

Totipotency: The capacity of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues and germ cells, and thus to (re)generate an organism. In mammals, with rare exceptions, only the zygote and early cleavage blastomeres are totipotent.

Pluripotency: The capacity of a single cell to give rise to differentiated cell types within all three embryonic germ layers and thus to form all lineages of an organism. A classic example is pluripotent embryo-derived stem cells (ESCs). However, some species differences can occur; for example, mouse ESCs do not give rise to extraembryonic cell types, but human ESCs can give rise to trophoblasts.

Multipotency: Ability of a cell to form multiple cell types of one or more lineages. Example: hematopoietic stem cells in adults and neural crest cells in developing embryos

Unipotency: Ability of a cell to give rise to cell types within a single lineage. Example: spermatogonial stem cells can only generate sperm or sperm-precursor intermediate cells.

Lineage: Differentiated cells in a tissue related to each other by descent from a common precursor cell.

Reprogramming: Change in phenotype of a cell so that its differentiation state or potency is altered. At least two kinds of reprogramming have been described. In one, the term refers to a process that involves an initial process of dedifferentiation to a state with greater potency, as in the formation of iPSCs from a differentiated cell such as a fibroblast. Alternatively, the concept of "direct reprogramming" refers to a switch in phenotype from one lineage to another without going through a multipotent or pluripotential intermediate state. This usually involves genetic manipulation (e.g., fibroblast to neuronal cell or liver cell) by expression of a few transcription factors or may occur in injury, for example conversion of pancreatic exocrine cells to hepatocytes in copper deficiency. The ability of Scgb1a1^{+} club cells to give rise to type 2 alveolar epithelial cells after certain kinds of lung injury may be another example of reprogramming in response to injury.

Dedifferentiation: Change in phenotype of a cell so that it expresses fewer differentiation markers and changes in function, such as an increase in differentiation potential (e.g., reversion of a differentiated secretory cell to a basal stem cell in the tracheal epithelium and blastema formation during tissue regeneration in amphibians). In most respects, this is synonymous with reprogramming.

Transdifferentiation: The process by which a single differentiated somatic cell acquires the stable phenotype of a differentiated cell of a different lineage. The classic example is the differentiation of a pigmented epithelial cell of the amphibian iris (neurectoderm) to a lens cell (ectoderm). May involve transition through a dedifferentiated intermediate, usually but not necessarily with cell proliferation. The distinction between transdifferentiation and reprogramming may be semantic.

Epithelial–mesenchymal transition: A developmental process in which epithelial cells acquire phenotypic and functional attributes of mesenchymal-origin cells, usually fibroblastic cells. Whether this process occurs in adult lungs (or other adult tissues) remains controversial. In cancer biology, epithelial cells can change shape, polarity, and migratory capacity characteristic of other cell phenotypes, but whether they have undergone a full lineage transition remains unclear.

Plasticity: Ability of a cell to change its phenotype through the process of dedifferentiation, reprogramming, or transdifferentiation. Mature differentiated cells may be more difficult to dedifferentiate into an iPSC than are immature cells or tissue stem cells. Another use of the term plasticity is to describe normally adaptive changes in cell phenotype as they adapt to different environmental conditions.

Embryonic stem cells (ESCs): Cell lines developed from the inner cell mass of a blastocyst stage embryo. ESCs have the capacity for self-renewal and are pluripotent, having the ability to differentiate into cells of all three germ layers and all adult cell types. Mouse (but not human) ESCs cannot form extraembryonic tissue such as trophectoderm.

Adult stem cell: Cells from adult tissues, such as bone marrow, intestine, nervous tissue, and epidermis, that have the capacity for long-term self-renewal and differentiation into cell types specific to the tissue in which they reside. These cells can also regenerate the tissue after transplantation or injury. In general, adult stem cells are multipotent, having the capacity to differentiate into several different mature cell types of the parent tissue. The differentiation potential of a single adult stem cell may change after transplantation to a new environment or in response to local injury/inflammation or after culture. For example, MSCs from adipose tissue can give rise to smooth muscle, cartilage, or bone when cultured under different conditions and/or in response to specific signaling factors. Although easy to track in *in vitro* culture systems using isolated cells, demonstrating this change in potential *in vivo* requires single cell lineage tracing.

Induced pluripotent stem cell (iPSC): Reprogrammed somatic cells that have undergone a resetting of their differentiated epigenetic states into a state reminiscent of embryonic stem cells after the expression of reprogramming molecules, such as the transcription factors Oct 3/4, Sox2, c-Myc, and Klf4. iPSCs are similar to ESCs in morphology, proliferation potential, pluripotent differentiation repertoire, and global transcriptomic/epigenomic profiles. *In vivo* implantation of iPSCs results in formation of tissues from all three embryonic germ layers. iPSCs have been generated from both mouse and human cells.

Progenitor cell: A general term traditionally used to describe any relatively immature cell that has the capacity to proliferate, giving rise to mature postmitotic cells within a given tissue. More recent evidence suggests that differentiated epithelial cells in the lung can act as progenitors under certain conditions. Unlike stem cells, progenitor cells are generally believed to have limited or no self-renewal capacity and may undergo senescence after multiple cell doublings. The literature continues to blur distinctions between uses of the terms "stem" and "progenitor."

Transit-amplifying cell: The progeny of a tissue stem cell that retains a relatively undifferentiated character, although more differentiated than the parent stem cell, and demonstrates a finite capacity for proliferation. One recognized function of transit-amplifying cells is the generation of a sufficient number of specialized progeny for tissue maintenance or repair. There may be other as-yet-unknown functions.

Obligate progenitor cell: A cell that loses its ability to proliferate once it commits to a differentiation pathway. Intestinal transit amplifying cells are a traditional example. However, it has recently been demonstrated that some intestinal transit amplifying cells can give rise to Lgr5^{+} intestinal stem cells after ablation of the resident Lgr5^{+} population.

Facultative progenitor cell: A cell that exhibits differentiated features when in the quiescent state yet has the capacity to proliferate for normal tissue maintenance and in response to injury. Bronchiolar club cells are an example of this cell type. However, it is becoming apparent that there are likely multiple populations of club cells, not all of which may function in this respect.

Classical stem cell hierarchy: A stem cell hierarchy in which the adult tissue stem cell actively participates in normal tissue maintenance and gives rise to transit-amplifying progenitor population. Within this type of hierarchy, renewal potential resides in cells at the top of the hierarchy (i.e., the stem and transit-amplifying cell), and cells at each successive stage of differentiation become less potent.

(Continued)

Table 1. (*Continued*)

Nonclassical stem cell hierarchy: A stem cell hierarchy in which the adult tissue stem cell does not typically participate in normal tissue maintenance but can be activated to participate in repair after progenitor cell depletion. A related concept is that of population asymmetry or neutral drift, in which there is no dedicated slow-cycling stem cell but rather a pool of equipotent cells that can give rise to clones of differentiated progeny. This has been shown for intestine, interfollicular epidermis, testis, and human airway basal cells.

Rapidly renewing tissue: Tissue in which homeostasis is dependent on maintenance of an active mitotic compartment. Rapid turnover of differentiated cell types requires continuous proliferation of stem and/or transit-amplifying cells. A prototypical rapidly renewing tissue is the intestinal epithelium.

Slowly renewing tissue: Tissues in which the steady state mitotic index is low. Specialized cell types are long lived and some, perhaps all, of these cells, the facultative progenitor cells, retain the ability to enter the cell cycle in response to injury or changes in the microenvironment. The relative stability of the differentiated cell pool is paralleled by infrequent proliferation of stem and progenitor cells. The lung is an example of a slowly renewing tissue.

Hematopoietic stem cell: Cell that has the capacity for self-renewal and whose progeny differentiate into all of the different blood cell lineages, including mature leukocytes, erythrocytes, and platelets.

Endothelial progenitor cell: This term has been replaced with the following two categories of cells.

Proangiogenic hematopoietic cell: Bone marrow–derived hematopoietic cells that display the ability to functionally augment vascular repair and regeneration principally via paracrine mechanisms. Most evidence indicates that the recruited proangiogenic hematopoietic cells circulate to sites of tissue injury and facilitate resident vascular endothelial cell recruitment to form new vessels but lack direct vessel-forming ability. In general, most prior uses of the term endothelial progenitor cell have now been demonstrated to be more appropriately described as effects emanating from proangiogenic hematopoietic cells.

Endothelial colony–forming cell: Rare circulating blood cells that display the ability to adhere to tissue culture plastic or matrix proteins *in vitro*, display robust clonal proliferative potential, and generation of cells with endothelial lineage gene expression and *in vivo* blood vessel forming potential when implanted in a variety of natural or synthetic scaffolds. Endothelial colony–forming cells have also been termed blood or late outgrowth endothelial cells and, in some cases, have also been referred to as endothelial progenitor cells.

Mesenchymal stromal (stem) cells: Cells of stromal origin that can self-renew and give rise to progeny that have the ability to differentiate into a variety of cell lineages. Initially described in a population of bone marrow stromal cells, they were first described as fibroblastic colony-forming units, subsequently as marrow stromal cells, then as mesenchymal stem cells, and most recently as multipotent mesenchymal stromal cells or MSCs. MSCs have now been isolated from a wide variety of tissues, including umbilical cord blood, Wharton's jelly, placenta, adipose tissue, and lung. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has published the minimal criteria for defining (human) MSCs in 2006 (116). However, this definition is being reinvigorated as it has become clear that the functional attributes of MSCs, (i.e., potency in any given application), in combination with cell surface markers, differentiation capacity, source, or culture conditions, will provide a more relevant framework for study and potential therapeutic use of MSCs (38).

Fibrocyte: A cell in the subset of circulating leukocytes that produce collagen and home to sites of inflammation. The identity and phenotypic characterization of circulating fibrocytes is more firmly established than that for EPCs. However, whether fibrocytes originate from bone marrow lymphoid or myeloid progenitors remains unclear. These cells express the cell surface markers CD34, CD45, CD13, and MHC II and also express type 1 collagen and fibronectin.

Airway basal stem cells: Cells present within the pseudostratified airway epithelium that are rich in hemidesmosomal connections that anchor the epithelium to the basement membrane. These cells are characterized by the variable expression of p63 and cytokeratins (K)5 and 14. There are more K5-positive basal cells in the airway than p63-positive cells. Not all K5-positive cells express p63 at steady state. K14 is expressed in self-renewing basal cells and is only present in rare basal cells at steady state. In the pseudostratified proximal airway epithelium, basal cells function as stem cells that give rise to ciliated and secretory cells. Recently, cells with some features of basal cells were described in the distal lung. The extent of the molecular and function similarity of these cells to basal cells of the upper airways is not clear.

Bronchiolar stem cell: A term applied to a population of naphthalene-resistant Scgb1a1^{lo}, Scb3a2^{hi} expressing cells that localize to neuroepithelial bodies and the bronchoalveolar duct junction of the rodent lung. These cells proliferate infrequently in the steady state but increase their proliferative rate after depletion of mature club cells by naphthalene. Lineage tracing studies indicate that these cells have the ability to self-renew and to give rise to Scgb1a1 club cells and ciliated cells after injury. Apart from naphthalene resistance, there is no evidence that these cells have a higher capacity for functioning as facultative progenitors than Scgb1a1^{+} club cells. Human correlates have not yet been identified.

Bronchioalveolar stem cell (BASC): A term applied to a rare population of cells ($<$1 per terminal bronchiole) located at the bronchoalveolar duct junction in the mouse lung identified *in vivo* by dual labeling with Scgb1a1 and Sftpc and by resistance to destruction with naphthalene or bleomycin. In culture, dual-positive cells can be enriched by FACS by selecting for cells that also express Sca1 and CD24. However, these markers can also be expressed on other cells. The BASCs can self-renew and give rise to progeny that express either alveolar epithelial lineage markers such as Sftpc, or aquaporin 5, or progeny that express airway epithelial lineage markers such as Scgb1a1. Currently, it is unknown if BASCs or club cells have any true phenotypic or functional distinction, as there is no evidence that the dual-positive cells are any more likely than single-positive Scgb1a1 club cell abilities to give rise to type 2 and type 1 cells either in culture or *in vivo* after injury. Notably, there are currently no known BASC-specific markers to distinguish them from club cells *in vivo*. However, in three-dimensional cocultures, single BASCs are multipotent, with the ability to produce alveolar or airway lineages. Human correlates have not yet been identified.

Definition of abbreviations: FACS = fluorescence-activated cell sorter; iPSC = induced pluripotent stem cell; MHC = major histocompatibility complex; MSC = mesenchymal stromal cell.

Modified by permission from Reference 1. The authors gratefully acknowledge input and discussion toward updating of this table from the following individuals: Christina Barkauskas, Brian Davis, Massimo Dominici, John Engelhardt, Amy Firth, Brigitte Gomperts, Erica Herzog, Carla Kim, Darrell Kotton, Laertis Ikonomou, Luis Ortiz, Darwin Prockop, Susan Reynolds, Duncan Stewart, Barry Stripp, and Mervin Yoder.

understanding of the underlying cell biology in the tracheal scaffolds and use these to advance efficacy of clinical applications.

Each of these areas is reviewed in the following sections, followed by overall recommendations for ongoing and future studies, depicted in Table 2. The overall consensus of conference participants was strongly positive and supportive of continued extensive and intensive investigations into each of these areas.

Table 2. Overall conference summary recommendations and questions for further study

Fundamental/basic
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, and pulmonary vascular cells, advanced histologic imaging techniques (e.g., confocal microscopy, deconvolution microscopy, electron microscopy, laser capture dissection, etc.) 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 for an unbiased assessment of engraftment efficiency.
Continue to elucidate 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 of immunomodulation.
Continue to encourage new research to elucidate molecular programs for development of lung cell phenotypes. Incorporate technological advances including single cell sorting and analyses, CRISPR/Caspase, and advanced microarray approaches.
Continue to refine the nomenclature used in study of endogenous and exogenous 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 and progenitor cells are regulated in normal development and in diseases.
Identify and characterize putative lung cancer stem cells and regulatory mechanisms guiding their behavior.
Continue to elucidate mechanisms by which embryonic and induced pluripotent stem cells develop into lung cells/tissue.
Comparative assessments of different ESC and iPSC differentiation protocols. Should protocols be standardized?
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?
Continue to develop disease-specific populations of ESCs and iPSCs, for example for cystic fibrosis and alpha-1 antitrypsin deficiency, with the recognition that no strategy has yet been devised to overcome the propensity of ESCs and iPSCs to produce tumors. Expand use of these cell populations for drug screening and as tools for probing basic disease-specific molecular and cellular pathophysiology.
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, 3D culture systems, 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.
What is the optimal environment for growing and/or maintaining lungs *ex vivo*? Develop advanced bioreactor systems for doing this. Evaluate effect of environmental influences, including oxygen tension, and mechanical forces, including stretch and compression pressure, on development of lung from stem and progenitor cells.
Incorporate studies of pulmonary nervous and lymphatic structure and function in *ex vivo* lung bioengineering.
Strong focus must be placed on understanding immunomodulatory and other mechanisms of cell therapy approaches in different specific preclinical lung disease models.
Improved preclinical models of lung diseases are necessary.
Disseminate information about and encourage use of existing core services, facilities, and web links.
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 NHLBI Production Assistance for Cellular Therapies (PACT), NCRR stem cell facilities, GMP Vector Cores, small animal mechanics, and computed tomography scanner facilities at several pulmonary centers.
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 where 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 be initially studied.
Develop uniform criteria for outcome measures and clinical assessments in cell therapy trials and in patients who receive engineered tracheal implantations or lung implantations 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 NCRR/NIH Center for Preparation and Distribution of Adult Stem Cells (MSCs; http://medicine.tamhsc.edu/irm/msc-distribution.html), coordination among multiple centers, and 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 biologic materials such as lung tissue, bronchoalveolar lavage fluid, blood, etc. 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.

(Continued)

Table 2. (*Continued*)

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 ARDSNet or ACRC, 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 MSCs in graft vs. 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 stem cell medical tourism and be familiar with the resources available to patients, caregivers, and all involved health care professionals on the websites of respiratory disease and patient advocacy groups as well those of the leading stem cell societies, the International Society for Cellular Therapy (ISCT), and the International Society for Stem Cell Research (ISSCR).

Definition of abbreviations: 3D = three-dimensional; ACRC = Asthma Clinical Research Centers; CRISPR = clustered regularly interspaced short palindromic repeats; EPC = endothelial progenitor cells; ESC = embryonic stem cells; GMP = good manufacturing practice; iPSC = induced pluripotent stem cell; MSC = mesenchymal stromal cells; NCRR = National Center for Research Resources; NIH = National Institutes of Health.

The growing problem of stem cell medical tourism in the United States and globally was also addressed. This is a significant issue that will require 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. A statement prepared by the American Thoracic Society (ATS) Respiratory Cell and Molecular Biology Assembly Stem Cell Working Group was included in the conference program and discussed further at the conference. An updated version of that statement is now posted on the ATS website at http://www.thoracic.org/members/assemblies/assemblies/rcmb/working-groups/stem-cell/resources/statement-on-unproven-stem-cell-interventions-for-lung-diseases.pdf and is included as an online supplement to the conference report.

Methods

The conference was divided into five sessions, each featuring a plenary speaker, research talks presented by leading international investigators, and a panel-led debate and discussion. There was a particular focus on talks featuring up-and-coming junior investigators. As such, each session featured two research presentations given by junior investigators selected by the conference organizing committee and also two talks by junior investigators chosen by a competitive abstract review blinded for both authors and institutions. An expanded number of trainee travel awards supporting both oral and poster presentations by junior investigators and trainees was provided. This included a new trainee travel award specifically targeting women and underserved minority participants. A continued feature of the 2015 conference was a session devoted to career development led by representatives from the Lung Division of the NHLBI. This session also featured a mentoring lunch with junior investigators and trainees having one-on-one or small-group time with senior investigators and a dedicated forum for the challenges and need for increased women and diversity in the field. A further continued successful feature of the conference was the preconference hands-on training session. These have all been highly successful additions to the conference and will continue to be included in future conferences. The conference also included an expanded vibrant poster session. The complete conference program and list of speakers, oral presentation abstracts, poster abstracts, trainee travel award winners, organizing committee, sponsors, and attendees can be found in the online supplement.

The conference report is a summation of the research presentations and accompanying discussions. Each section was written by the moderator of that particular section, with introduction and conclusions written by the conference co-chairs, Drs. Wagner and Weiss. Drs. Wagner and Weiss collated and edited the final sections to produce the completed draft. The information in Tables 1 and 2 was based on comparable tables in previous conference reports and was updated with contributions from each author on the basis of discussions that occurred at the conference.

The conference was accredited and monitored by the University Of Vermont Office Of Continuing Medical Education, which received Accreditation with Commendation from the Accreditation Council for Continuing Medical Education for their efforts to provide evidence-based education that is free from commercial influence.

In keeping with the Accreditation Council for Continuing Medical Education Standards of Commercial Support, the following safeguards were implemented for this program to ensure an environment free of commercial bias: Speakers were sent faculty letters instructing them on the Standards of Commercial Support, and each speaker signed a disclosure and attestation statement identifying possible conflicts of interest and attesting that they would provide evidence-based research/education free of bias. Four faculty members were identified with conflicts. Two of these conflicts were resolved. Two were employees of a commercial interest speaking on research related to their companies' interests; as such, no credit was awarded for their lectures. Their information was deemed worthy of participation in the conference because of the importance of discovery and sharing research. However, because their employment created a conflict that could not be resolved, credit was withheld from their lectures.

Participants were notified of the presence of commercial support, the lack of credit for the two lectures, and the disclosures of the faculty by a notice in the front of the syllabus packet. In addition, a disclosure slide was shown at the start of each day. This allowed the audience to be a critical judge of the information presented and make their own conclusions about the data that were shared.

Session 1: Advances and Controversies in Tracheal Bioengineering

Clinical transplantation of a tissue-engineered airway almost a decade ago (7) spurred huge interest and a number of parallel investigations. Complex airway replacement cases are infrequent, but clinically challenging, and often fatal without innovative solutions. Lessons learned from early applications, approved as compassionate use for single patients, are broadly relevant to respiratory system regenerative medicine. In the current era, scientific underpinnings, ethical review, and clinical results are being rigorously scrutinized. The first evening of the Conference examined the field of tracheal bioengineering and focused on the limitations of current approaches and the promise of new strategies.

Professor Martin Birchall, University College London, provided a review of the field and traced the history of tracheal replacement surgery. He highlighted the importance of historical lessons, such as the Neville prosthesis (8, 9); discussed recent approaches, including aortic autografting (10) and two-stage chimeric allografts (11); and reviewed his clinical experience, including several fascinating individual cases. In general, the initial approach of reseeding epithelial and mesenchymal cells into a decellularized trachea and culture in a bioreactor before implantation have been replaced, in part due to clinical urgency, by use of a decellularized scaffold concurrently seeded with freshly isolated bone marrow mesenchymal and airway epithelial cells. Postoperative courses were recounted, and Professor Birchall then shared lessons learned during development of RegenVOX, a planned prospective clinical trial of tracheal replacements (ClinicalTrials.gov identifier: NCT01977911).

Professor Emmanuel Martinod, Assistance Publique Hôpitaux de Paris, described clinical experience with aortic homografting for tracheal or bronchial replacement, while also providing a complementary view of overall progress. Aortic homografting stemmed from decades of work by Dr. Carpentier, a father of contemporary cardiac surgery. The size, elasticity, and infection resistance of the aorta are counterbalanced by its tendency to collapse, a shortcoming managed with intraluminal stenting. The clinical surgical experience was preceded by step-wise rabbit and sheep studies with autografts and allografts (12). Further experimental work documented the superiority of cryopreserved over decellularized or glutaraldehyde-fixed grafts (13). Aortic allografts have promise to allow lung- or lobe-sparing resections of the major bronchi as well as tracheal replacement. The successful use of a cryopreserved human aortic allograft supported by a custom-designed silicon/nitinol stent for a lobe-sparing sleeve lobectomy operation of a patient with cancer was described. Multicenter studies of this approach are underway in the TRACHBRONCHAR study (ClinicalTrials.gov identifier: NCT01331863) being conducted by the French Group for Airway Transplantation.

Dr. Glenn Green, University of Michigan, a pediatric otolaryngologist, presented his work on the management of tracheobronchomalacia. The traditional approach is tincture of time, minimally invasive or invasive mechanical ventilation, tracheostomy, various reconstructive surgical procedures, and internal stenting. Dr. Green detailed the confluence of advances in complementary fields supporting development of a stabilizing, bioresorbable exoskeleton for abnormally collapsible airways. The process was first developed in a porcine model (14) and is based on imaging and 3D reconstruction of the lesion, followed by virtual design and 3D printing of the external splint and finally testing of physical properties. Dr. Green reported on the successful use of the splinting procedure in three clinical cases meeting U.S. Food and Drug Administration (FDA) emergency use exemption criteria (15). One question that arose was whether the external stents had erosion potential similar to early prostheses, which seemed to be minimal due to the nature of the stent material. Future directions include seeking generalized FDA approval; defining indications, contraindications, and complications; outcome after bioresorption; long-term tissue effects; and endoscopic approaches.

Based on prior success with vascular grafts (16), Dr. Laura Niklason, Yale University, presented current work on trachea, first outlining criteria for success, including lateral rigidity, longitudinal flexibility, easy implantation, avoiding immunosuppression, development of a mature respiratory epithelium, and host integration/vascularization. The innovative approach first encases nitinol struts in polyglycolic acid, which are then seeded with vascular smooth muscle cells and cultured in a bioreactor. During bioreactor culture, the polyglycolic acid dissolves and is eventually replaced with a complex vascular smooth muscle cell–derived matrix. Decellularization creates the final product in which physical and biochemical properties were characterized. When implanted orthotopically in rats and vervet monkeys, ingrowth of respiratory epithelium and vascularization was observed, although not uniformly, pointing to areas for further development.

It is broadly appreciated that successful airway grafts will require an epithelial lining to prevent secretion retention, granulation tissue formation, and restenosis. Professor Samuel Janes, University College London, presented the work of his group toward expanding epithelial cell populations from small initial biopsies and highlighted the need for high seeding densities in either synthetic or native tissue constructs. Conventional culture methods appear insufficient for the task, but culture with irradiated feeder cells in the presence of a rho kinase inhibitor, as previously reported (17), enabled dramatic expansion of cells that remained diploid and competent for mucociliary differentiation in an orthotopic tracheal graft model. There was spirited discussion about identification of somatic airway epithelial stem cells and the need for accurate models of progenitor–progeny relationships.

Session 2: Endogenous Lung Progenitor Cells

A breadth of respiratory epithelial progenitor cell types were discussed in this session, including submucosal cells, basal cells, neuroendocrine cells, and nasal basal cells. Comparisons were made between the stem cell properties of progenitors during pulmonary homeostasis and disease and the initiation of cancer. A universal theme of the presentations was the need for developing methods to evaluate and understand the signaling events that maintain progenitors in their naive state, including their niches.

The session began with Dr. John Engelhardt, University of Iowa, providing an overview of the respiratory tree and corresponding epithelial progenitor niches, followed by his work detailing how

canonical Wnt signaling (herein referred to as Wnt signaling) within the adult proximal airway stem cell compartments regulated progenitor expansion after injury. Two niches in the proximal airway were evaluated: the intercartilaginous zones of surface airway epithelium (SAE) and the tracheal submucosal gland (SMG) (18, 19). During development, Wnt signaling induces Lef-1 and decreases Sox2 to initiate primordial glandular placode formation, required for SMG development. Using Wnt-specific lacZ and green fluorescent protein (GFP) reporter mice, Wnt-active SMG cells were confined to specific niches in the SMG and SAE during homeostasis. In response to naphthalene injury, SMG and SAE proliferation increased significantly; however, SMG was fourfold greater. Wnt-active SMG cells were also found to be clustered with label-retaining cells, whereas label-retaining cells were not Wnt activated. SMG cells were described as distinct from a basal cell phenotype, and grafting of SMG cells demonstrated their multipotent nature and ability to differentiate to both surface epithelium as well as submucosal structures. Taken together, these data suggest that regulation of Wnt signaling plays an important role in glandular stem cell lineage specification and proliferation. However, the precise regulation of Wnt signaling in glandular stem cells by ligand expression or otherwise is unclear, highlighting the necessity for studying epithelial interactions with the mesenchyme and their specific niches.

Dr. Renat Shaykhiev, Weill Cornell Medical College, focused on basal cells and how the environment influences global gene expression signatures and how these signatures relate to cancer. He first presented a comparison of global gene expression patterns from proximal to distal airway epithelium obtained from healthy nonsmoking individuals and healthy smokers to define region-specific pathogenesis associated with disease. Smoking is the major risk factor for cancer and induces epithelial hyperplasia accompanied by genomic alterations. Oxidant stress was identified as a regulator of abnormal specification of distal to proximal epithelium. Previous genomic basal cell studies identified genes unique to airway basal cells and highlighted similarities between ESC genetic signatures, lung cancer, and basal cells analyzed from the lungs of smokers (20, 21). A significant application of these studies applies the knowledge gleaned from these genetic signatures to identify precancerous lesions as well as personalize treatment for disease.

Concepts and conflict associated with the theory of cancer stem cells were presented by Dr. Mark Onaitis, Duke University, followed by a review of his recently published work determining the cell of origin and subtype of K-ras–induced lung cancer (22, 23). He began by posing the question "What do we call stem cells in cancer?" The first hierarchical organization of cancer and the existence of a stem cell were proposed in 1997 by Bonnet and Dick, describing human acute myeloid leukemia (24). Early studies used markers such as CD133, aldehyde dehydrogenase (ALDH), and Hoechst dye efflux (termed side population or SP) to identify cancer stem cells (25–27), and more recent studies have demonstrated the importance of genotype in cancer stem cell–initiating capacity (28, 29). This theory is controversial, because the assays used to evaluate initiating cell potential typically involve severely immunocompromised mice, in which many cells will form tumors (30). K-Ras activation in club cell secretory protein (CC10) (Scgb1a1)–expressing epithelium leads to adenocarcinoma in a subset of type II alveolar cells (ATIIs) and hyperplasia in the bronchioalveolar duct region in the absence of bronchiolar tumors (23). These studies were designed to assess the difference between bronchiolar and alveolar epithelium, specifically with regard to Notch signaling. Xu and colleagues performed an elegant study, modulating Notch or Sox2 along with K-Ras in CC10-expressing epithelium (28). Inhibition of Notch signaling strongly abrogated alveolar K-Ras–induced adenocarcinoma formation, and activation of Notch in bronchiolar cells promoted K-Ras–induced tumor formation. Sox2 expression reduced the expression of Notch transcripts. These studies defined novel cell-specific mechanisms by which Sox2-regulated Notch signaling regulated tumor initiation and tumor progression in K-Ras–activated respiratory epithelial cells.

Trainee travel award winner Dr. Laura Succony, University College London, presented her recent studies using lineage tracing of leucine-rich repeats and immunoglobulin-like domains (LRIG) in the lung. LRIG proteins regulate signal transduction and are highly expressed in skin and gut epithelial stem cells (31). LRIG1 is known to be an endogenous inhibitor of receptor tyrosine kinases, including epidermal growth factor receptor. In the lung epithelium, the expression of LRIG1 is down-regulated by tobacco smoking and further down-regulated in lung squamous cell carcinoma (32). Basal cells are the hypothesized origin of squamous cell carcinoma; however, it is unknown whether or not they express LRIG1. Using lineage reporter mice, LRIG1-expressing cells were labeled with GFP and sorted by flow cytometry and localized with immunofluorescent staining. LRIG1 expression did not enrich a basal cell population, and expression was limited to 40% of basal cells. However, the LRIG1-positive basal cells were enriched for colony-forming unit potential, and clonal expansion was observed in a spheroid assay. LRIG1 therefore labels a proliferative subpopulation population of basal progenitor cells in lung, similar to skin and gut, further demonstrating heterogeneity within lung epithelial stem cell populations.

Dr. Christina Barkauskas, Duke University, reviewed her recently published work (33–35) on the concept of epithelial mesenchymal crosstalk in lung fibrosis and alveolar homeostasis. Although the FDA has recently approved drugs that slow the progression of pulmonary fibrosis, the underlying mechanisms of disease remain unclear. Understanding the mechanisms of disease will help to design more effective therapies. Dr. Barkauskas and colleagues identified that ATIIs were stem cells in the adult lung. These cells are able to self-renew and give rise to additional ATII and type I cells. They were the first to demonstrate that growth and differentiation of these cells occurred more readily when cultured with platelet-derived growth factor receptor-α lung mesenchymal cells, characterized as lipofibroblasts (34), illustrating the importance of mesenchymal cells in alveolar biology. The ability of type I alveolar epithelial cells (AECs) to "dedifferentiate" to an ATII lineage in response to pneumonectomy and tissue regrowth was presented and challenges existing paradigms (35). Last, she presented data linking telomere dysfunction and alveolar stem cell senescence (33). Telomerase mutations have been identified in patients with heritable pulmonary fibrosis. Shortened telomeres, due to deletion of the telomeric repeat binding

factor 2 (Trf2) in AECs, resulted in decreased colony-forming ability *in vitro*, and ATIIs underwent senescence and acquired a proinflammatory phenotype *in vivo*. In response to bleomycin challenge, deletion of Trf2 in AEC resulted in 100% mortality. Taken together, these studies illustrate the importance of maintenance of ATII stem cell function in lung repair and regeneration.

Dr. Mitsuru Morimoto, Riken Center Japan, presented his studies dissecting the role of Notch signaling in the three-dimensional structure formation of the neuroepithelial body (NEB) (36). NEBs are clusters of neuroendocrine (NE) cells, which are frequently observed at the bifurcation points of branching bronchioles. These NEBs have been characterized as a progenitor niche for multiple cell types (37). Stepwise deletion of Notch receptors from the developing lung epithelium, combined with Sonic hedgehog–Cre used to lineage label NE cells, revealed that three Notch receptors coordinately regulated the abundance of NE cells and the size of the neuroendocrine body (NEB) via interactions between the NE and Stage-specific embryonic antigen-1^{+}, peri-pNEB, intracellular domain of $NOTCH^{+}$, CC10 cells, cells adjacent to the NE in the NEB. Two-photon microscopy tracked labeled NE cells in E13.5 explanted lung organ cultures as they migrated to the bifurcations. The conclusion from these studies was that Notch signaling regulates a stepwise developmental process in the formation of the NEB and NE cells localized at bifurcation points. Future studies will be necessary to dissect the molecular mechanisms underlying NEB size regulation by Notch signaling.

Trainee travel award winner Dr. Dawn Bravo, Stanford University, translated what has been discovered with regard to lung basal epithelial progenitors to the murine and human nasal epithelium. The human nasal epithelium harbors an accessible source of progenitors with potential for cell therapy, the nasal epithelial basal cell (NEBC) (38). NEBC progenitors were found to express both p63 and Keratin5 and localized to the nasal floor crypt. The isolated p63 NEBCs were found to be highly proliferative. However, the number of these cells decreased with age and mitotic index. After sulfur dioxide or trichloroacetic acid injury, lineage-tracing analyses demonstrated that the NEBC completely regenerated the upper airway epithelium. Both human and murine NEBCs also formed nasospheres at a clonal level. This exciting work indicates the potential redundancy in airway stem cell niches, which is an important concept for tissue regeneration and repair.

Drs. Brigitte Gomperts, Mattell Children's Hospital UCLA, Susan Reynolds, Nationwide Children's Hospital, and Barry Stripp, Cedars Sinai, led the panel discussion and summary. They raised important questions, including: How do different injury models and aging impact the different stem cell populations? Can and should we use endogenous progenitor cells before we know everything about them? Although it may seem counterintuitive to study a small population of progenitors as a major contributor to disease pathologies and repair, the innate characteristics that define them as a progenitor cell highlight them as ideal candidates. These characteristics include the ability to self-renew or divide asymmetrically and give rise to a clonal stem cell offspring as well as a second daughter that can amplify itself exponentially (39). Last, the multipotent differentiation capacity of epithelial progenitors highlights them as an ideal candidate to potentially influence abnormal remodeling and subsequent function, either through influencing surrounding mesenchymal and vascular cells or their direct involvement in the remodeling process. As an undifferentiated cell population, reactivation of developmental programs may be relevant to the activation of adult stem cells during disease. A comparison of homeostatic function versus repair or disease phenotypes, as well as epithelial–mesenchymal cell interactions, will be paramount to the development of therapeutics to treat disease, promote tissue regeneration, and develop functional graft systems.

Session 3: ESCs, iPSCs, and Lung Regeneration

Recent progress and increasing sophistication in the methodologies to generate and characterize lung progenitor cells from ESCs and iPSCs have opened new perspectives to study lung development and regeneration. This session was moderated by Dr. Wellington Cardoso, Columbia University, and the lectures encompassed diverse topics ranging from technical challenges in genome editing and in obtaining mature cellular phenotypes to novel applications of reprogramming to overcome aging of endogenous progenitors, among others. The major recurring themes from this session were (*1*) the development of the next generation of tools for researchers in determining factors necessary for directed differentiation of iPSCs (e.g., screening platforms, lung lineage reporters, etc.); and (*2*) the new opportunities afforded by genome editing technologies (clustered regularly interspaced short palindromic repeats-caspase [CRISPR-Cas] and transcription activator-like effector nucleases [TALEN]) for disease modeling, drug development, and potential cell-based therapies in the future.

Dr. Paul Gadue, University of Pennsylvania, presented an overview of the field of human pluripotent stem cell differentiation into lung lineage cells, highlighting the advancements, pitfalls, and challenges the field faces. He commented on the potential therapeutic perspectives opened by the feasibility of using CRISPR-Cas gene editing–based approaches. However, difficulties remain in the management of this technology, given the long time and labor-intensive requirements and the challenge of generating fully differentiated lung-specific cell subtypes. Although studies from several groups have demonstrated the feasibility of generating NK2 homeobox 1 $(NKX2.1)^{+}$ lung precursors (40–42), establishing robust protocols to generate mature end-stage cells has proven more challenging. To help overcome this problem, he described a novel robotic screening platform, developed in collaboration with Dr. Jan Jensen (Cleveland Clinic) to better define in a step-wise manner the inductive signals that drive lung development from human pluripotent stem cells. As proof of principle, he presented evidence that this platform could define signaling pathways that regulate patterning of early endoderm from human ESCs. These results, derived completely from a software algorithm to optimize for the induction of anterior $(SOX2^{+})$ or posterior $(CDX2^{+})$ cell fates, agreed well with our knowledge of signaling in endoderm patterning from various model organisms. He proposes that these screening platforms can help identify programs that generate specific lung cellular phenotypes.

Dr. Gadue also discussed the difficulties arising from species-specific differences in programming and highlighted the importance of using the human pluripotent cell system when mouse models do not replicate the human biology and disease. He illustrated some fundamental differences between human and mouse developmental programs. He showed that although in mice the transcription factor GATA6 is dispensable for gut tube formation, it is crucial for induction of definitive endoderm in human pluripotent cells (Paul Gadue, unpublished). He emphasized the importance of better understanding the differences in the various model systems available.

Dr. Chad Cowan, Harvard University, presented a comprehensive review of genome-editing technologies (TALEN, CRISPR/Cas9) and cell-based approaches for disease modeling in ESCs and iPSCs (43). His laboratory is using these technologies to advance our knowledge on disease mechanisms and therapeutic targeting of metabolic conditions such as type 2 diabetes mellitus and coronary artery disease. He stressed the potential of using these approaches to gain novel insights into the mechanisms and management of pulmonary diseases. He described the overall strategy, emphasizing the importance of linking human genotypes to phenotypes. Key steps included performing human genome editing (CRISPR, TALEN) to introduce disease-associated gene mutations and DNA variants into human iPSCs, followed by using established protocols for induction of differentiation of the engineered human iPSCs into relevant cell types for studies. With the development of these iPSC-based models, functional assays can be performed to gain insights into pathogenic mechanisms relevant to disease. The identification of disease-relevant phenotypes in these human cell–based models ultimately provides the opportunity to perform genetic and drug screens to develop novel therapies. When combined with resources such as genome-wide association studies and the identification of novel DNA variants by exome sequencing, this approach can be used to interrogate relevant questions, such as how naturally occurring human genetic variation influences susceptibility of only some patients to pulmonary disease.

Dr. Finn Hawkins, Boston University, presented his work on the use of TALENs to generate reporters for the study of specific lung cell lineages derived from human iPSC-derived lung progenitors. Dr. Hawkins provided an overview of the recent progress in deriving putative lung epithelium from iPSCs (40–42) and the advantages of using iPSCs to study early aspects of human development, previously inaccessible to researchers. iPSC-based platforms can provide insights into the transcriptional and epigenetic programs that control human lung specification and subsequent patterning into the full repertoire of the cells that constitute the postnatal lung epithelium.

Dr. Hawkins also highlighted the current hurdles in dissecting the genetic program of lung development and using this knowledge to generate clinically relevant, functional lung epithelium from iPSCs. Major issues he presented were the heterogeneity of the cells derived from current directed differentiation protocols, the paucity of lung-specific markers, and the lack of robust functional assays to test efficiency of these protocols.

Dr. Hawkins then presented new unpublished work in which he generated and characterized a TALEN-targeted reporter NKX2.1-GFP iPSC line. He showed that the TALEN-targeted NKX2.1-GFP line allowed identification and purification of lung progenitor cells generated through a directed differentiation protocol. Importantly, global transcriptional profiling of these cells at key stages of the directed differentiation program defined the kinetics of gene expression that leads to lung lineage specification in this iPSC model. The work was developed in collaboration with Drs. Darrell Kotton (Boston University) and Brian Davis (University of Texas, Houston).

Dr. Amy Firth, Salk Institute, described her efforts to develop a robust and reproducible *in vitro* model of lung epithelial cell differentiation and how this model can be used to recapitulate human lung disease in a dish. She discussed the step-wise iPSC differentiation protocol passing through $SOX17^+$ and $FOXA2^+$ definitive endoderm, $SOX2^+$ and $FOXA2^+$ anterior foregut endoderm, with $NKx2.1^+$ emerging as an early marker of commitment to a lung progenitor (44). She used an air–liquid interface to mature these cells to a pseudostratified polarized layer of endodermal-derived epithelial cells. This polarized layer included club cells with $CC10^+$ vesicles, Mucin $5AC^+$ goblet cells, and tumor protein 63^+ basal epithelial cells. The robust multiciliogenesis observed occurred only when Notch signaling was inhibited and was identified by (*1*) the assembly of multiple pericentrin-stained centrioles at the apical surface, (*2*) the expression of transcription factor FOXJ1, and (*3*) the presence of multiple acetylated tubulin labeled cilia projections in individual cells (44).

Dr. Firth also presented some of her most recent data showing a footprint-free approach to gene correction of cystic fibrosis transmembrane conductance factor (CFTR) ΔF508 mutant iPSCs using state-of-the-art CRISPR gene editing technology (45). She was able to show apical cell surface CFTR localization and demonstrate functional correction by recording cyclic adenosine monophosphate–activated and CFTRinh-172–sensitive CFTR currents in isolated epithelial cells from both wild-type and the CRISPR gene–corrected CFTR mutant, not seen in the uncorrected mutant iPSC-derived cells. The CRISPR donor vector was designed to be seamlessly excised using piggyBac transposase. This allowed for increased efficiency of correction without leaving a genomic footprint from the donor vector other than the desired correction of the target ΔF508 mutation. Her results open new perspectives for the use of this approach in cell-based therapy for patients with lung disease. Dr. Firth is now moving forward using iPSC and refining her differentiation model system to identify novel therapeutic targets for lung diseases, such as primary ciliary dyskinesia, which has been difficult to study in a human model from a developmental perspective (46).

Dr. Lily Guo, trainee travel award recipient from Toronto General Hospital, presented on "rejuvenation" of aging endogenous progenitors by reprogramming. Aging is known to be accompanied by a marked decline of stem and progenitor cell function. In the lung, aging has been associated with telomerase dysfunction and reduction of the proliferative and clonogenic capacities of these cells, ultimately leading to impaired tissue maintenance and repair. She described an optimized protocol in which transient doxycycline-mediated induction of iPS reprogramming factors turns aging ATIIs into young ATII-like cells, turning off inductive factors before the pluripotent state. This "interrupted reprogramming" approach enables expansion of a selected mature cell type and preserves the

differentiation potential of the parental population to generate functional progeny. She proposes that this novel approach can potentially address the fundamental issue of how to overcome the aging of available cellular resources and enhance regeneration.

Dr. Mirabelle Ho, trainee travel award recipient from Ottawa Hospital Research Institute, discussed her research findings on the cooperative interaction between iPSC-derived vascular smooth muscle cells (SMCs) and endothelial cells (ECs) in vascular network formation. The work was motivated by the need for better understanding conditions that improve vascular development *in vitro* and endothelialization of lung scaffolds. Dr. Ho presented evidence that the integrity of vascular networks formed in Matrigel through a combination of ECs and SMCs persisted for at least 72 hours, compared with 24 hours when ECs alone were plated. She showed that this holds true for human umbilical vein endothelial cells used in studies to support iPSC-derived SMCs, and for iPSC-ECs. She found that a 3:1 ratio of ECs to SMCs was critical for optimal network formation and stability. Network persistence may also be attributed to increases in expression of genes regulating endothelial function, such as TIE2, CD34, and Kruppel-like factor 2 in ECs and angiogenic genes such as vascular endothelial growth factor. In her discussion, she stressed that vessel stability can often be a function of the ratio between angiogenic angiopoietin Angpt1 and its antagonist Angpt2. The former affords a stabilizing effect, whereas the latter reduces stability. An increase in Angpt1:Angpt2 ratio, observed on coculture of iPSC-SMCs with ECs, favored network stability. She proposes that approaches to revascularize decellularized lungs/organ scaffolds will benefit from the combined delivery of both SMCs and ECs to allow more stable vessels.

The session ended with a lively panel discussion moderated by Dr. Darrell Kotton, Boston University, and Dr. Peter Lelkes, Temple University. A number of the issues and questions described above were addressed in more detail, and directions for focused future studies are summarized in Table 2.

Session 4: Bioengineering Approaches to Lung Regeneration: Julia M. Polak Memorial Session

The fourth session on bioengineering approaches has been named the Julia M. Polak Memorial Session in honor of the many contributions Dame Julia brought to the field. Moderated this year by Dr. Harald Ott (Massachusetts General Hospital, Harvard Medical School), the session addressed several key questions related to the bioengineering of lung tissue for the treatment of end-stage lung disease. In the featured presentation, Dr. Gordana Vunjak-Novakovic, Columbia University, introduced several key concepts of lung engineering based on decellularized scaffolds. She showed the design and construction of a moderate-throughput bioreactor system enabling culture of multiple organ scaffolds under controlled biomimetic conditions. As a group, they have generated substantial, but sometimes anecdotal, data on decellularization and recellularization. Such an approach as what Dr. Vunjak-Novakovic presented would enable direct comparison of different culture protocols and conditions and might enable us to build on each other's data. In the second part of her presentation, Dr. Vunjak-Novakovic shared her recently published experimental results on site-specific seeding of microvolumes into rodent lung scaffolds via control of droplet size (47). Site-specific delivery of cells, growth factors, and small molecules is highly relevant to our community, because it may enable the control of site-specific tissue formation and maturation on the acellular lung scaffold. To date, the generation of respiratory epithelium in appropriate proximo-distal distribution has proven to be challenging. In the third part of her presentation Dr. Vunjak-Novakovic discussed a novel approach to selective *in vivo* decellularization in large animals. In this approach, the contralateral lung and/or extracorporeal membrane oxygenation can be used to support the animal while the right upper lobe is selectively intubated and decellularized via perfusion through the selected pulmonary artery.

In the second feature presentation, Dr. Bryan Brown, University of Pittsburgh, shared his work on extracellular matrix (ECM) scaffolds as an inductive template for *in vivo* tissue engineering. They have examined the immune response to implantable ECM scaffolds with a special focus on the macrophage polarity and role in tissue remodeling (48, 49). Dr. Brown highlighted the fact that mononuclear cells are the hallmark of early ECM remodeling and that the macrophage phenotype is a predictor of histomorphologic outcome of matrix implants. Dr. Brown went on to explore the role of ECM age and ECM degradation products in the programming of macrophages between M1 and M2 phenotypes (50). Dr. Brown closed his talk suggesting that the sourcing of tissues for decellularization, and control of host response in any particular graft, will be key to achieving constructive graft remodeling and tissue homeostasis postimplantation.

Dr. Jenna Balestrini, Yale University, from Dr. Laura Niklason's group, presented data on the comparative biology of decellularized lung matrix. Several publications have provided some degree of compositional analysis of decellularized lung matrix (51–56). Unfortunately, direct comparative data between protocols are somewhat limited, and findings and conclusions are not always consistent between different research groups. In her presentation, Dr. Balestrini discussed a modified perfusion decellularization regimen currently applied by Dr. Niklason's group, which provides optimized matrix preservation. This protocol is based on sodium deoxycholate and Triton X perfusion via the lung's vasculature. Dr. Balestrini also explored two topics relevant to clinical translation. She showed species-specific differences in decellularization and their influence on recellularization with species-matched and unmatched endothelial cells. With the understanding that the ECM serves as a reservoir for growth factors potentially relevant to cell differentiation and tissue maturation, and knowing that some of these signals will be species specific, they will have to put more weight on gaining a deeper understanding of species-specific cell fate on ECM scaffolds.

Dr. Charlie Xi Ren, Massachusetts General Hospital, Harvard Medical School, from Dr. Harald Ott's group, presented his work in bioengineering a functional pulmonary vascular bed using human iPSC-derived cells (57). Dr. Ren discussed characteristics of the vascular bed of acellular lungs and their implications on seeding techniques. Decellularization leads to loss of barrier function and hydrostatic perfusion pressure across the capillary bed (58). In line with these findings, Dr. Ren showed that bidirectional seeding from the artery and vein is necessary to reach the entire vascular bed. In translating his findings from a primary cell approach to

iPSC-derived cells, Dr. Ren showed that two distinct types of vascular cells, CD140b-expressing pericytes and CD31-expressing endothelial cells, could be derived from undifferentiated iPSCs using a single protocol of directed differentiation. He also described a novel organ culture protocol that directed the resulting recellularized constructs from an angiogenic phase to a stabilization phase and thereby enabled functional maturation. These lungs showed high levels of endothelial coverage, decreased thrombogenicity, partial reconstitution of barrier function, and perfusability *ex vivo* and *in vivo*. Last, Dr. Ren showed how this technology was successfully applied to human-scale lungs, highlighting the translational importance of his results for the field of lung bioengineering.

Collin Stabler, Temple University, from Dr. Peter Lelkes' group, shared data on anatomical and integrin-specific reendothelialization of acellular rodent lungs. They have focused on physical methods to optimize cell delivery, including an integrin-based approach to improve endothelial cell adhesion and maturation. In rat lungs, endothelial delivery was superior when acellular lungs were kept in the supine position compared with lungs hanging vertically. In the second part of his presentation, he examined the integrin-specific binding of rat microvascular endothelial cells to ECM proteins, lung matrix slices, and acellular whole lungs and concluded that a5b1, a2b1, and a1b1 integrins mediate endothelial cell adhesion on acellular ECM. These results complement earlier findings of Dr. Lelkes' group highlighting the importance of understanding both the integrin receptor of seeded phenotypes and the cell-binding motifs of acellular lung matrix (59).

Travel award recipient Dr. Andrew Le, Yale University, from Dr. Laura Niklason's group, shared advanced methods for endothelial and epithelial cell seeding and culture for whole-lung tissue. In his experiments, Dr. Le examined the phenotypical fate of primary endothelial cells, and confirmed the stability of their phenotype, including important functional features such as expression of endothelial nitric oxide synthetase and prostaglandin I2 synthase after seeding onto acellular lung matrices. These findings resulted in sustained perfusability after orthotopic implantation in rats.

Dr. Thomas Petersen, United Therapeutics Regenerative Medicine, shared recent advances in moving lung tissue engineering closer toward its clinical application from an industry perspective. Dr. Petersen shared results of successful implementation of fully automated perfusion decellularization, showing how this platform could move toward a future device production phase. He went on to discuss issues related to scalable cell expansion, cell seeding of porcine scaffolds, and a novel systematic tissue analysis technique that enables quantitative analysis of human-scale tissue.

Dr. Franziska Uhl, University of Vermont, from Dr. Daniel Weiss' group, shared her results on how stiffness and stretch influence cell fate during lung culture and might translate to biomimetic culture conditions. In the first part of her presentation, she shared data on Wnt/b-catenin–mediated lung repair in a slice model of intermediate-term *ex vivo* lung culture and the difficulty in keeping healthy, native lung slices along for long time points (60). She shared unpublished results on the influence of two-dimensional and 3D stretch on gene expression profiles of bronchoalveolar stem cell, type 2 alveolar epithelial cell, epithelial cell adhesion molecule^{+} epithelial progenitor cells, and mesenchymal stem cells. She also showed that cyclical stretch in a high-throughput 3D model of human lung matrix culture promoted high levels of surfactant protein-C expression in primary epithelial cells, highlighting the importance of physiologic biomechanical signals in maintaining mature cellular phenotypes, and potentially developing advanced tissue function (61).

In the final talk of the bioengineering session, travel award recipient Qi (Eric) Tan, Mayo Clinic, shared interesting data on developing a self-assembling vascularized airway organoid as a step toward lung regeneration. Dr. Tan showed self-assembly and organization of constructs derived from primary mesenchymal, epithelial, and endothelial cells. The resulting constructs matured over several days in culture and showed organization similar to what has been shown in liver constructs. He went on to explore the role of Notch signaling on epithelial phenotype and examined the mechanical properties of the resulting constructs. In a final transplant experiment, Dr. Tan showed feasibility of implantation and engraftment of the self-assembled constructs under the kidney capsule in rats.

The session concluded with a panel discussion on current progress and future directions led by Drs. Christine Finck (Connecticut Children's Medical Center), Peter Lelkes, (Temple University), Laura Niklason (Yale University School of Medicine), and Angela Panoskaltsis-Mortari (University of Minnesota). As a group, we identified the need for a concerted regulatory strategy to enable the development of translational strategies for iPSC-derived constructs, with special considerations on autologous versus allogeneic (banked, matched) cell sourcing. We discussed the need for preclinical models enabling maturation and testing of necessary higher-level function of regenerated constructs. From a developmental perspective, epithelial maturation and site-specific tissue formation will be key areas of research in the coming years. From the very engaging comments from the audience and the panelists, it became clear that the jury is certainly still out on which cell type and scaffold will ultimately enable first-in-man implants. In summary, the session showed the substantial progress the lung-regeneration community has made over the past years. The acellular lung matrix has become an important platform for many disciplines interested in repair and regeneration, and there is continued support to further pursue this approach.

As part of this session, talks were also presented by Drs. John West, University of California–San Diego, and Graham Parker, Wayne State. Dr. West, a pioneer in lung physiology and comparative lung physiology, gave a fascinating presentation on the evolution and function of avian lungs. Potential applications to *ex vivo* lung engineering were discussed. Dr. Parker, editor of *Stem Cells and Development*, gave an educational presentation on the good, bad, and ugly in publishing stem and progenitor cell studies.

Session 5: Careers in Stem Cells, Cell Therapies, and Lung Bioengineering

After the successful introduction of this session in the 2013 conference, this year's conference hosted two speakers from the National Institutes of Health to provide an update on academic careers in the field, with a special focus on fostering early career development.

The presentations given by Dr. Timothy Moore, Chief of the Lung Biology and Diseases Branch at the National Institutes of Health (NIH)/NHLBI and Dr. Ghenima Dirami, Scientific Review Officer, Lung Injury Repair and Remodeling Study Section, Center for Scientific Review, NIH, were structured around the different grants and training opportunities offered by the NHLBI. Dr. Dirami first gave an overview of the NIH's mission as a funding body and how to navigate the grant process. Importantly, she explained the general structure of the NIH and outlined the different institutions and centers within the NIH and their corresponding applications—including the Small Business Innovation Research and Small Business Technology Transfer awards. She then provided an excellent step-by-step guide to submitting a grant and how to select the appropriate study section, which she identified as a key step in a successful grant process. She explained the general structure of the peer-review process, including the different divisions and integrated review groups, and provided important insight into study sections and the official review criteria used to score applications. The NIH Reporter system was also highlighted as a tool for young investigators to access abstracts from previous successful grant applicants and to gain insight into the types of grants that have been previously funded by the different study sections. An overview of the resubmission policy and process after unsuccessful submissions was also provided. Finally, a general overview of the mechanisms and opportunities available for new and early-stage investigators was discussed. This included the various career-development awards and fellowships available at the NIH, and the NIH Early Career Reviewer Program, which aims to train and educate early scientists to become critical and well-trained reviewers.

Dr. Moore's presentation provided more detail on the training mechanisms available at the NIH for those pursuing academic careers in lung stem cells, cell therapies, and bioengineering. In addition to its mission to advance scientific knowledge and human health, a key mission of the NIH is to promote the development of the next generation of researchers and physician-scientists. He gave a historical overview of the T32 training grants in lung biology, including a breakdown of the number of predoctoral and postdoctoral students who have been or are currently been trained using this mechanism. In addition, he provided statistics on the high success rates of T32 trainees who transition to an F32 or a K award. This aspect was discussed in more detail in the discussion session. The average number of years that postdoctoral students are training has increased dramatically over the past decade, but the NIH has not adjusted the number of years at which a trainee can be on a T32/F32 fellowship (total of 3 yr) or the number of years that a trainee can be a postdoctoral fellow before applying for a K award (4 yr). This highlights a significant training gap in the NIH's program, where there are few mechanisms to sustain the training period of young scientists, outside of applying for extremely limited and competitive private foundation grants. Although the NIH is aware of this gap, there are currently no upcoming changes or programs to address this gap in training. This is further complicated by the increasing age of first-time research grant awardees, which has increased by about 3 years over the past decade. Although K awardees have higher chances of success in receiving their first research grant, the aforementioned problems remain a significant hurdle for the future (62, 63). It was therefore a recommendation of this session that the NIH continues to monitor its training mechanisms and to identify areas where change could be most effective. Dr. Moore also discussed the K99/R00 award program in detail and discussed the special initiatives for training physician-scientists under this mechanism. This initiative would provide a longer period of support during the R00 phase, a stronger enforcement of the minimum 75% research effort, and sufficient salary support. He also highlighted the various potential leverage points for young investigators, including alignment with the current consortia and programs. He concluded his talk by discussing strategies for writing the training plan in young investigator grants.

The discussion that followed focused on the realities of training the next generation of scientists. Although the NIH has a set of mechanisms in place, and there is no doubt that getting on this track of funding enhances your chance of success, the landscape is changing. There seem to be some critical mechanisms missing in terms of training the next generation of scientists, including training mechanisms for dual business-scientist or business-physician-scientists. There is significant funding available for Small Business Innovation Research and Small Business Technology Transfer grants, yet there are no training mechanisms available at the NIH for scientists or physician-scientists to obtain business or entrepreneurial training in parallel to their clinical or research programs. Thus, a second recommendation coming from this session was that the NIH should look at initiating new training programs to fill the current gaps in knowledge and training timelines for the next generation of scientists and physician-scientists.

After the formal presentations and the poster discussion session, all conference participants were invited to participate in the Women's and Diversity reception. For the second year, this successful networking and career session was again led by junior investigators Drs. Darcy Wagner, Comprehensive Pneumology, Munich, Germany and Sarah Gilpin, Harvard University, who moderated a meaningful discussion of what diversity means in the context of research and innovation. This year's panel was composed of senior leaders in the field, who each provided a narrative of their personal experiences and advice on navigating academic careers. Unique and engaging perspectives were provided by Drs. Susan Reynolds, Gordana Vunjak-Novakovic, Bernard Thebaud (University of Ottawa), Angela Panoskaltsis-Mortari, and Luis Ortiz (University of Pittsburgh). The lively and informative discussion touched on many important elements of diversity in scientific careers.

The recurrent advice to build a supportive team of mentors was evident throughout the discussion, as panelists urged more junior investigators to find mentors for various aspects of their professional and personal lives. All of the senior investigators reiterated that one mentor is likely not enough to enhance success and that mentors are not limited to those at your own institution or even necessarily in your exact field of research. A uniqueness of experience was emphasized, reminding young investigators that success is defined and can be accomplished in diverse ways. On the basis of positive feedback, the organizers are hoping to expand this session to a full evening program for the next conference. It is clear

that there is an ongoing need for a forum to address questions of mentorship, diversity, and career development. This important opportunity for researchers from diverse backgrounds to interact and develop supportive relationships will remain a critical component to future conferences and will provide an opportunity for young trainees to ask questions and for senior investigators to reflect on their mentorship practices.

Session 6: Endothelial Progenitor Cells, MSCs, and Cell Therapy Approaches for Lung Diseases

The main advance since the last edition in 2013 was the initiation/completion of early-phase clinical trials to test the feasibility and safety of MSCs in acute inflammatory diseases. Other highlights included presentations on the therapeutic potential of human amnion epithelial cells (hAECs), the quest for cell-free–based therapies via microvesicles, superior bioengineering approaches, and a better understanding of resident lung MSCs and distal epithelial progenitor cells in health and disease.

Dr. Duncan Stewart, University of Ottawa, a pioneer in translating cell therapies into the clinic for cardiopulmonary diseases, presented very preliminary data on the use of MSCs in his most recent Cellular Immunotherapy for Septic Shock (CISS) phase I trial. Dr. Stewart introduced the business concept of "headroom," which estimates the potential value of innovative new therapies on the basis of a patient's quality of life improvement. He highlighted the strong economic rationale for cell therapy in severe sepsis on the basis of its large headroom (high mortality/high disease burden) compared with myocardial infarction (low mortality/mid-to-high disease burden).

Dr. Stewart then presented the scientific rationale for testing MSCs in septic shock, on the basis of (*1*) their established antiinflammatory and immune-modulatory function (64), (*2*) a systematic review of all preclinical MSC studies in sepsis models (n = 21) demonstrating benefit in reducing overall mortality (L. McIntyre and colleagues, unpublished results), and (*3*) a systematic review and metaanalysis of more than 100 patients enrolled in clinical trials with MSCs for various indications suggesting safety of MSCs (65).

The current CISS study is a phase I single-center, open-label safety and dose-escalation trial of allogeneic bone marrow–derived MSCs in septic shock in up to nine patients with an observational arm with no intervention (n = 18–24). The primary objectives are to define the safety profile and a tolerable dose of MSCs. Secondary objectives include the biological effects of MSCs on serial biomarkers of inflammation and measures of feasibility related to trial implementation and conduct. Recruitment began in May 2015. At the time of presentation, two patients had been enrolled in the low-dose group without adverse events. Patient 1 showed a dramatic decrease in need for inotropic support within 8 hours of MSC administration.

Next, Dr. Euan Wallace, Monash Institute, Melbourne, presented extensive preclinical data on hAECs for lung injury (66, 67). hAECS attenuated lung injury and inflammation in bleomycin-induced fibrosis in wild-type mice (68, 69). Similar to MSCs, lung engraftment of human AF stem cells is extremely low, suggesting an alternate mechanism of action of these cells (70). In transgenic mice, Dr. Wallace demonstrated the crucial interactions of hAECS with immune modulatory cells. The lack of efficacy of hAECs to mitigate bleomycin-induced lung injury in surfactant protein C–deficient mice—known to have impaired macrophage function—suggests that hAECs require normal host macrophage function to exert their reparative effects (71). Indeed, hAECs decreased lung macrophage influx, promoted the M2 healer macrophage phenotype, and, *in vitro*, decreased macrophage migration and increased macrophage phagocytic activity (72). hAECs also require regulatory T cells (Tregs) to exert their beneficial effect. Only the combined administration of hAECs and $CD45^{+}/FoxP3^{+}$ Tregs or $CD45^{+}/FoxP3^{-}$ non-Tregs adoptive transfer into bleomycin-exposed $Rag1^{-/-}$ mice attenuated lung fibrosis and polarized macrophages into an M2 phenotype (73). hAECs also promote bronchioalveolar stem cell proliferation in a coculture system (Wallace and colleagues, unpublished results). Furthermore, hAECS attenuated pulmonary hypertension and loss of lung vessel density in a combined hyperoxia + LPS neonatal mouse model of BPD, the chronic lung disease of prematurity (Zhu and colleagues, unpublished results). Finally, Dr. Wallace reported efficient lung injury prevention in bleomycin-induced fibrosis of conditioned medium (74) and reduced repair activity of preterm hAECs compared with term hAECs. A phase I study of hAECs in premature infants with established severe BPD has recently started.

Dr. Fernanda Cruz, Federal University of Rio de Janeiro, Brazil, provided an overview of preclinical and clinical activity on bone marrow–derived mononuclear cells (BMDMC) for lung repair and regeneration. Putative advantages of using this mixture of cells include the low cost, same-day acquisition of autologous cells (75), and high lung entrapment of cells after intravenous infusion (76). First, Dr. Cruz demonstrated efficacy of BMDMC in elastase-induced murine emphysema (77) and possibly the superiority of intratracheal versus intravenous administration (Cruz, unpublished). Second, in LPS-induced ARDS, BMDMC improved survival and static lung elastance; the number of green fluorescent protein–positive cells was higher in the direct lung injury group (intratracheal LPS) compared with extrapulmonary (intraperitoneal LPS) (5 vs. 2%) (76). BMDMC obtained from ARDS mice were equally effective as BMDMC obtained from control mice (78). Third, in murine silicosis, BMDMC improved lung mechanics and histology after intratracheal instillation of BMDC at 30 days (15 d after treatment) but not at 60 days. A phase I trial of endobronchial instillation of autologous BMDMC in five patients with silicosis showed feasibility and safety (79). Plans for a phase II study are underway. In ovalbumin-induced asthma, BMDMC decreased eosinophilic inflammation and airway remodeling (80), independent of the route of administration (intravenous or intratracheal) (81).

More recent work attempted to identify which cell(s) among the mixture of BMDMC promote(s) the beneficial effects in experimental asthma. Extensive analysis of lung inflammation, airway histology, bronchoalveolar lavage cell count, and cytokines revealed that the monocyte and MSC fraction contribute to the beneficial effects of BMDMC (82). Finally, Dr. Cruz outlined the design of a phase I/II trial of intravenous autologous BMDMC in severe asthma, nonresponsive to steroid, bronchodilators, and/or anti-IgE.

Dr. Kathleen Liu, University of California–San Francisco, updated the audience on her group's progress in translating allogeneic human MSC therapy for ARDS into the clinic. In preclinical

studies, new mechanisms of action of MSCs were identified: in mice, human bone marrow–derived MSCs improved survival and promoted the resolution of LPS-induced lung injury in part through the proresolving lipid mediator Lipoxin A4 (LXA4). In an *ex vivo* perfused human lung preparation in which lung injury was induced by endotoxin or live *Escherichia coli*, intratracheal or intravenous delivery of allogeneic, clinical-grade human MSCs or MSC-conditioned media attenuated endothelial and epithelial injury, accelerated alveolar edema resolution, and enhanced bacterial killing. These effects were in part mediated through keratinocyte growth factor (KGF) (83). The clinical translation was greatly facilitated by the access to NIH Production Assistance of Cellular Therapies (PACT) program clinical-grade human MSCs. To obtain the investigational new drug/device, the safety and efficacy of MSCs were completed in a large animal model in sheep with severe lung injury from cottonwood smoke inhalation and intrapulmonary instillation of live bacteria (84). Importantly, MSCs lost their therapeutic efficacy if the washing step after thawing and before administration was removed. Dr. Liu then presented the results of a phase 1 multicenter open-label dose-escalation trial assessing the feasibility and safety of human bone marrow–derived allogeneic MSCs in moderate to severe ARDS (ClinicalTrials.gov identifier: NCT01775774) (85). No prespecified infusion-associated events or treatment-related adverse events were reported in any of the nine patients. On the basis of these results, a phase II trial is currently underway.

Dr. Antoine Monsel, Hôpital Pitié Salpêtrière, Paris, exploited the paracrine effect of MSCs through the use of MSC-free conditioned media (CM) (86). A host of preclinical studies have demonstrated the therapeutic benefit of MSC-derived CM in experimental acute lung injury (87, 88), BPD (89–95), asthma (96, 97), and COPD (97). Classic questions remain: best dose, route and timing of administration, best manufacturing approach (concentrated CM, cell starvation, preconditioning), and entire cocktail administration versus selected molecules. In this respect, recent insight into microvesicle biology (98) has offered interesting alternate approaches for cell-free therapy, including the use of exosomes, nanosize (30–120 nm) circular membrane fragments released from the endosomal compartment and shed from the surface membranes, which contain biologically active growth factors such as KGF and miRNAs.

Preclinical evidence for the therapeutic benefit of MSC-derived exosomes in lung diseases was first demonstrated in hypoxia-induced pulmonary hypertension in mice (99). In *E. coli* endotoxin–induced acute lung injury in mice, human MSC-derived microvesicles reduced lung permeability and inflammation in part through the expression of KGF mRNA and increased bacterial clearance and monocyte phagocytosis (100, 101). In the *ex vivo* human lung perfusion model, microvesicles derived from human MSCs increased alveolar fluid clearance in a dose-dependent manner, decreased lung weight gain, and improved pulmonary vascular resistance. Thus, microvesicle-based therapies are an interesting cell-free strategy. Hurdles to be overcome include the standardization of the isolation methods, large-scale manufacturing, and quality monitoring.

Dr. Charles Drinnan, University of Connecticut Health Center, presented work on AF stem cells for bioengineering purposes. AF stem cells, first described in 2007, are multipotent stem cells that can also differentiate into lung cells (102). A subset of these cells are MSCs that can undergo osteo-, adipo-, myo-, neuro-, and vasculogenesis (103). Dr. Drinnan showed in a series of *in vitro* experiments how various culture conditions such as (*1*) culture media (standard AF-MSC medium, modified small airway growth medium, and a definitive endoderm + anterior foregut epithelium + lung epithelium cocktail adapted from various pluripotency schemes) or (*2*) culture support (plastic, matrigel, fibronectin, collagen 1, decellularized rat lung slurries) modify the acquisition of lung-specific genes by AF-MSCs. Decellularized rat lung slurries seem to be potent in enhancing the expression of surfactant protein C and NKX2.1.

Likewise, physical interactions with the extracellular matrix in controlling stem cell fate are important (104, 105). In the lung, physiological stretch promotes differentiation of alveolar epithelial cells (106). Using the FlexCell device, Dr. Drinnan demonstrated how different stretch regimens combined with the aforementioned culture conditions affect the acquisition of distal lung cell markers by AF-MSCs. Last, Dr. Drinnan showed the feasibility of seeding undifferentiated AF-MSC into decellularized lungs and the acquisition of markers such as TTF-1, SP-C, neuron-specific class III beta-tubulin, and paired box gene 8. In summary, refining various culture conditions may optimize the lung differentiation potential of AF-derived MSCs for use in acute and chronic lung diseases.

Trainee travel award recipient Kathleen Atkins, Ottawa Hospital Research Institute, focused on resident lung MSCs in the developing murine lung (107–109). This is relevant to BPD, the chronic lung disease of prematurity characterized by interrupted alveolar and lung vascular growth (110, 111). Because of the critical window during which lung injury occurs, BPD may have long-term respiratory health consequences reaching into adulthood (112). Exogenous bone marrow– and cord-derived MSCs improve lung structure and function in neonatal rodents exposed to chronic hyperoxia (113). It remains unclear why resident lung MSCs do not contribute to lung repair (83, 114). Using a clinically relevant model in which neonatal mice were mechanically ventilated for 8 hours and exposed to 40% hyperoxia (115), the function of resident lung $Sca1^{+}CD31^{-}CD45^{-}EpCAM^{-}$ MSCs was explored. The chondro- and adipogenic differential potential were unchanged between control and lung MSCs from ventilated mice. Compared with nonventilated control mice, lung MSCs isolated from ventilated mice showed higher levels of insulin growth factor–binding protein 2, 3, 9, and 10; the antiangiokines tissue inhibitor of metalloproteinase, thrombospondin 2, Serpin E1, and Serpin F1; and the proinflammatory cytokines Pentraxin-3, monocyte chemoattractant protein-1, and stromal cell–derived factor 1. Conditioned media generated from lung MSCs of ventilated mice were less efficient in wound closure in an alveolar epithelial cell scratch assay compared with nonventilated controls. Preliminary RNA seq analysis also suggests differences in the transcriptome of lung MSCs of ventilated mice. A better understanding of resident lung MSCs in lung health and disease may enable the development of enhanced cell products.

Trainee travel award recipient Dr. Carole Schmoldt, University of Giessen, Germany, presented original work on

lung epithelial progenitor cells (EpiSPC) for lung regeneration after virus-induced lung injury (116, 117). On the basis of previous work by McQualter and colleagues (118), Dr. Schmoldt isolated two populations from the distal alveolar epithelial cell pool from murine lungs: $CD49^{+}CD31^{-}CD45^{-}$EpCamhigh EpiSPCa, and the EpCamlow fraction–labeled type 2 AECs (AEC II). EpiSPC also expressed the cell surface markers CD104 and Sca-1 and form organoids in culture that express proSPC and CC10. A genome array on EpiSPC and AEC II isolated from mock-infected mice and mice infected with the PR8 strain of influenza virus revealed a clear antiviral response by EpiSPC on the basis of increased gene expression IFN signaling, virus resistance, and cytokine pathways.

Wnt/β-catenin is important for stem cell regulation and repair (119). In a series of *in vitro* and *in vivo* experiments in influenza-infected mouse lung epithelial cell line 12 cells and AEC II and in EpiSPC isolated from infected mice, Wnt/β-catenin–dependent transcription impairment seems to serve as an immune evasion therapy, because the Wnt/β-catenin activation led to a decrease in viral replication, whereas Wnt/β-catenin inhibition increased viral replication (120). Fibroblast growth factor 10 (FGF10)/fibroblast growth factor receptor 2b (Fgfr2b), downstream of Wnt/β-catenin, a crucial growth factor in lung development and repair (121), promotes the proliferation of EpiSPC *in vitro*. Furthermore, influenza virus decreased Fgfr2b expression in mouse lung epithelial cell line 12 cells after *ex vivo* infection and EpiSPC Fgfr2b expression and EpiSPC proliferation after *in vivo* infection. Inactivation of FGF10/Fgfr2 after influenza virus infection in transgenic $Rosa26rtTA/^{+}$;$tet(O)sFgfr2b/^{+}$ mice that express a dominant-negative soluble Fgfr2b lead to decreased EpiSPC proliferation, increased lung permeability, and decreased body weight. Thus, Wnt/β-catenin signaling is activated in response to influenza virus infection and enhances the antiviral response. Wnt/β-catenin also participates in lung repair through activation of FGF10/FGFR2b. Influenza virus infection impairs Wnt/β-catenin–dependent transcription as an immune evasion mechanism, which likely impacts the regenerative EpiSPC response.

Session 7: Summation and Directions

The above session was followed by intense discussions that summarized the evolution of the field. Unlike in previous years, the question was not "if" clinical trials should be initiated, but "when." To illustrate the appropriate timing for a phase I trial, Dr. Donald Fink from the FDA used Dr. Duncan Stewart's efforts as a state-of-the-art example of careful and successful clinical translation of cell-based therapies through the provision of a rationale, reproducible results, a mechanism of action and preclinical toxicity, and safety data. Regulatory issues around cell-free products were also discussed: conditioned media or exosomes are not considered cell products. Dr. Fink also emphasized the need for disease-specific potency assays early on for quality control and to predict *in vivo* efficacy of the cell product. Heated discussions around stem cell tourism for lung diseases also took place, emphasizing the clear need for an international strategy on how to best inform patients and family members about cell-based therapies.

The conference directors, Drs. Weiss and Wagner, then worked through a detailed summary of key points of each session and invited further comment from the audience. Key points for future research and funding directions are summarized in Table 2.

Summary

A continuing accumulation of data in both animal models and clinical trials suggests that cell-based therapies and novel bioengineering approaches may be potential therapeutic strategies for lung repair and remodeling after injury. In parallel, further understanding of the role of endogenous lung progenitor cells will provide further insight into mechanisms of lung development and repair after injury and may also provide novel therapeutic strategies. Remarkable progress has been made in each of these areas since the last conference 2 years ago. We hope that the workshop recommendations (Table 2) will spark new research that will provide further understanding of mechanisms of repair of lung injury and further provide a sound scientific basis for therapeutic use of stem and cell therapies in lung diseases. ■

This Workshop Report was prepared by an *ad hoc* subcommittee of the ATS Assembly on Respiratory Cell and Molecular Biology.

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A list of participants, travel award winners, executive summaries of speaker presentations, and poster abstracts are included in the accompanying online supplement.

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