

# AMERICAN THORACIC SOCIETY DOCUMENTS

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

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THIS OFFICIAL WORKSHOP REPORT OF THE AMERICAN THORACIC SOCIETY (ATS) WAS APPROVED BY THE ATS BOARD OF DIRECTORS, DECEMBER 2014

### Abstract

The University of Vermont College of Medicine and the Vermont Lung Center, in collaboration with the NHLBI, Alpha-1 Foundation, American Thoracic Society, European Respiratory Society, International Society for Cell Therapy, and the Pulmonary Fibrosis Foundation, convened a workshop, "Stem Cells and Cell Therapies in Lung Biology and Lung Diseases," held July 29 to August 1, 2013 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 conference was a follow-up to four previous biennial conferences held at the University of Vermont in 2005, 2007, 2009, and 2011. 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.

The conference was supported in part by R13 HL097533 from the NHLBI (D.J.W.), the Alpha-1 Foundation, the American Thoracic Society, the European Respiratory Society, the International Society for Cell Therapy, the Pulmonary Fibrosis Foundation, the University of Vermont, the University of Vermont College of Medicine, ACell Inc., Athertsys Inc., Biogen Idec, Flexcell International Corporation, Harvard Apparatus Regenerative Technologies, Organovo Inc., and the United Therapeutics Corporation.

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This article has an online supplement, which is accessible from this issue's table of contents at [www.atsjournals.org](http://www.atsjournals.org)

Ann Am Thorac Soc Vol 12, No 4, pp S79–S97, Apr 2015

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DOI: 10.1513/AnnalsATS.201502-086ST

Internet address: [www.atsjournals.org](http://www.atsjournals.org)

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### Overview

This workshop report is based on the fifth 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 both the lung epithelium and pulmonary vasculature; (2) progress in understanding the steps necessary to have both embryonic and induced pluripotent stem cells differentiate into airway and alveolar epithelial cells; (3) increased delineation of the potential roles of mesenchymal stem 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 stem 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 in *ex vivo* tracheal bioengineering; and (7) progress in development of decellularized whole lungs as scaffolds for *ex vivo* lung bioengineering.

However, there remain many questions in each of these areas. One additional area that still remains problematic is the nomenclature of the different stem and progenitor cell populations involved. 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.

This conference was a follow-up to four previous biennial conferences held at the University of Vermont in 2005, 2007, 2009, and 2011 (1–5). Since the last conference in 2011, investigations of stem cells, cell therapies, and *ex vivo* bioengineering in lung biology and diseases have continued to rapidly progress. Exciting advances have occurred in studies of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS), 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 (6–11). 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 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 suggest an increasingly complex network of cellular repair after injury (reviewed in [12–19]). Recent data have broadened this beyond consideration of epithelial progenitors to also include endogenous pulmonary vascular and interstitial progenitors (20–22). However, ongoing challenges are to better define, access, and manipulate the appropriate niches and to continue to devise more refined lineage tracing and other study mechanisms 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 is that most studies of endogenous progenitor cells continue to use mouse models. For example, although evidence from several laboratories suggest that p63<sup>+</sup>Krt5<sup>+</sup> basal cells are a heterogeneous progenitor cell population in the human lung as in many other epithelial tissues, correlative information in human lungs remains less well defined, with varying degrees of rigor in the available literature (23–29).

Stem and progenitor cell nomenclature remains a thorny issue, although some progress has been made. Despite suggested guidelines from previous conferences and from other sources, precise definitions and characterizations of specific cell populations, notably the putative endogenous cell populations in the lung as well as MSCs and EPCs, are not agreed on. In many respects this reflects more sophisticated knowledge and increasing appreciation that the phenotypic and functional attributes of cells are context dependent (12–19). Cells previously considered to be differentiated airway or alveolar epithelial cells can proliferate and differentiate into other lung epithelial cell types under varying circumstances. As such, paradigms of lung cell behavior are in evolution.

However, these are evolving concepts, and 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. Similar observations about epithelial cell plasticity in tissue repair have been made in other organs, notably skin (30).

A growing number of preclinical studies of immunomodulation and paracrine effects of adult 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, chronic obstructive pulmonary disease (COPD), sepsis, ventilator-induced lung injury, and other lung diseases (reviewed in References 2, 31). A growing number of investigations with other cell populations, including bone marrow mononuclear cells and amniotic fluid-derived cells, show efficacy in ameliorating injury in mouse models of lung diseases (reviewed in Reference 31). In parallel, more sophisticated understanding of the mechanisms by which these cells can act has provided growing insight into their potential applicability for clinical lung diseases (2, 31). An initial multicenter double-blinded randomized placebo-controlled trial of MSCs in patients with moderate to severe COPD conducted in the United States, although underpowered for efficacy, established safety and demonstrated a decrease in a circulating inflammatory mediator, C-reactive protein, in treated patients (32). 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, and idiopathic pulmonary fibrosis (IPF), as well as continuing trials in COPD listed on clinicaltrials.gov that are taking place in the United States, Canada, Brazil, Europe, and Australasia. However, as further discussed below in the section on EPCs, MSCs, and Cell Therapy Approaches for Lung Diseases, some of these trials have provoked controversy as to applicability of cell therapy approaches, notably for pulmonary fibrosis (33, 34).

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* (reviewed in

References 35 and 36). 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 (reviewed in References 37–39). A significant challenge will be to develop a fuller understanding of the underlying cell biology in the tracheal scaffolds and use these to best advantage in clinical applications.

## 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. Particular focus was on featuring talks by 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 to both authors and institutions. An expanded number of trainee travel awards supporting both oral and poster presentations by junior investigators and trainees was provided. A new feature of the 2013 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, which allowed junior investigators and trainees to have focused one-on-one or small group time with senior investigators. Another new feature of the conference was a dedicated forum for women and diversity development. These were all highly successful additions to the conference and will 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 first author, Daniel J. Weiss, M.D., Ph.D. Dr. 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 based on discussions that occurred at the conference.

## Session 1: Emerging Topics in MSC Biology

This first session, moderated by Armand Keating, M.D. (University of Toronto), addressed several key issues in MSC biology and, in particular, focused on MSC potency and cell communication. Darwin Prockop, M.D., Ph.D. (Texas A&M) identified wide variations in the potency of different preparations of human MSCs, despite the application of the same criteria for progenitor cell frequency (CFU-F), immunophenotype, doubling time, and the ability to undergo differentiation (further reviewed in [31, 40, 41]). He demonstrated that in a corneal injury model, the efficacy of MSCs correlated with the transcription levels of the antiinflammatory molecule, tumor necrosis factor-inducible gene 6 protein (TSG6) (42). His work highlighted the importance of finding appropriate markers of efficacy against inflammation that can be easily assayed and that appear to be distinct from the cell surface markers and other criteria that are currently used to define MSCs (40). The considerable variation documented among some of the characteristics of MSCs derived from different donors highlighted one of the dilemmas facing the clinical translation of this cell population and raised concerns among some about the need for reproducible potency assays. In the general discussion that followed, it was emphasized that choosing a potency assay will depend on the putative mechanism by which the cells act in a particular clinical indication. Nonetheless, there was enthusiasm for a simple polymerase chain reaction assay such as for TSG6 as an indicator of the immunosuppressive properties of the MSC product.

Jason Aliotta, M.D. (Brown University) highlighted the potential importance of cell communication via small circular fragments of membrane released from the endosomal compartment or from the cell membrane containing various mixtures of proteins, cell organelles,

mRNA, microRNAs (miRNAs), and other substances, variously known as extracellular vesicles, exosomes, microvesicles, or microsomes, in addition to the better known mechanisms of cell–cell interaction and paracrine release of bioactive molecules. This is a rapidly expanding area of investigation that has significant implications for cell-based therapies (43). Different types of extracellular vesicles have been described, but it appears that the term exosomes is gaining wider usage. However, investigators in this field face challenges regarding definitions and nomenclature similar to those confronting researchers describing MSCs. Nonetheless, studies are now underway to investigate the role of MSC-derived extracellular vesicles in mediating tissue repair and modulating the inflammatory response. Specific extracellular vesicle components that carry proteins/polypeptides or miRNA may be directly implicated in MSC-mediated processes. For example, Stella Kourembanas, M.D. (Harvard University) showed that MSC-derived exosomes mitigate the development of pulmonary hypertension in a murine neonatal lung injury model of bronchopulmonary dysplasia (44). Other studies have also described a role of exosomes in mediating MSC action in other lung injury models, including ARDS (45). The mechanisms by which the exosomes act are likely to be multifactorial and could be as basic as involving signaling pathways that induce changes in mRNA expression and/or epigenetic change. This will be a rapidly expanding field.

Almost a decade ago, mitochondrial transfer between MSCs and cultured airway epithelial cells was demonstrated to rescue anaerobic respiration in cells with mitochondrial dysfunction (46). Luis Ortiz, M.D. (University of Pittsburgh) has extended this notion and reported on progress in testing his hypothesis that MSCs modulate innate immune responses by the transfer of MSC-derived mitochondria to macrophages. He showed that MSC-derived microvesicles can contain mitochondria and have specific miRNAs that reduce inflammation and fibrosis in a mouse lung fibrosis model. This talk was followed by a presentation by Jahar Bhattacharya, M.D., D.Phil. (Columbia University) demonstrating live imaging

**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 (ES cells). However, some species differences can occur; for example, mouse ES cells do not give rise to extraembryonic cell types, but human ES cells 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 iPS cells 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<sup>+</sup> club cells to give rise to Type2 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 (EMT):** 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 iPS cell 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 (ES) cell:** Cell lines developed from the inner cell mass of a blastocyst stage embryo. ES cells 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) ES cells 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, mesenchymal stem (stromal) cells 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:** 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. iPS cells are similar to ES cells in morphology, proliferation potential, pluripotent differentiation repertoire, and global transcriptomic/epigenomic profiles. *In vivo* implantation of iPS cells results in formation of tissues from all three embryonic germ layers. iPS cells have been generated from both mouse and human cells.

(Continued)

Table 1. (Continued)

- 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 use of the term “stem” vs “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<sup>+</sup> intestinal stem cells after ablation of the resident Lgr5<sup>+</sup> 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.
- 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 (ECFC):** 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. ECFC 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) cell:** 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 (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 (114). 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 produces collagen and homes 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.

(Continued)

**Table 1.** (Continued)

**Basal epithelial cell:** Cells present within pseudostratified and stratified epithelia that are rich in hemidesmosomal connections that anchor the epithelial to the basement membrane. These cells are characterized by the expression of p63 and (variably) cytokeratins 5 and 14. In the pseudostratified proximal airway epithelium, these 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 functional 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<sup>lo</sup>, Scb3a2<sup>hi</sup> 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<sup>+</sup> 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 fluorescence-activated cell sorter 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 cells' abilities to give rise to Type2 and Type1 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 abbreviation:* iPS = induced pluripotent stem cells.

The authors gratefully acknowledge input and discussion toward updating of this table from the following individuals: Adam Giangreco, Ph.D., Erica Herzog, M.D., Brigid Hogan, Ph.D., Carla Kim, Ph.D., Darrell Kotton, M.D., Bethany Moore, Ph.D., Laertis Okonomou, Ph.D., Anglea Panoskaltis-Mortari, Ph.D., Emma Rawlins, Ph.D., Susan Reynolds, Ph.D., Jason Rock, Ph.D., Barry Stripp, Ph.D., Mervin Yoder, M.D.

techniques of the lung that can help address the challenges of identifying the means by which mitochondria transferred by MSCs to alveolar epithelial cells are able to repair experimentally induced acute lung injury (47, 48).

The session concluded with an enthusiastic call to undertake further studies on the role of extracellular vesicles/exosomes in dissecting out their contribution to tissue repair. The longer-term goal was to consider the use of extracellular vesicles themselves as a therapy to repair injured lung tissue. It was of interest that some expressed the need for caution and the execution of careful preclinical studies of extracellular vesicles, in part because of their considerable heterogeneity of composition and potentially high potency. It was argued that unlike cells, which uncommonly have dose-limiting toxicity, extracellular vesicles may behave more like drugs and exhibit significant off-target effects.

## Session 2: Endogenous Lung Progenitor Cells

Moderated by Adam Giangreco, Ph.D. (University College, London), this year's session on endogenous lung progenitor

cells included reports from leading researchers investigating epithelial and mesenchymal progenitor cells in airway development, homeostasis, and disease. A number of themes emerged during these presentations that suggest a consensus has now been reached regarding several salient points in lung progenitor cell biology. These include the observation that both epithelial and mesenchymal compartments contain multiple cell types that can be classified as "endogenous progenitor cells," that lung injury models are often needed to interrogate the growth and differentiation potential of individual progenitors, and that both the type and severity of lung injury are of paramount importance in determining lung repair outcomes (12–19). Emerging themes included the growing recognition that epithelial–mesenchymal interactions are not only important during lung development but remain relevant for maintaining adult lung homeostasis, repair, and regeneration and that these are functionally distinct processes that likely involve different cell populations and signaling pathways.

Hal Chapman, M.D. (UCSF) opened the session with an overview of his work on alpha6-beta4 ( $\alpha6\beta4^+$ )-expressing epithelial cells in distal murine lung regeneration (49). He mentioned that multiple epithelial

cell types contribute to lung repair and regeneration: integrin  $\alpha6\beta4^+$ , cytokeratin 5<sup>+</sup> basal cells of the trachea,  $\alpha6\beta4^+$ , k5/CC10/surfactant protein C (SPC)–negative distal airway and alveolar cells, CC10<sup>+</sup> bronchiolar cells that are ck5 negative, and finally SPC<sup>+</sup> type 2 cells that are normally  $\alpha6\beta4$  and ck5 negative. He then demonstrated that *ex vivo* techniques such as kidney capsule transplantation can be effective for establishing the growth and differentiation capacities of prospectively isolated airway progenitors (49).

Dr. Chapman went on to present data indicating that, after bleomycin injury,  $\alpha6\beta4$  cells express keratins 5 and 14, exhibit a surprisingly mesenchymal-like morphology, and are highly motile. These findings agreed with a recent report that keratins 5 and 14 are expressed in a subset of progenitor cells localized to the alveolar region after influenza infection (50). However, it remains unclear whether these cells were initially present in the alveolar region or migrated there post injury. In either case, these data suggest that a process involving loss of some epithelial characteristics may be associated with successful distal airway repair. Overall, this work highlighted new areas for investigation in alveolar repair and clarified some of the difficulties in establishing

**Table 2.** Overall conference summary recommendations**Fundamental/basic**

For studies evaluating putative engraftment, 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 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.

Continue to refine the nomenclature used in study of endogenous and exogenous lung stem cells.

Comparatively identify and study endogenous stem/progenitor cell populations between different lung compartments and between species.

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.

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.

Continue to develop disease-specific populations of ES and iPS, for example for CF and  $\alpha_1$ -antitrypsin deficiency with the recognition that no strategy has yet been devised to overcome the propensity of ESCs and iPS cells to produce tumors.

Continue to explore lung tissue bioengineering approaches such as artificial matrices and three-dimensional culture systems for generating lung *ex vivo* and *in vivo* from stem cells, including systems that facilitate vascular development.

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.

Identify additional cell surface markers that characterize lung cell populations for use in visualization and sorting techniques.

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), NCCR stem cell facilities, GMP Vector Cores, small animal mechanics, and computed tomography scanner facilities at several pulmonary centers.

**Translational**

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).

**Clinical**

Continue with design and implementation of initial exploratory safety investigations in patients with lung diseases where appropriate, such as ARDS/ALI, asthma, and others. This includes full consideration of ethical issues involved, particularly which patients should be initially studied.

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 NCCR/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.

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.

*Definition of abbreviations:* ALA-ACRC = American Lung Association Airways Clinical Research Centers; ALI = acute lung injury; ARDS = acute respiratory distress syndrome; ARDSNet = Acute Respiratory Distress Syndrome Network; CF = cystic fibrosis; EPC = endothelial progenitor cell; ESC = embryonic stem cell; iPS = induced pluripotent stem cells; GMP = good manufacturing practice; MSC = mesenchymal stem cells; NCCR = National Center for Research Resources.

definitive lineage relationships among airway epithelial cells.

Moving proximally in the airway, Barry Stripp, Ph.D. (Cedars Sinai) reminded the audience of the extensive cellular plasticity present during lung development and contrasted this with the region-specific segregation that exists among tracheobronchial, bronchiolar, and bronchial regions of normal adult lungs (17, 51, 52). He then discussed recent unpublished studies in which his laboratory identified and generated new genetically modified mouse (GMM) models to label regionally distinct proximal and distal conducting airway cells. These GMM Cre-recombinase models will be used for lineage tracing airway cell populations in the context of homeostasis, during injury, and after lung repair and regeneration. He also highlighted the usefulness of *ex vivo* transplantation and *in vitro* organoid assays for interrogating the maximal growth and differentiation capacities of putative lung stem and progenitor cells (53, 54). Echoing the work of Dr. Chapman, Dr. Stripp demonstrated that although ozone injury models do not induce multipotent progenitor cell activation, more severe forms of airway injury, such as influenza infection, bleomycin, and naphthalene exposure, can trigger multipotent progenitor cell growth and lineage plasticity (48, 55–59).

Vibha Lama, M.D., M.S. (University of Michigan) then shifted the focus of the session toward adult human lung MSCs. She described her work investigating female lung transplant patients who had received sex-mismatched donor lungs. An assessment of Y chromosome abundance in the lungs of these patients allowed her to identify the long-term, lung-specific origin of resident MSCs (60). Dr. Lama found that, in contrast to bone marrow-derived MSCs, lung resident MSCs (LR-MSCs) exhibit increased *Foxf1*, *Hoxa5*, and *Hoxb5* transcription factors and are preferentially located near endothelial tissues adjacent to alveolar septae (61). Although the function of these cells in lung homeostasis and repair remains incompletely understood, Dr. Lama suggested that LR-MSCs may provide a supportive microenvironment for epithelial progenitor cells while also contributing to the pathogenesis of fibrotic lung disease (61, 62). Finally, she provided evidence that patients at increased risk of developing bronchiolitis obliterans

exhibited elevated LR-MSc abundance in their bronchiolar lavage fluid. These data indicate a potential usefulness for measuring LR-MSc abundance as an early biomarker for lung transplant failure (63).

Echoing a statement by Dr. Lama, Anne Karina Perl, Ph.D. (University of Cincinnati) began by highlighting the fact that many different stromal and fibroblast populations exist in the lung that exhibit a wide variety of phenotypes, functions, and responses to pathogenic stimuli (64). Dr. Perl then presented data in which she interrogated the role of platelet-derived growth factor alpha (PDGF $\alpha$ )-expressing fibroblasts in lung regeneration. Five days after left lung pneumonectomy, Dr. Perl found that PDGF $\alpha$ (+) fibroblasts expanded in number and began to express  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) (65). This process was age dependent, required downstream fibroblast growth factor receptor 2 (FGFR2) signaling, and was positively associated with increased alveolar regeneration and elastin deposition in the remaining lung. Dr. Perl suggested that this finding highlights an important role for PDGF $\alpha$  and PDGF $\alpha$ -expressing fibroblasts in promoting lung regeneration post pneumonectomy. She indicated that PDGF $\alpha$  expression in stromal cells may function by activating processes similar to those found during early branching morphogenesis (14, 51).

After a brief overview of his previous work on tracheal epithelial basal cells (66, 67), Jason Rock, Ph.D. continued on the theme of lung regeneration post-pneumonectomy raised by Dr. Perl. Using an SPC lineage tracing model he helped develop while in Brigid Hogan's laboratory (56), Dr. Rock demonstrated that SPC-expressing type 2 alveolar epithelial cells are multipotent progenitors for the alveolar epithelium that give rise to type 1 alveolar epithelial cells under steady state conditions, after bleomycin injury, and post-pneumonectomy (56, 66). He found that stretch and cellular tension are major factors in determining regeneration outcomes post-pneumonectomy and provided new evidence that inhibition of lung macrophages significantly impairs lung regeneration. Overall, these findings highlight emerging roles for matrix tension and immune and inflammatory cell activity as extrinsic mediators of lung epithelial progenitor cells.

The final talks of the session were selected from submitted abstracts and presented by junior investigators and travel award recipients Elizabeth Hines, B.A. and Marcin Wlizla, Ph.D. Ms. Hines described her recent work with GMM models in which either *Srf* or *Sox9* were conditionally deleted during lung development. She found that conditional deletion of *Srf* resulted in smooth muscle and cartilage agenesis and early embryonic lethality, whereas *Sox9* mutants lacked cartilage development, retained SMA expression, and exhibited abnormal tracheal epithelial cell differentiation. Similarly, Marcin Wlizla found that targeted knockdown of *Foxf1* in the anterior plate mesoderm during early *Xenopus* embryogenesis reduced mesenchyme-derived Wnt and retinoic acid signaling and impaired respiratory progenitor cell specification. Together, these talks provided new mechanistic evidence of how epithelial–mesenchymal crosstalk regulates airway growth and differentiation (51).

After these speaker presentations there was a lively, interactive panel discussion where Barry Stripp, Andrew Hoffman, D.V.M., D.V.Sc. (Tufts Veterinary College), and Jan Kajstura, Ph.D. (Harvard) addressed two timely and controversial aspects of lung progenitor cell research: whether murine models are appropriate for understanding human lung disease, and how current research can be translated to improve human health. Dr. Hoffman opened this discussion by pointing out that many animal species, including mice and humans, experience comparable pathological lung disorders, with evidence increasingly suggesting that similar types of injury will elicit comparable repair and regeneration outcomes among multiple species. Despite this, a clear caveat to most animal research is that current models typically reflect only the earliest stages of disease pathogenesis, whereas most human lung diseases present as end-stage conditions. Dr. Kajstura also raised the point that differences between human and murine lung physiology may complicate the direct clinical translation of murine progenitor cell research. Barry Stripp then suggested that an improved understanding of mechanisms regulating normal murine lung homeostasis and disease could be used as a means to establish and identify new targets for pharmacological interventions in human disease. He, along with Drs. Hoffman and Kajstura, agreed that

although animal models are inevitably imperfect, they are nonetheless important for establishing broad paradigms in lung regenerative medicine. All three panelists additionally believed that researchers should continue working toward improving recently established human *in vitro* and *ex vivo* lung models, including differentiated airway organoid and externally ventilated lung perfusion systems. In the end, both the panel and audience reached the consensus that a long-term, balanced approach should be pursued, in which researchers identify paradigmatic pathways and cellular targets using a mix of animal, *in vitro*, and *ex vivo* human research models. These targets can then be used to help develop novel interventional therapies through strategic industrial partnership agreements.

### Session 3: Embryonic Stem Cells, iPSCs, and Lung Regeneration

Since the past Vermont Lung Stem Cell Meeting, several advances were published in the pursuit of deriving lung epithelium *de novo* from pluripotent stem cells, both embryonic and adult (6–11). Moderated by Darrell Kotton, M.D. (Boston University), speakers discussed the contributions of both human (ESC and iPSC) and nonhuman (mouse and *Xenopus*) model systems to the pursuit of the directed differentiation of pluripotent stem cells to generate lung epithelium *de novo*.

Laertis Ikononou, Ph.D. (Boston University) reviewed his recent publication of a protocol that enables the directed differentiation of mouse ESCs and iPSCs into Nkx2-1<sup>+</sup> endodermal “primordial” lung and thyroid progenitors (7). The directed differentiation of these cells involves induction or inhibition of signaling pathways sequentially in feeder-free, serum-free culture conditions. After the efficient induction of definitive endoderm using Activin A, cells are later specified to lung/thyroid lineages using brief exposure to inhibitors of the bone morphogenetic protein (BMP) and transforming growth factor (TGF)- $\beta$  signaling pathways, as recently published by Snoeck and colleagues (6, 10). Induction of lung and thyroid progenitors was accomplished with 20% efficiency using combinatorial stimulation of the BMP, FGF, and Wnt signaling pathways using media

supplemented with BMP4, FGF2, and Wnt3a (7). A knock-in green fluorescent protein (GFP) reporter gene, targeted to the Nkx2-1 locus, allowed the monitoring of differentiation efficiency and the sorting of endodermal Nkx2-1<sup>+</sup> progenitors to purity for further outgrowth in conditions. This enabled further patterning and maturation of these Nkx2-1<sup>+</sup> cells, indicated by subsequent up-regulation of markers of lung (SPC, surfactant protein B [SPB], club cell secretory protein, Foxj1, cystic fibrosis transmembrane conductance regulator, and p63) and thyroid lineages (thyroglobulin and thyroid-stimulating hormone receptor).

A key question remains regarding how closely the Nkx2-1<sup>+</sup> primordial progenitors resemble naturally occurring primordial progenitors specified *in vivo* during normal development. Dr. Ikononou presented an approach, using a knock-in Nkx2-1–GFP reporter mouse that enables the first isolation of primordial Nkx2-1<sup>+</sup> progenitors close to their moment of initial specification in developing embryos. Dissecting out the primordial domains of lung, thyroid, and forebrain in this system provides material for sorting to purity the GFP<sup>+</sup> cells of each domain and should allow the profiling of the global transcriptome of *in vivo* primordial lung progenitors at E9.5 in the years ahead.

Aaron Zorn (University of Cincinnati) presented work recently published on signaling pathways controlling lung lineage specification from endoderm (68). Using the *Xenopus* model of development, Dr. Zorn found that suppression of Bmp4 signaling by the Odd-skipped-related (Osr) zinc-finger repressors Osr1 and Osr2 is required for Wnt/ $\beta$ -catenin–mediated lung specification. Recent publications have revealed that mesenchymal FGF and Wnt2b signaling are implicated in specification of mammalian pulmonary progenitors from the ventral foregut endoderm, but their epistatic relationship and downstream targets were largely unknown until the *Xenopus* model system was used to identify these targets. This model system revealed that Osr1 and Osr2 are redundantly required for *Xenopus* lung specification in a molecular pathway linking foregut patterning by FGFs to Wnt-mediated lung specification and retinoic acid (RA)-regulated lung bud growth. FGF and RA signals were required for robust *osr1* and *osr2* expression in the

foregut endoderm and surrounding lateral plate mesoderm (lpm) before respiratory specification. Depletion of both Osr1 and Osr2 (Osr1/Osr2) resulted in agenesis of the lungs, trachea, and esophagus. The foregut lpm of Osr1/Osr2-depleted embryos failed to express Wnt2, Wnt2b, and *raldh2*, and consequently Nkx2-1<sup>+</sup> progenitors were not specified. These findings suggest that Osr1/Osr2 normally repress *bmp4* expression in the lpm and that BMP signaling negatively regulates the Wnt2b domain. These results significantly advance an understanding of early lung development and are now impacting the strategies used to differentiate respiratory tissue from human ESCs and iPSCs.

Hans Snoeck, M.D., Ph.D. (Columbia University) then presented his recently published work developing a protocol to direct the differentiation of human pluripotent stem cells (ESCs and iPSCs) into respiratory epithelial lineages (6, 10). The Snoeck lab’s seminal discovery revealed that ESC or iPSC-derived human definitive endoderm requires patterning toward anterior foregut endoderm before endoderm is rendered competent to specify to lung. Inhibition of BMP and TGF- $\beta$  signaling pathways in human endodermal ESC/iPSC-derived cells allowed the highly efficient induction of Nkx2-1 in this endodermal population with up to 60% efficiency in response to a cocktail of growth factors, including epidermal growth factor (EGF), keratinocyte growth factor (KGF), BMP4, RA, FGF10, and a canonical Wnt pathway inducer. A similar patterning approach was subsequently used to differentiate mouse pluripotent stem cells as well as human cells in the publications of Longmire and colleagues (7) as well as Mou and colleagues (8). Dr. Snoeck presented new data demonstrating the further differentiation and maturation of the human ESC/iPSC-derived endoderm into lung lineages expressing gene markers of most known lung lineages, including SPB, p63, CCSP, FOXJ1, and mucins. Kidney capsule transplantation of the resulting cells revealed outgrowth of complex 3D structures representing epithelial-lined lumens expressing a broad diversity of lung epithelial markers. These studies now pave the way for the lung research community to derive a variety of lung epithelial lineages from the human ESC and lung disease-specific iPSCs that have been banked by investigators.

The final talks of the session were selected from submitted abstracts and presented by junior investigators and travel award recipients Lily Guo, M.D. M.Sc. and Pimchanok Pimton, Ph.D. Dr. Guo presented an optimized, controlled, doxycycline-mediated transient induction scheme for iPS reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) to generate an induced progenitor population (iPP) from a highly purified EpCAM<sup>high</sup>-club cell population. The functionality of the generated iPP cells was evaluated by both *in vitro* differentiation assay and *in vivo* animal studies. These studies demonstrated that transient induction of reprogramming factors induced quiescent EpCAM<sup>high</sup> cells to proliferate, which can be regulated by withdrawal of the inductive factors. EpCAM<sup>high</sup>-derived, transiently induced cells were found to have the capability of returning to their original phenotype on withdrawal of reprogramming factors. *In vitro*, they can differentiate to generate functional cystic fibrosis transmembrane conductance regulator (CFTR)-expressing ciliated epithelium and repopulate CFTR-knockout epithelium *in vivo* after a recipient conditioning regimen.

Dr. Pimton presented data suggesting that reduced oxygen tension might coordinate and enhance the *in vitro* differentiation of embryonic stem cells into definitive endoderm and then into SPC-expressing distal lung cells. Using an established definitive endoderm differentiation protocol using Activin A, hypoxic conditions known to exist during early embryonic development were incorporated into a novel differentiation protocol that appeared to improve expression and yield of definitive endoderm by about a factor of 10. The effects of hypoxia appear to be mediated by HIF-1 $\alpha$ .

As further elaborated and explored during the panel discussion led by Brian Davis, Ph.D. (University of Texas) and Wellington Cardoso, M.D. (Columbia University), these recent findings open a door on accelerated progress using induced pluripotent cells in lung regenerative medicine schemes. One focus of discussion was how best to define and determine the phenotype of the stem cell-derived putative lung lineages being engineered *in vitro*, given that histologic architecture, 3D structure, and polarized cell-cell interactions are typically not present in the two-dimensional (2D) *in vitro* models being used at present. Because one defining and unique feature of

lung epithelial cells is their structure and function in lung tissue, this question is likely to be an increasing challenge as the stem cell field advances. Clearly, the gene expression programs of these newly engineered cells are being increasingly defined with impressive results, and the field will now need to turn to engineering structure and function into these new model systems to harness their full potential. Most discussants agreed that lung epithelial lineage specification of ESC and iPSC-derived cells has now been convincingly demonstrated by a variety of new reports, but true maturation of these lung epithelia to a degree that resembles postnatal lung cells remains a challenge.

#### Session 4: Bioengineering Approaches to Lung Regeneration

Since the topic of Bioengineering Approaches to Lung Regeneration was first introduced in 2009, the field has made significant progress, trailblazing in part, such as proof-of-concept implantations of “breathing” decellularized/recellularized lungs in rodents and the first clinical implants of engineered trachea (69–71). Some of the progress has been more incremental (e.g., the optimization of protocols for using decellularized and recellularized lung scaffolds, both in rodents and more recently in larger animal models and in human lungs (7, 72–90). Moderated by Peter Lelkes, Ph.D. (Temple University), the main aim of this session was to review recent progress in terms of the techniques used for tissue processing, both decellularization and recellularization, as well as in enhancing our necessary mechanistic understanding necessary to, perhaps, bring bioengineering approaches to lung regeneration into the realm of clinical reality.

The first speaker, Stephen Badylak D.V.M., Ph.D., M.D. (McGowan Institute for Regenerative Medicine in Pittsburgh), discussed some of the mechanisms by which biologic scaffold materials composed of extracellular matrix proteins contribute to improved healing, include regeneration of bioactive molecules that recruit endogenous stem/progenitor cells to modulate the innate immune response and provide a favorable microenvironmental niche that can help direct constructive and functional tissue

repair (91). In presenting more recent studies on the use of organ-specific decellularized extracellular matrix (ECM) scaffolds for engineering a variety of whole visceral organs, (e.g., lung, liver, heart, and kidney) (92), Dr. Badylak stressed the importance of fine tuning harvesting methods, including decellularization techniques. The types of detergent, proteases, and fluid dynamics used in decellularization all have important effects on residual structures, such as vascular basement membranes, and topographic features and ECM composition, all of which impact the suitability for such scaffold material for subsequent recellularization (93).

Bela Suki, Ph.D. (Boston University) gave a refresher on how to assess lung mechanics in bioengineered lungs. Although traditional lung mechanics are characterized by measuring the pressure-volume curves, a much more elegant method is based on the forced oscillation technique (94), which provides information on the mechanical properties characterized by airway resistance (Raw), tissue resistance (G), and elastance (H). Careful analysis of these three key parameters, obtained experimentally or modeled *in silico* (95), can provide information on pathological changes in the nonlinearity of the lung and inform about alterations of microscopic properties in the wake of tissue remodeling in fibrosis or tissue breakdown in emphysema. Although currently little is known about the actual values of Raw, G, and H in bioengineered whole lungs, analysis of decellularized lung strips by mechanical and optical tools in combination network models suggests that the mechanical properties of the residual ECM scaffolds depend on the decellularization methods used (96). Dr. Suki stressed the need for multimodal assessment of the mechanical properties of decellularized scaffolds, because this information will be essential for restoring healthy organ function after repopulation.

Elizabeth A. Calle, M.Phil. (Yale University) described the tribulations associated with repopulating decellularized lung scaffolds with distal lung epithelium. The Yale group was one of the first to reimplant recellularized lung scaffolds in a rodent model in 2010 and has been refining the methodology ever since (36, 69). Specifically, they suggested the existence of a “zip code” effect, originally proposed by Pasqualini and colleagues (97), in the

context of drug targeting and vascular heterogeneity. In the context of lung recellularization, this concept stipulates that cells introduced to the matrix may adhere to the substrate and express proteins in a regionally specific manner. In this lecture, Ms. Calle first described the isolation of neonatal distal epithelium by using an adult rat type II marker, RTII-70, previously identified by the Dobbs group at UCSF (98). These cells were then seeded at different degrees of purity into the decellularized matrices and tracked over time. Cultured cells were assayed for epithelial, mesenchymal, and pluripotency markers. Although the isolated epithelium did populate the alveolar regions of the lung, current culture conditions do not support the ability of neonatal RTII-70<sup>+</sup> cells to repopulate the alveolar compartment with a full complement of alveolar epithelium. Interestingly, the RTII-70<sup>-</sup> population may promote the expression of epithelial markers in other cells within the lung matrix. However, whether these cells are the ones expressing these markers or whether they support expression in the original RTII-70 population is not clear at this time. This is also true of the interactions between the RTII-70 cells and the extracellular matrix. When RTII<sup>-</sup> cells are present, there is active remodeling of the matrix, whereas the near-absence of these cells leaves the matrix relatively undisturbed. These data suggest that functional repopulation of decellularized ECM scaffolds will have to take into account the hitherto poorly understood complex interactions between the various cell populations.

The lecture by Darcy Wagner, Ph.D. (University of Vermont) focused on the role of mechanotransduction in functional *ex vivo* lung tissue regeneration. The Vermont group is testing the hypothesis that mechanical stretch will be a critical parameter in regenerating functional lung tissue. The effects of mechanical stretch, well characterized in terms of surfactant expression in cultured ATII cells, remain largely unexplored in terms of promoting distal lung phenotypes in stem and endogenous lung epithelial progenitor cells, especially after repopulation of decellularized ECM scaffolds. To test the respective and synergistic contributions of ECM proteins and mechanical stretch on the expression of SPC and SPB, mouse mesenchymal stem cells (mMSCs,

control cells), and murine lung-specific C10 alveolar epithelial cells, ATIIs, and endogenous distal lung epithelial progenitor cells were seeded on different ECM substrates and stretched in 2D using the Flexcell System. Cells were also seeded intratracheally into decellularized whole lungs (3D) and exposed to positive pressure ventilation (75, 76, 79, 80, 87, 88, 90). Both mechanical stretch and ECM substrates contributed to the up-regulation of SPC and SPB in 2D and 3D. Specifically, cyclic mechanical stretch dramatically altered SPC gene expression in endogenous distal lung epithelial progenitor cells. Tidal volume and frequency were found to be important parameters in promoting SPC and SPB expression as well as controlling epithelial or myofibroblast phenotypes in 3D. In the search for potential mechanistic understanding, these studies identified for the first time activation/nuclear translocation of YAP/TAZ transcription factors (99) as potential mediators of mechanotransduction in lung repair, regeneration, and surfactant production. Taken together, these data indicate that, like in other contractile cardiovascular tissues, such as heart and blood vessels, mechanical stretch will be a necessary factor in an *ex vivo* lung regenerative scheme.

Thomas Gilbert, Ph.D. (University of Pittsburgh and ACell, Inc.), recipient of one of the travel awards, described how processing of tracheal ECM impacts ECM remodeling and functionality on transplantation in a rodent model (100). To date, several trachea decellularization methods using a variety of detergents (sodium deoxycholate [SDC], sodium dodecyl sulfate, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, and Triton X-100), have been described, but this is the first study to compare the effects of processing on the host response to the scaffold. After decellularization with the various detergents/protocols, all grafts were quite similar *in vitro*, except for a loss of elastin on SDS exposure. *In vivo*, only grafts treated with SDC or Triton X-100 showed the presence of ciliated cells, although their numbers were less than in the normal trachea. Motile cilia were only observed in the Triton group. Disinfection with peracetic acid enhanced the number of ciliated cells and their motility. As such, these data demonstrate that the choice of detergents changes the remodeling

responses, for better or for worse. In the case of tracheal implants, functional host remodeling can be enhanced with the choice of Triton over SDC or with the addition of a disinfection step with peracetic acid.

Asaf Presente, Ph.D., the other travel award winner for this session (University of California, San Diego), reported on the self-replicating RNA (replicon)-assisted directed development of lung progenitors. Although recent advances informed by our understanding of lung development have substantially improved our ability to generate lung progenitors from stem cell populations *in vitro*, these protocols have wide-ranging efficiencies, critical timing requirements, and/or rely on specific marker lines for sorting (7). Self-replicating RNA reagents (Replicons) can be used for prolonged expression of key transcription factors in any stem cell line after a single transfection of *in vitro* transcribed RNA, facilitating directed differentiation without the use of viruses or a DNA intermediate. This talk described work in progress on novel Replicons that will be able to deliver transcription factors key to lung development with subsequent assays for improved differentiation on decellularized lung scaffolds and in rodent models of lung disease. Importantly, Replicons that improve directed differentiation without genetic alterations might enable efficient generation of lung progenitors from patient-derived iPS (101). The hunt is on for suitable Replicons incorporating the direct expression of key transcription factors that will allow the field to improve sources of difficult to derive endodermal organ stem cells while preserving their clinical potential.

In the final talk of this session, Martin Birchall, M.D., F.R.C.S., F.Med.Sci. (University College London) reflected on the reality and hype of current tissue engineered airways. Although considerable optimism has been raised by the preclinical application of partly functional hearts, lungs, and livers, tracheal tissue engineering is also advancing rapidly (71). To date, a number of patients have been treated with a natural or synthetic scaffold and autologous bone marrow-derived stem cells. The intense press coverage of these surgeries further added to the hype and to considerable investment by universities, funding bodies, and governments. In a critical self-reflective step backward, Dr. Birchall reported that

only one of their patients has been reported formally, pending follow-up of others (102). Observations of the problems encountered, such as failures of certain biomaterials and surgical complications, have not been highlighted as much as the high-profile life-saving success, but they are just as important. They lead to iterative improvements in our ability to deliver functional organs and generate the critical questions biotechnologists and clinicians may address together to achieve our goal of providing practical alternatives to organ transplantation. In assessing the true place of regenerative therapies in airway replacement and regeneration, along with consideration of technical, ethical, and commercial challenges faced before such therapies can be considered established (103), Dr. Birchall delineated a clear route forward, but at the same time predicted that it will be many years before “routine” products will match the current hype.

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

For the first time, the conference included an entire session devoted to career development, which included sessions led by the National Institutes of Health (NIH)-NHLBI, a mentoring luncheon, and a women’s and diversity forum. Small groups of young investigators were each paired with senior investigators in their relevant or closely related field of stem cells, cell therapies, or lung bioengineering during the mentoring lunch. This mentoring session was well received by both the young and senior investigators and provided a forum for discussion about careers in the field and how to navigate and evaluate different career trajectories in both academia and industry. An added benefit of the small group sessions was peer mentoring among young investigators and peer sharing of the different mentoring and training environments at different institutions.

The presentations given by Dr. Sara Lin, Ph.D., Program Director Developmental Biology and Pediatrics, Division of Lung Disease, NHLBI/NIH and Dr. Ghenima Dirami, Ph.D., 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. Lin’s presentation was on the NIH’s role in academic careers in lung stem cells, cell therapies, and bioengineering. In addition to supporting and communicating research results to the medical community, a significant part of the NIH’s mission is in training early career investigators. She discussed the current funding rates and how early career investigators can find a path to funding. Specific NIH training programs, such as Pathways to Independence Award (K99/R00), were outlined as well as strategies for transitioning to independence. Specific details of qualifications for early-stage investigators R01 applications were also discussed. There was a brief overview of the different scientific programs supported by the NHLBI—Lung Repair and Regeneration Consortium (LRRC), Progenitor Cell Biology Consortium, and the Cardiovascular Cell Therapy Research Network—as well as recent request for applications supported by NHLBI, including “New Strategies for Growing Tissues,” “Next Generation Genetic Association Studies,” and “Molecular Atlas of Lung Development.” The presentation also included a significant section on the important steps a young investigator should take to enhance their funding chances—developing a compelling scientific question, understanding the peer review process and reviewers, the role of mentors in the development of the proposal, and resources offered by the NHLBI, including the website, meeting summaries, and workshops.

Dr. Dirami gave an overview of the peer review process at the NIH and discussed strategies for enhancing chances for successful funding. She discussed specifics of the peer review process, such as getting your grant to the right study section, study section details, review criteria, and the NIH scoring system. She also gave an overview of what reviewers are typically looking for in applications and discussed common problems in applications. She then gave the relevant resources for asking questions and tracking your grant throughout the review process with the eRA commons website as well as the use of Program Officers and Scientific Review Officers at the different stages of review (web link provided in reference 104). There was also a discussion on grants specifically designed and offered for new and early-stage investigators and career development and fellowship awards and a description of the Early Career Reviewer Program, which aims to train

and educate qualified scientists in the peer-review process. Further grant offerings, such as small business innovation research and small business technology transfer applications, were also explained.

The final component to the career development session was a Women’s and Diversity Forum and Networking Session led by junior investigators Dr. Darcy Wagner and Dr. Sara Gilpin, Ph.D. All participants of the conference were invited to attend, and the session began with a networking session. A group of senior women and diversity investigators were stationed around the room to help facilitate discussion. After the open networking session there was a panel discussion on past and present hurdles to overcome for enhancing recruitment and retention of women and minorities in the field as well as strategies that the panelists have personally used to overcome these hurdles. The panel consisted of Polly Parsons, M.D., Patricia Rocco, M.D., Ph.D., Eva Mezey, M.D., Ph.D., Diane Krause, M.D., Ph.D., and Diego Alvarez, M.D., Ph.D. The discussion was centered around both individual and institutional struggles to create equal work environments and the strategies that different institutions use. The overwhelming majority of panel members and senior mentors reiterated the importance of patience and perseverance in addition to hard work for enhancing chances for success.

Overall, the career development session was overwhelmingly successful in its inaugural year and will be a critical component to future conferences.

### Session 6: EPCs, MSCs, and Cell Therapy Approaches for Lung Diseases

Moderated by Daniel Chambers, M.B.B.S., M.R.C.P., F.R.A.C.P., M.D. (University of Queensland), the overall themes of this session were the definitions for, place in, and pathogenesis and evolving use of MSCs and EPCs for the prevention and/or treatment of important diseases of the lung, ranging from acute lung injury/ARDS to pulmonary fibrosis. A recurrent principle throughout the session was the capacity of these cellular populations, and/or perhaps critical cytoplasmic elements delivered via vesicles, to function in immunomodulatory and/or tissue repair roles, rather than regenerative medicine capacities *per se*.

The first speaker in the session was Amer Rana, Ph.D., (University of Cambridge) who provided a state-of-the-art overview of EPCs. Dr. Rana highlighted the absence of specific surface markers for EPCs and hence the heterogeneity in cellular populations under study. Each EPC population may in fact represent cells with very different embryological origins or cells at different stages on the same developmental continuum. Therefore, these similarly named cells may in fact have very different identities and properties.

In this talk, Dr. Rana focused on blood-derived EPCs, which can be broadly categorized into early-EPCs (E-EPCs/MACs/CACs), which are CD31<sup>+</sup>CD14<sup>+</sup>CD45<sup>+</sup> and which have a monocytic/alternately activated M2 phenotype, and late-EPCs/blood outgrowth endothelial cells (L-EPCs/BOECs/OECs), which are CD31<sup>+</sup>CD34<sup>+</sup>CD133<sup>-</sup>CD146<sup>+</sup> and which have an endothelial cell phenotype. E-EPCs are not endothelial cells and are not able to be incorporated into endothelial networks. However, E-EPCs do contribute to endothelial network assembly and repair via paracrine effects and have the potential to act as vehicles for gene therapy. In contrast, L-EPCs are endothelial cells; they express endothelial cell-specific markers and are able to contribute to endothelial networks. Although it is likely that E-EPCs have a bone marrow origin, the origin of L-EPCs is not clear, and there may be several tissue resident sources.

The rest of the presentation focused on issues related to the translation of L-EPCs and their derivatives to the clinic and their potential applications. These included using circulating EPCs as biomarkers for disease, as vehicles to deliver gene therapies, to promote revascularization via paracrine effects and/or via incorporation/neovascularization, to extend their proliferative capacity by reducing IL8 signaling, and finally to generate iPSCs to make isogenic vasculature and pulmonary tissues, including airways. L-EPCs are a very attractive starting cell type for the generation of iPSCs as they are easy to isolate, propagate well *in vitro*, can be generated at clinical grade, are genetically stable, and, as demonstrated by Dr. Rana and his colleagues, can be readily reprogrammed.

The next speaker in this session was Professor Patricia Rieken Macêdo Rocco, M.D., Ph.D. (Federal University of Rio de Janeiro, Brazil), who provided an overview

of preclinical studies and cell therapy clinical trial activity for lung disease in Brazil. In the ovalbumin asthma model, murine bone marrow-derived mononuclear cells, which were isolated using gradient centrifugation and administered intravenously, attenuated eosinophilic inflammation, airway remodelling, and physiologic parameters (105). These effects were independent of the route of administration (intravenous or intratracheal) (106). Next, her group compared their bone marrow-derived mononuclear cells to murine bone marrow-derived MSCs. The two cell types were equally effective in the ovalbumin model; however, the mononuclear cells were more effective at reducing lung tissue levels of TGF- $\beta$  and vascular endothelial growth factor (107). Finally, Professor Rocco presented unpublished data suggesting that bone marrow-derived mononuclear cells from ovalbumin-treated mice have an attenuated effect when delivered to syngeneic ovalbumin-treated mice. She then outlined her group's plans for a phase I trial of intravenous autologous bone marrow-derived mononuclear cell therapy in severe asthma.

Next, Professor Rocco provided preclinical data for the efficacy of bone marrow-derived cells in elastase-induced murine emphysema before describing clinical trial plans in humans with cigarette smoke-induced emphysema. Her group plans a randomized (n = 5/group), placebo-controlled, phase I/II trial of endobronchial administration of  $1 \times 10^7$  human leukocyte antigen-unmatched bone marrow-derived MSCs before the delivery of endobronchial valves in patients with emphysema.

Finally, Professor Rocco outlined her lab's preclinical data in murine silicosis. In this model, intravenous or intratracheal murine bone marrow-derived mononuclear cells attenuated the fibrotic response. These data have been translated into a phase 1 trial of endobronchial administration of autologous bone marrow-derived mononuclear cells for silicosis (ClinicalTrials.gov identifier: NCT01239862). Her team has treated five patients thus far (all men, aged 37–45 yr) with mild to moderate lung function abnormalities, with satisfactory short-term safety.

In the next session, Professor Michael Matthay, M.D. (University of California – San

Francisco) updated the audience on his group's progress in developing allogeneic human MSC therapy for ARDS. In preclinical studies, mice were injured with endotoxin or live *Escherichia coli* and then treated with bone marrow-derived mouse or human MSCs, either by the intratracheal or intravenous route (108–111). Additional experiments were done in an *ex vivo* perfused human lung preparation in which lung injury was induced by endotoxin or live *E. coli* to test the therapeutic effects of intratracheal or intravenous delivery of allogeneic, clinical-grade human MSCs or of conditioned media from the MSCs (112). Experiments were also completed in a 24-hour sheep model of severe lung injury from smoke inhalation and intrapulmonary instillation of live bacteria to test for safety and efficacy of two doses of human MSCs (5 or  $10 \times 10^6$  human MSC/kg). In mice, intratracheal or intravenous administration of mouse or human bone marrow-derived MSCs reduced mortality compared with fibroblast and phosphate-buffered saline controls (108–110). Treatment with MSCs reduced proinflammatory cytokines and the quantity of pulmonary edema. Intravenous human MSCs were also effective in reducing mortality in a sepsis model of *Pseudomonas aeruginosa*-induced peritonitis in mice (111). Also, human MSCs exerted an antimicrobial effect in the lungs (pneumonia model) and in the blood (peritonitis model), in part from enhanced release of the antimicrobial factor LL-37 and increased monocyte phagocytosis (110, 111). In the perfused human lung studies with lung injury induced by endotoxin or live *E. coli*, intrabronchial or intravenous human MSCs induced a more rapid resolution of alveolar edema, reduced alveolar neutrophil influx, and accelerated bacterial clearance (112). In sheep, severe lung injury was induced by inhalation of hot cotton smoke and instillation of live *P. aeruginosa* bacteria. Intravenous delivery of cryopreserved human MSCs 1 hour after the induction of lung injury demonstrated safety as well as therapeutic efficacy over 24 hours for improved oxygenation with both doses of MSCs, and a reduction in extravascular lung water with the higher dose ( $10 \times 10^6$  human MSCs/kg).

Professor Matthay concluded that several mechanisms contribute to the therapeutic benefit of human MSCs for experimental lung injury, including a decrease in lung endothelial and alveolar epithelial injury, a decrease in acute inflammation, enhanced resolution of alveolar edema, and antimicrobial effects. The preclinical data support the potential value of human MSC therapy for patients with severe ARDS. Phase I and II clinical trials supported by an NHLBI U01 grant are planned to begin soon.

Next, Professor Duncan Stewart, M.D., F.R.C.P.C. (University of Ottawa) outlined his group's plans for the Cellular Immunotherapy for Septic Shock (CISS) phase I trial. Professor Stewart noted that although many humans have been exposed to MSC therapy in clinical trials for multiple indications, MSC therapy has not yet been evaluated in humans with septic shock. The specific evidence gaps that need to be addressed before a randomized controlled trial are the safety and optimal dose of MSCs in this setting. The CISS trial will address these objectives and be the first clinical trial to evaluate safety, tolerability, and maximum tolerable dose of MSC therapy in this vulnerable population. Professor Stewart proposes a single-center, open-label phase I safety and dose-escalating trial with a control population with no intervention ( $n = 24$ ). MSCs will be administered after stabilization of hemodynamic and pulmonary parameters and with a pulmonary artery catheter *in situ*. Patients ( $n = 3$  for each dose) will receive MSCs at each of three dose panels (low dose:  $0.3 \times 10^6$ /kg; mid dose:  $1.0 \times 10^6$ /kg; high dose:  $3 \times 10^6$ /kg). Impressively, these patient cohorts will be followed to 10 years to monitor specifically for the development of malignancy.

In the next session, Argyris Tzouveleakis, M.D., Ph.D., M.Sc. (Democritus University of Thrace, Greece), described the results of a phase 1b study of endobronchial administration of autologous adipose-derived stromal cells in patients with IPF. Cells were obtained by lipoaspiration followed by centrifugation to isolate the stromal vascular fraction. This cellular fraction was then treated with platelet-rich plasma and photo-exposure to alter the cellular phenotype. A total of  $0.5 \times 10^6$  cells/kg body weight were then introduced endobronchially into 14 subjects with moderately severe IPF. Technetium labeling confirmed pulmonary

retention of cells to 24 hours. Follow-up to 12 months confirmed an excellent safety profile for this approach to cellular therapy, with no significant changes in lung function and a modest improvement in respiratory-specific quality of life (113).

There followed a discussion involving Professor Marilyn Glassberg, M.D., Director, Interstitial Lung Disease Program, University of Miami, who was recently awarded NHLBI funding to conduct a phase I/II study of intravenous allogeneic bone marrow-derived MSC therapy in patients with IPF. Professor Glassberg outlined her study protocol and compared it with a recently completed, but at the time unpublished, Australian trial of allogeneic placenta-derived MSC in eight patients with IPF. The principal investigator for this trial, which has since been accepted for publication, was this session's moderator, Daniel Chambers (114). There was spirited and extensive discussion between the audience and the three investigators. This included deliberation on whether IPF is indeed a rational target for therapeutic actions of MSCs and whether the available preclinical data support this. Discussion also centered on the differences between these three trials, particularly with respect to cell types used, as each trial used MSCs obtained from different tissues. There was consensus that carefully done safety trials of MSCs in IPF will add useful information and that use in the early phases of IPF, or perhaps in acute exacerbations of IPF, may demonstrate benefit and that these should perhaps be the patient populations investigated in future trials. As such, there was agreement between the inclusion/exclusion criteria across these three clinical trial protocols, wherein patients with moderately severe but not end-stage IPF were targeted. At present, there is no consensus about potential dose or dosing schedules.

Next, Claudia dos Santos, M.D. (University of Toronto) presented her laboratory's work on the use of MSCs in experimentally induced sepsis. The background to her talk was that MSC treatment is known to significantly reduce sepsis-induced organ injury and mortality in mice receiving appropriate antibiotic therapy; however, the mechanisms remain poorly defined. To characterize MSC-

dependent mechanisms of protection from sepsis, her group analyzed gene expression in five sepsis-target organs (lung, liver, kidney, spleen, and heart) from mice exposed to experimental polymicrobial sepsis induced by cecal ligation and perforation treated with either placebo or MSCs. In parallel, they also profiled the expression of regulatory miRNAs in selected sepsis-target tissues. A bioinformatic analysis strategy designed to identify "common" gene expression patterns in all sepsis-target organs in response to MSC administration was exploited. MSC administration resulted in a broad range of transcriptional reprogramming amounting to approximately 13% of the murine genome. Network analysis identified three prominent effects of MSC administration that were common to all five organs: (1) reconstituted transcription of mitochondrial related genes, (2) down-regulation of innate immune proinflammatory pathways, and (3) coordinated expression of endothelial and vascular smooth muscle-related genes. Promoter analysis identified enrichment for specific transcription factor binding sites among MSC-responsive genes, and miRNA profiling identified potential target miRNAs (115).

Next, Jae-Woo Lee, M.D. (University of California, San Francisco) presented his data on the opportunity to use human mesenchymal stem cell microvesicles, rather than whole cells, for the treatment of acute lung injury. Microvesicles are circular fragments of membrane released from the endosomal compartment as exosomes or shed from the surface membranes. He proposed that human MSC microvesicles are biologically active, perhaps through transfer of mRNA from the microvesicle to the injured lung epithelium and endothelium. His vision is to use microvesicles to avoid the risks and drawbacks (potential tumor formation *in vivo*, immunogenicity, and the difficulty storing the cells for clinical therapy) of whole cell therapy.

Dr. Lee shared his data demonstrating abrogation of neutrophilia, reduced bronchoalveolar lavage (BAL) MIP-2 levels, and restoration of lung protein permeability during murine endotoxin-induced lung injury by MSC-derived, but not fibroblast-derived, microvesicles (45). The therapeutic effect was evident regardless of whether

microvesicles were delivered intravenously or intratracheally. These *in vivo* studies were elaborated on in a primary human alveolar epithelial type II culture model, where again MSC microvesicles restored protein impermeability. The suggested mechanisms of action of MSC microvesicles included increased expression of KGF, potentially through direct delivery of mRNA, because KGF protein appeared in greater amounts in BAL after microvesicle treatment, an effect abrogated by inhibitory RNA; coadministration of a blocking antibody to KGF attenuated the therapeutic effect of microvesicles; and recombinant exogenously delivered KGF mirrored the therapeutic effect. However, MSC microvesicles also altered the murine macrophage phenotype by down-regulating TNF- $\alpha$  expression and up-regulating IL-10 expression.

The next speaker in the session was travel award winner Diego Alvarez (University of South Alabama), who revisited the biology of EPCs. Dr. Alvarez again highlighted the distinction between early- and late-outgrowth EPCs, which had been extensively outlined in the talk by Dr. Rana. He also introduced the idea of vascular-resident EPCs, which display a phenotype more similar to late-outgrowth EPCs, are able to generate secondary endothelial colonies, and are vasculogenic (116). These cells exist in distinct niches within the various segments of the vascular tree. Next, Dr. Alvarez outlined the therapeutic potential of resident microvascular EPCs in a murine model of *Pseudomonas* pneumonia, where they engrafted and restored vascular barrier integrity, and as a cellular source to create bioengineered pulmonary vasculature. To close, Dr. Alvarez demonstrated that the nucleosomal assembly proteins (NAP) like 1 (NAP1) and like 2 (NAP2) are molecular determinants of progenitor cell capacity and may prove useful as markers to identify EPCs.

Finally Susan Majka, Ph.D., (Associate Professor of Medicine, Allergy Pulmonology, and Critical Care, Vanderbilt) presented on behalf of Trainee travel award recipient Melissa Matthews, who could not be present. Dr. Majka's talk focused on how resident lung mesenchymal cells may be required for lung architecture homeostasis. Her laboratory has noted that primitive mesenchymal cells are

identifiable in adult tissues and that they adopt a perivascular location. She and her team hypothesized that dysfunction of these cells in adult lung may lead to organ dysfunction through vascular rarefaction. They define lung MSCs using the side population phenotype and expression of the ATP-binding cassette subfamily G member 2 (ABCG2) (117). Support for this population representing lung MSCs includes their typical surface marker expression, trilineage differentiation potential, colony-forming capacity, and high level of telomerase expression (117, 118). In addition to these MSC-like characteristics, the population has the capacity to differentiate into a perivascular endothelial and pericyte precursor population (118). Of great interest, lung MSCs were found to be depleted in a number of animal models of lung disease, including the bleomycin fibrosis, hypoxia, and EC-SOD knockout-induced pulmonary arterial hypertension and hyperoxia lung simplification models (117–119). Similar depletion was also seen anecdotally in human pulmonary arterial hypertension.

To further investigate the potential role of lung MSCs in organ function, Dr. Majka and her team used an ABCG2 knockout mouse model. They found that the knockout was associated with accentuated vessel rarefaction and that MSC from these animals lost stemness and were more likely to develop a contractile phenotype characterized by  $\alpha$ -SMA expression. Dr. Majka concluded her talk by outlining data suggesting that the WNT/ $\beta$ -catenin pathway may be central to lung MSC dysfunction.

Vigorous discussion followed each of the talks in this packed session, which concluded with the speakers and audience at once exhausted and energized by the exciting data presented. A recurrent talking point was the appropriate timing and design of first-in-human clinical trials. A broad range of opinions were expressed on this front, no doubt reflecting the complexity and relative immaturity of the lung cell therapy field. Some participants believed that any clinical trial activity was premature, and others advocated a cautious approach to bedside translation for selected cell products and indications. The discussion was generally shaped around four key themes, which provide a framework for bench-to-bedside translation. These key considerations were: the strength of the

preclinical data and the robustness of the model used to recapitulate the human disease, the known and potential risk of harm of the cell product, the clinical need/availability and risk of alternative treatment options, and the likelihood that early-phase human studies will be able to provide the feedback to the bench required to speed product development.

## Session 7: Summation and Directions

Dr. Prockop opened the session by suggesting that research on therapies for lung diseases with stem/progenitor cells is developing in a manner similar to the development of successful bone marrow transplantation (BMT). Clinical trials with BMT in patients with terminal illnesses were initiated primarily by Dr. E. Donnall Thomas before many of the critical questions in the field were answered. But the data from patients helped drive the basic research. Success in the end depended on many subsequent discoveries and especially on quantitative assays and biomarkers that predicted the *in vivo* efficacy of the administered cells. Similar assays will probably be required for successful therapies for lung diseases with stem/progenitor cells (120).

Edward Morrissey, Ph.D. (University of Pennsylvania) discussed the NHLBI Lung Repair and Regeneration Consortium (LRRC). The field of lung stem cell and regeneration has grown tremendously in recent years. In contrast to some tissues, such as the heart and neural system, the lung has significant repair and regenerative capacity. However, the molecular mechanisms that promote this process are poorly understood. In response to this burgeoning field, the NHLBI established the LRRC in 2012 to investigate the mechanisms of lung repair and regeneration. The LRRC is a consortium of six research sites with an administrative coordinating center, which are charged with uncovering the basic mechanisms by which the respiratory system reacts to injury and promotes repair and regeneration. The six research sites represent diverse approaches to tackling the challenges presented by the LRRC. From the basic understanding of epigenetic pathways controlling lung gene expression to the use of decellularized matrices to explore the ability of lung cells generated

*in vitro* to engraft, these six sites represent the full span of basic to translational research in the lung field. Dr. Morrissey discussed what the LRRC plans to offer the lung research community through sharing of new reagents and tools generated with the consortium. These will include development of useful databases such as gene expression databases that could prove useful to the community in general. The ultimate goal for the LRRC is to provide deep insight into the molecular pathways that can be harnessed through new therapeutic interventions to promote lung repair and regeneration in humans.

Mahendra Rao, M.D., Ph.D., Director, Center for Regenerative Medicine, NIH, discussed development of stem cells to evaluate lung function. The NIH funds a variety of research related to diagnosis, evaluation, and treatment of lung disorders. Dr. Rao provided an update on our efforts related to developing a microfluidic device to simulate organ on a chip, efforts to develop assays to evaluate primary cells for screening, and development of engineering techniques to develop reporter lines to enhance analysis of transplanted cells and cells in culture. He expressed the hope that these efforts, combined with our efforts to make clinical grade cells available via the Production Assistance for Cellular Therapies (PACT) centers, both MSC and PSC, will help enable researchers to move forward in a cost effective fashion.

Robert Deans, Ph.D. (Athersys Inc.) discussed the role of the International Society for Cell Therapy in developing cell-based

therapies for lung diseases. The Society is working with investigators and a variety of organizations to develop criteria for the quality of cells being used for clinical trials.

## 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. It is hoped 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 official ATS Workshop Report was prepared by an *ad hoc* subcommittee of the ATS Assembly on Respiratory Cell and Molecular Biology.

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**Author disclosures:** D.J.W. reported receiving research support from United Therapeutics, paid to institution (\$100,000–\$249,999) and Athersys Inc., also paid to institution (\$50,000–\$99,999). D.J.P. reported that he chairs the scientific advisory committee of Temple Therapeutics LLC and holds an interest of less than 5% in equities of this start-up company, which is not publicly traded. D.C. reported serving as a consultant for United Therapeutics (amount not reported). A.G., A.K., D.K., P.I.L., and D.E.W. reported no relevant commercial interests.

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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.

**Acknowledgment:** The organizers thank the staffs of the University of Vermont Continuing Medical Education and University of Vermont College of Medicine Communications Offices, notably Terry Caron, Natalie Remillard, Jennifer Nachbur, and Carole Whitaker for organizational support and Gwen Landis for administrative support.

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