Stem Cells and Cell Therapies in Lung Biology and Lung Diseases

Daniel J. Weiss[1], Ivan Bertoncello[2], Zea Borok[3], Carla Kim[4], Angela Panoskaltsis-Mortari[5], Susan Reynolds[6], Mauricio Rojas[7], Barry Stripp[8], David Warburton[9], and Darwin J. Prockop[10]

[1]University of Vermont College of Medicine, Burlington, Vermont; [2]University of Melbourne, Victoria, Australia; [3]University of Southern California, Los Angeles, California; [4]Boston Children's Hospital, Boston, MA; [5]University of Minnesota, Minneapolis, MN; [6]National Jewish Health, Denver, CO; [7]University of Pittsburgh, Pittsburgh, PA; [8]Duke University Medical Center, Durham, NC; [9]Children's Hospital of Loas Angeles, Los Angeles, CA; and [10]Texas A&M Health Science Center, Temple, TX

EXECUTIVE SUMMARY

The University of Vermont College of Medicine and the Vermont Lung Center, with support of the National Heart, Lung, and Blood Institute (NHLBI), the Alpha-1 Foundation, the American Thoracic Society, the Emory Center for Respiratory Health, the Lymphangioleiomyomatosis (LAM) Treatment Alliance, and the Pulmonary Fibrosis Foundation, convened a workshop, "Stem Cells and Cell Therapies in Lung Biology and Lung Diseases," held July 26–29, 2009 at the University of Vermont, 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 approaches for lung diseases. These are rapidly expanding areas of study that provide further insight into and challenge traditional views of the 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 two previous conferences held at the University of Vermont, "Adult Stem Cells, Lung Biology, and Lung Disease" sponsored by the NHLBI, the Cystic Fibrosis Foundation, the University of Vermont College of Medicine, and the Vermont Lung Center in 2005 and "Stem Cells and Cell Therapies in Lung Biology and Diseases sponsored by the NHLBI, Alpha-1 Foundation, American Thoracic Society, Pulmonary Fibrosis Foundation, University of Vermont College of Medicine, and the Vermont Lung Center in 2007. Those conferences have been instrumental in helping to guide research and funding priorities (1, 2).

Since the 2007 conference, investigations of stem cells and cell therapies in lung biology and diseases have continued to expand rapidly. However, there continue to be changes in focus and direction, particularly with respect to cell-based therapy approaches. Recent studies of immunomodulation and paracrine effects of adult stem and progenitor cells, notably adult mesenchymal stromal (stem) cells (MSCs) derived from bone marrow, adipose, and other tissues, have increasingly provided evidence of efficacy in animal models of acute and fibrotic lung injuries as well as in asthma, bronchopulmonary dysplasia, chronic obstructive pulmonary disease (COPD), sepsis, and other lung diseases. Although the mechanisms of MSC effects in these models are not yet fully understood, growing evidence implicates both soluble mediators released by the MSCs as well as cell to cell contact of MSCs with different inflammatory and immune effector cells. These studies have recently been extended to human lung explant models, and it is anticipated that clinical investigations of initial safety and efficacy of MSCs in acute lung injury will occur in the near future. In parallel, a 6-month interim analysis of a current clinical trial in the United States assessing systemic administration of MSCs in patients with moderate to severe COPD has demonstrated safety and has yielded promising results with respect to efficacy. This trial has completed its 2-year observation period, and data is expected to be released in late 2010 or early 2011. Circulating endothelial progenitor cells (EPCs) can contribute to regeneration of diseased pulmonary vasculature, and two recent clinical investigations in China have suggested the efficacy of autologous bone marrow–derived EPC administration in both adult and pediatric patients with pulmonary hypertension. A comparable trial of autologous EPC administration in pulmonary hypertension, the Pulmonary Hypertension: Assessment of Cell Therapy (PHaCET) trial, is ongoing in Canada. Circulating endothelial progenitor cells may also play roles in both acute lung injury and in fibrotic lung diseases.

Engraftment of systemically or intratracheally administered cells remains a controversial issue. Although most available evidence argues against significant engraftment, publications and abstracts presented at the conference suggest that several newly investigated cell types, including those derived from placental tissues or novel cell populations derived from adult bone marrow, may demonstrate a more robust ability to engraft and participate in lung repair. Further, significant advances continue to be made in novel areas of investigation including increasing exploration of 3-dimensional culture systems and bioengineering approaches to generate functional lung tissue *ex vivo* and *in vivo*. This has culminated in the first successful clinical use of a bioengineered trachea. In parallel, several recent reports demonstrate the potential feasibility of using decellularized whole lungs as scaffolds for recellularization and subsequent implantation. These areas are predicted to be of intense investigation over the next several years.

Comparably, progress continues in studies of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS). Several

(*Received in original form December 22, 2010; accepted in final form February 3, 2011*)

Conference was supported by: Alpha-1 Foundation, American Thoracic Society, Emory Center for Respiratory Health, LAM Treatment Alliance, National Heart, Lung, and Blood Institute, R13 Conference Grant (HL097533), Pulmonary Fibrosis Foundation, University of Vermont Department of Medicine, and the Vermont Lung Center.

Correspondence and requests for reprints should be addressed to Daniel J. Weiss, M.D., Ph.D., Associate Professor of Medicine Pulmonary and Critical Care, Vermont Lung Center, University of Vermont College of Medicine, Burlington, VT 05405. E-mail: dweiss@uvm.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Proc Am Thorac Soc Vol 8. pp 223–272, 2011
DOI: 10.1513/pats.201012-071DW
Internet address: www.atsjournals.org

groups have presented protocols for deriving definitive endoderm from either ESCs or iPS cultured *in vitro* and some have further demonstrated the ability to generate cells with phenotypic markers of type 2 alveolar cells from either mouse or human ESCs. In addition, a recent study demonstrated the potential ability of type 2 alveolar epithelial cells, derived in culture from human embryonic stem cells, to engraft in lung and ameliorate experimentally induced lung injury in a rodent model. Nonetheless, many challenges remain to generating functional lung cells from either ESCs or iPS. One potentially fruitful area of investigation derives from the recently demonstrated generation of disease-specific iPS from tissue samples obtained from patients with cystic fibrosis, α-1 antitrypsin deficiency, and other genetic or acquired lung diseases.

Significant progress continues to be made in investigations of local (endogenous) stem and progenitor cells residing in the lungs. Advances in lineage-tracing approaches and other techniques have provided important insights into understanding the identity and lineage expansion properties of previously identified putative endogenous progenitor populations and suggest an increasingly complex network of cellular repair after injury. However, the study of endogenous lung stem and progenitor cells remains complicated by the role of the specific microenvironmental niches in which these cells reside. Alteration of the niches with experimental protocols or removal of cells from the niches can change their identifying characteristics and biologic activities. One of the challenges continuing to face the field is 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. Correlative information in human lungs remains poorly defined.

A continuing issue of confusion is that of terminology. 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 mesenchymal stromal cells and endothelial progenitor cells, are not agreed upon. 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 the comparison of different investigative approaches. A glossary of relevant working definitions applicable to the lung, originally presented in the report of the 2007 conference, is depicted in Table 1. This glossary does not necessarily reflect a consensus for the definition of each term and will undergo continuing revision as an overall understanding of the cell types and mechanisms involved in lung repair continue to be elucidated. Nonetheless, it is a useful framework.

The first session, "Endogenous Lung Progenitor Cells/Lung Cancer Stem Cells," following an overview of the field by Paul Simmons (University of Texas) and respective presentations by Susan Reynolds (National Jewish), Ed Morrisey (University of Pennsylvania), Barry Stripp (Duke), Majd Mouded (University of Pittsburgh), and Kerstin Sinkevicius (Boston Children's Hospital), reviewed the current state of knowledge of endogenous progenitor cell populations, mechanisms regulating their behavior, and their potential to initiate or augment repair. This included lessons learned from lung development, the role of the local microenvironmental niches, and consideration of lung cancer progenitor cells. Key points emphasized during this session were that stem cells are operationally defined not solely by their intrinsic developmental potential but by their interaction with the microenvironments in which they reside. Further, the stem cell niche is a dynamic "temporal" niche with the capacity to modify stem cell behavior/readout in different contexts. Moreover, stem cell–associated markers are not uniquely expressed by stem cells and are unreliable predictors of the "stem" or "progenitor" cell potential of isolated cells. Validation by functional assays and lineage-tracing studies, particularly when interrogating isolated cells where histomorphometric spatial and positional cues are lost, are increasingly valid and necessary.

The second session, "Embryonic Stem Cells, iPS, and Lung Regeneration," included a featured talk on nuclear reprogramming and pluripotency by Konrad Hochedlinger (Massachusetts General) that was followed by presentations from Carolyn Lutzko (Los Angeles Children's Hospital), Darrell Kotton (Boston University), Rick Wetsel (University of Texas) and Peter Lelkes (Drexel University), which highlighted developments in these areas. One of the notable advances in this area is the improved sophistication in directing ESCs *in vitro* through stages involved in generation of definitive endoderm and subsequently into cells with some phenotypic characteristics of type 2 alveolar epithelial cells. Comparable data demonstrates that iPS can be similarly manipulated toward definitive endoderm and potentially toward lung epithelial cells. However, full phenotypic and functional characterization of putative lung cells derived from ESCs or iPS remains controversial and is also an area where more rigorous methods are required. Novel data was presented demonstrating for the first time that *in vivo* administration of ESC-derived cells could mitigate experimentally induced lung injury. Whether this reflects engraftment of the cells in the lung or a heretofore unrecognized paracrine effect is not yet clear.

The third session, "Bioengineering Approaches to Lung Regeneration," featured an overview by Dame Julia Polak (Imperial College London) followed by presentations from Christine Finck (University of Connecticut), David Hoganson (Massachusetts General), Edward Ingenito (Brigham and Women's), Viranuj Sueblinvong (Emory University), and Charles Vacanti (Brigham and Women's), which explored new and developing areas in bioengineering approaches for cell therapies of lung diseases. Advances in scaffold systems, understanding the role of cyclic mechanical forces, and other related areas were discussed. The fourth and fifth sessions, "MSC Immunomodulation of Immune and Inflammatory Responses," and "EPCs and Clinical Trials in Lung Diseases" highlighted recent advances in cell therapy approaches for lung diseases. After the featured presentations in each session given by, respectively, Armand Keating (University of Toronto) and Mervin Yoder (Indiana University), presentations by Ryang Hwa Lee (Texas A and M), Conrad Liles (University of Toronto), Michael Matthay (UCSF), Daniel Weiss (University of Vermont), Serpil Erzurum (Cleveland Clinic), Asrar Malik (University of Illinois), Judith Shizuru (Stanford), and Duncan Stewart (University of Ottawa) highlighted different areas of advance.

The final session, "Summation and Direction," featured a review of the current state of lung engraftment by Diane Krause (Yale) that was followed by perspectives given by representatives of the NHLBI, FDA, and each of the sponsoring nonprofit respiratory disease organizations, a presentation on ethics and policy issues in stem cell research by Jeffrey Kahn (University of Minnesota) and a summary by David Scadden (Harvard Stem Cell Institute). The conference concluded with vigorous discussion on future research and funding priorities led by Darwin Prockop (Texas A and M). As in previous conferences, discussion was spirited as to how and when to proceed to further clinical investigation in addition to the recent trials for COPD and for pulmonary hypertension. It was agreed that strong emphasis must continue be placed on animal models of human lung diseases, with a focus on studies that incorporate relevant functional outcome measures. Nonetheless, the safety and initial efficacy results obtained with the trial of MSCs in COPD and the

TABLE 1. GLOSSARY AND DEFINITION OF TERMINOLOGY

Potency	Sum of Developmental Options Available to Cell
Totipotent	Ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues, and thus to (re)generate an organism in total. In mammals only the zygote and the first cleavage blastomeres are totipotent.
Pluripotent	Ability of a single cell to produce differentiated cell types representing all three germ layers and thus to form all lineages of a mature organism. Example: embryonic stem cells.
Multipotent	Ability of adult stem cells to form multiple cell types of one lineage. Example: hematopoietic stem cells.
Unipotent	Cells form one cell type. Example: spermatogonial stem cells (can only generate sperm)
Reprogramming	Change in epigenetics that can lead to an increase in potency, dedifferentiation. Can be induced by nuclear transfer, cell fusion, genetic manipulation.
Transdifferentiation	The capacity of a differentiated somatic cell to acquire the phenotype of a differentiated cell of the same or different lineage. An example is epithelial–mesenchymal transition (EMT), a process whereby fully differentiated epithelial cells undergo transition to a mesenchymal phenotype giving rise to fibroblasts and myofibroblasts.
Plasticity	Hypothesis that somatic stem cells have broadened potency and can generate cells of other lineages, a concept that is controversial in mammals.
Embryonic stem cell	Cells isolated from the inner mass of early developing blastocysts. ES cells have the capacity for self renewal and are pluripotent, having the ability to differentiate into cells of all embryologic lineages and all adult cell types. However, ES cells cannot form extraembryonic tissue such as trophectoderm.
Adult stem cell	Cells isolated from adult tissues including bone marrow, adipose tissue, nervous tissue, skin, umbilical cord blood, and placenta that have the capacity for self renewal. In general, adult stem cells are multipotent, having the capacity to differentiate into mature cell types of the parent tissue. Some populations of adult stem cells, such as MSCs exhibit a range of lineage differentiation that is not limited to a single tissue type. Whether adult stem cells exhibit plasticity and can differentiate into a wider variety of differentiated cells and tissues remains controversial.
Adult tissue-specific stem cell	Same as adult stem cells, but with defined tissue specificity. A relatively undifferentiated cell within a given tissue that has the capacity for self-renewal through stable maintenance within a stem cell niche. Adult tissue-specific (endogenous) stem cells have a differentiation potential equivalent to the cellular diversity of the tissue in which they reside. The hematopoietic stem cell is a prototypical adult tissue stem cell.
Induced pluripotent stem cell	Reprogrammed adult somatic cells that have undergone dedifferentiation, such as dermal fibroblasts, reprogrammed by retroviral transduction to express four transcription factors: Oct 3/4, Sox2, c-Myc, and Klf4. iPS cells are similar to ES cells in morphology, proliferation, gene expression, and ability to form teratomas. *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.
Progenitor cell	A collective term used to describe any proliferative cell that has the capacity to differentiate into different cell lineages within a given tissue. Unlike stem cells, progenitor cells have limited or no self-renewal capacity. The term progenitor cell is commonly used to indicate a cell can expand rapidly, but undergoes senescence after multiple cell doublings. Terminology that takes into account the functional distinctions among progenitor cells is suggested below.
Transit-amplifying cell	The progeny of a endogenous tissue stem cell that retain relatively undifferentiated character, although more differentiated than the parent stem cell, and have a finite capacity for proliferation. The sole function of transit-amplifying cells is generation of a sufficient number of specialized progeny for tissue maintenance.
Obligate progenitor cell	A cell that loses its ability to proliferate once it commits to a differentiation pathway. Intestinal transit-amplifying cells are obligate progenitor cells.
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 Clara cells are an example of this cell type.
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 a transit-amplifying cell. Within this type of hierarchy, renewal potential resides in cells at the top of the hierarchy, that is, the stem and transit-amplifying cell, and cells at each successive stage of proliferation become progressively more differentiated.
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 following progenitor cell depletion.
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 broadly distributed, long-lived, and a subset of these cells, the facultative progenitor cell, retain the ability to enter the cell cycle. The relative stability of the differentiated cell pool is paralleled by infrequent proliferation of stem and/or transit amplifying cells. The lung is an example of a slowly renewing tissue.
Hematopoietic stem cell	Cell that has the capacity for self renewal and ability to differentiate into mature leukocytes, erythrocytes, and platelets. Whether HSCs exhibit plasticity and can differentiate into mature cells of other lineages remains controversial.
Endothelial progenitor cell	Circulating cells that have the potential to proliferate and differentiate into mature endothelial cells. Studies of EPCs have been complicated by the use of the same terminology to define at least two different cell populations that have different cell surface markers, different cell sources, and different abilities to differentiate into mature endothelial cells *in vitro* and *in vivo*. There is a critical need to develop a consensus definition of EPCs with particular emphasis on the functional capabilities of these cells.
Mesenchymal stromal (stem) cell	Cells of stromal origin that can self-renew and have the ability to differentiate into a variety of cell lineages. Initially described in a population of bone marrow stromal cells, they were first described as fibroblastic colony-forming units subsequently as marrow stromal cells, then as mesenchymal stem cells, and most recently as multipotent mesenchymal stromal cells or MSCs. MSCs have now been isolated from a wide variety of tissues, including umbilical cord blood, Wharton's jelly, placenta, adipose tissue, and lung. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has recently updated the minimal criteria for defining (human) MSCs (Table 3). MSCs have been described to differentiate into a variety of mature cells types and may also have immunomodulatory properties.

(*Continued*)

TABLE 1. (CONTINUED)

Potency	Sum of Developmental Options Available to Cell
Fibrocyte	A cell in the subset of circulating leukocytes that produce collagen and home to sites of inflammation. The identity and phenotypic characterization of circulating fibrocytes is more firmly established than that for EPCs. These cells express the cell surface markers CD34, CD45, CD13, MHC II and also express type 1 collagen and fibronectin.
Bronchiolar stem cell	A term applied to a rare population of toxin (i.e., naphthalene)-resistant CCSP-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 following depletion of transit-amplifying (Clara) cells. Lineage tracing studies indicate that these cells have the differentiation potential to replenish specialized cell types of the bronchiolar epithelium. Human correlates have not yet been identified.
Bronchioalveolar stem cell	A term applied to a small population of cells located at the bronchoalveolar duct junction in mice identified *in vivo* by dual labeling with CCSP and SPC and by resistance to destruction with toxins (i.e., naphthalene). In culture, some of the dual labeled cells also express Sca1 and CD34, self renew, and give rise to progeny that express either CCSP, pro-SPC, or aquaporin 5 leading to speculation that a single cell type has the capacity to differentiate into both bronchiolar (Clara cells) and alveolar (type 1 and 2 pneumocytes) lineages. At present, the relationship of the cells studied *in vitro* to those observed by dual labeling *in vivo* is unclear. Human correlates have not yet been identified.

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increasing body of data demonstrating efficacy in other inflammatory and immune-mediated lung injury disease models suggests a potential role in inflammatory and immune-mediated lung diseases even in the absence of a comprehensive understanding of the mechanisms by which the MSCs are acting.

All participants acknowledged that the role of endogenous lung progenitor cells and of cell therapy approaches for lung diseases remains a timely and exciting area of study. Nonetheless, there are many areas in which our understanding of the processes and mechanisms remain poorly understood. Recommendations for areas of continued and future investigation are presented in Table 2. Following a review of the current literature, more extensive details on each session are presented below. The conference program, executive summaries for each speaker, and abstracts from the poster sessions are included in the on-line supplement.

BACKGROUND AND REVIEW

A comprehensive summary of relevant published literature since the 2007 workshop through the fall of 2010 is presented below. Please see the reports from previous workshops for a comprehensive review of previous literature in the field (1, 2). Readers are also referred to a number of general reviews (3–48) and specific reviews of each of the topics below that have been published over the past approximately 3 years.

Endogenous Lung Stem and Progenitor Cells

Endogenous tissue stem cells are thought to contribute to tissue maintenance and repair. Best characterized in the intestine, these cells are rare, undifferentiated, and are localized to specialized niches within each tissue. Tissue-specific stem cells exhibit self-renewal capacity and give rise to daughter cells, termed as transit amplifying cells, which in turn give rise to the more specialized or differentiated cells specific to that organ. It has also been proposed that some of the differentiated cell types can be induced to a mitotically active state. In this capacity, these cells have been termed facultative progenitor cells. Such facultative progenitor cells perform general tissue functions on a daily basis but can enter the mitotic cell pool for tissue injury repair. Thus, a facultative progenitor cell pool functions as a large and broadly distributed pool of reparative cells and can supplement the reparative capacity of the tissue stem cell. Alternatively, the facultative progenitor pool may serve for routine tissue homeostasis and regeneration whereas the tissue stem cells only come to play in more extreme situations of injury. These topics are further explored in several recent reviews (9, 10, 22, 25, 26, 28, 29, 34, 37, 46).

It remains unclear if paradigms and hierarchies described for endogenous stem and progenitor cells in organs such as the intestine also apply to the lung. The lung is a complex organ containing many distinct cell types that are distributed in several different regional microenvironments along the pulmonary tract (Figure 1) (49). Consequently, whereas the identification of cells that can proliferate under steady state or injury conditions has been relatively straightforward, characterization and classification of mitotically active putative endogenous stem and progenitor cells into a hierarchy has been challenging. The difficult questions are *1*) if the cells should be arranged into a hierarchy; and *2*) if there is a hierarchy, how the cells should be arranged. Finally, if there is a hierarchy, what are the defining characteristics of cells at different levels of the hierarchy: is it differentiation and proliferation potential as in the intestine? Or are there other more important issues that are specific to the lung that might include cell cycle time and frequency? Further, analyses and interpretations of potential stem and progenitor cells in different regions of the lung have been complicated by lack of agreement on definition, terminologies, and functions of the putative stem cells populations. This continues to complicate the field despite efforts to come to an agreement on terminologies. A proposed list of terminologies was included in the report of the 2007 conference and is repeated here (Table 1). Although there is some degree of consensus with the proposed definitions, there is still disagreement and ongoing debate and discussion. Nonetheless, analyses of lung stem and progenitor cells in animal models, particularly the mouse, have led to important advances over the past 5 years. It seems most likely that distinct stem and/or progenitor cell populations maintain specific anatomic regions of the lung (Figure 1).

Evidence for distinct airway epithelial progenitor cell populations comes predominantly from studies in mice, but there is data from proliferation analyses in other species including hamster (50), rat (51), ferret (52), nonhuman primate (53, 54), and human (55). Much of the evidence for different airway progenitor cell populations and hierarchies has also come from studies in which selective ablation of epithelial cells was achieved through exposure of mice to toxic chemicals such as naphthalene or SO_2. Naphthalene selectively depletes facultative bronchiolar progenitor populations such as Clara cells, whereas SO_2 exposure can deplete upper airway cells allowing for assessment of underlying basal cell facultative progenitors. More recently these methods have been combined with genetic lineage tracing to evaluate differentiation potential of putative stem and progenitor cell populations (56–63). These analyses have identified five populations of airway epithelial cells in the mouse that have the ability to enter the cell cycle after injury to the lungs and thus be considered as facultative progenitor cells: basal (64), Clara-like, Clara, pulmonary neuroendocrine, and alveolar type 2 cells (56–58). Notably the difference between

TABLE 2. OVERALL CONFERENCE SUMMARY RECOMMENDATIONS:

Basic

- Strong focus must be placed on understanding immunomodulatory and other mechanisms of cell therapy approaches in different pre-clinical models.
- 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 analyses to maximize detection of rare events.
- 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.
- Encourage new research to elucidate molecular programs for development of lung cell phenotypes.
- Investigate the mechanisms and potential roles of epithelial/endothelial/mesenchymal transitions (EMT, MET, etc.) in lung injury and/or repair/remodeling.
- Comparatively identify and study endogenous stem/progenitor cell populations between different lung compartments and between species.
- Develop robust and consistent nomenclature for the endogenous cell populations.
- Develop more sophisticated tools to identify, mimic, and study *ex vivo* the relevant microenvironments for study of endogenous lung progenitor/stem cells.
- 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.
- Elucidate mechanisms by which embryonic and induced pluripotent stem cells develop into lung cells/tissue.
- Develop disease specific populations of ES and iPS, for example for CF and α_1-antitrypsin deficiency with the recognition that no strategy has yet been devised to overcome the propensity of ES and iPS cells to produce tumors.
- 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. This is predicted to be an area of rapid expansion.
- Evaluate effect of mechanical forces including stretch and compression pressure on development of lung from stem and progenitor cells.
- Identify additional cell surface markers which characterize lung cell populations for use in visualization and sorting techniques.
- Disseminate information about and encourage use of existing core services, facilities, and weblinks.
- Actively foster inter-institutional, multi-disciplinary research collaborations and consortiums as well as clinical/basic partnerships. Include a program of education on lung diseases and stem cell biology. A partial list includes NHLBI Production Assistance for Cellular Therapies (PACT), NCRR stem cell facilities, GMP Vector Cores, small animal mechanics and CT scanner facilities at several pulmonary centers.

Translational

- Support high-quality translational studies focused on cell-based therapy for human lung diseases. Pre-clinical 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/pre-clinical studies should include rigorous comparisons of different cell preparations with respect to both outcome and toxicological/safety endpoints. For example, it is not clear which MSC or EPC preparation (tissue source, laboratory source, culture scheme, etc.) is optimal for clinical trials in different lung diseases.
- Incorporate rigorous techniques to unambiguously identify outcome measures in cell therapy studies. Pre-clinical models require clinically relevant functional outcome measures (e.g., pulmonary physiology/mechanics, electrophysiology, and other techniques).

Clinical

- Proceed 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 NCRR/NIH Center for Preparation and Distribution of Adult Stem Cells (MSCs; http://medicine.tamhsc.edu/irm/msc-distribution.html), coordination among multiple centers, and registry approaches to coordinate smaller clinical investigations.
- Clinical trials must include evaluations of potential mechanisms and this should include mechanistic studies as well as assessments of functional and safety outcomes. Trials should include, whenever feasible, collection of biological materials such as lung tissue, BAL fluid, blood, and so on for investigation of mechanisms as well as for toxicology and other safety endpoints.
- 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 versus host disease will provide novel and invaluable information about potential MSC effects on development and the clinical course of bronchiolitis obliterans.
- Work with industry to have access to information from relevant clinical trials.

Clara cells and Clara-like cells further highlights the difficulty in identifying facultative progenitor cells. A Clara-like cell is a cell that expresses Clara cell secretory protein (CCSP) in the tracheobronchial epithelium rather than Max Clara's original definition for the Clara cell, which specified a terminal bronchiolar location. It is clear from ultrastructural analyses that there are epithelial secretory cells throughout the proximal to distal axis (54). However, they express a different repertoire of secreted proteins. Clara and Clara-like cells are also differentially sensitive to naphthalene as well as other toxic agents. Most importantly, the proximal Clara-like cells are derived from a different progenitor than the distal airway Clara cells (46).

Recent data suggests that, in mice, the facultative progenitor cell pool accounts for much of the airway epithelial cell replacement during normal homeostasis but can also significantly contribute to tissue repair after cellular injury (65). These studies have also been the basis for isolation of putative stem cells using flow cytometric analyses of lung homogenates to sort by cell surface markers. Cell populations isolated in this manner have been functionally tested *in vitro* (66, 67) or through ectopic transplantation approaches (68–70) and have suggested that these cells have the ability to regenerate various populations of airway and/or alveolar cells. However, reconstitution of the healthy or injured lung as a final, but critically important goal for the lung stem cell field, has not yet been achieved.

The trachea and large airway compartment contains two major epithelial cell lineages, basal and secretory/ciliated cells. A subpopulation of basal epithelial cells had been previously implicated as a tissue stem cell on the basis of morphology in combination with analysis of differentiation potential or label retention (69–71). Lineage tracing studies suggested that these cells express cytokeratins 5 or 14 and could serve as precursors for differentiated airway epithelial regeneration following injury (59–61). In contrast, pulse-chase studies have demonstrated that the Clara-like cell serves as a self-renewing cell type and as the progenitor for ciliated airway cells in rats (72–75). However, lineage tracing of Clara-like cells demonstrates that the Clara-like cells do not replenish all cell types in the tracheal and proximal airways (59, 60). This lack of consensus on whether a stem cell participates in repair of the upper airway epithelium

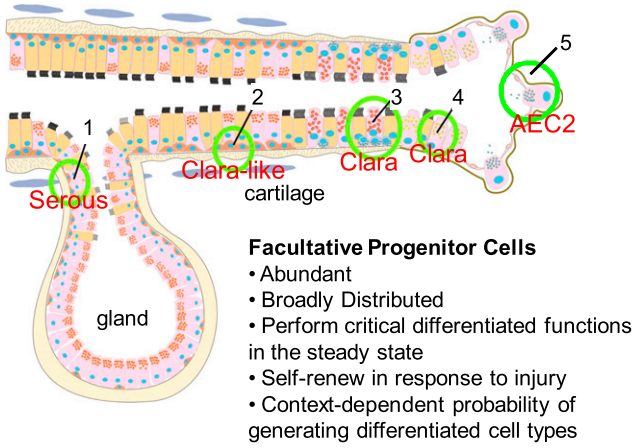

Figure 1. A graphic representation of putative stem cell niches in the airway epithelium. (*1*) Basal cells in the gland duct; (*2*) surface basal cells typically present in the intercartilaginous zone; (*3*) variant Clara cells associated with pulmonary neuroendocrine cell (PNEC) bodies; (*4*) variant Clara cells present at the bronchiolar–alveolar duct junction. *See* text for further explanation. Reproduced with permission from Reference 49.

reflects in part a failure to use all of the necessary stem cell analysis methods in a single study. This remains a significant limitation in the field. However, despite this, the use of multiple injury models *in vivo*, including exposure to SO_2, detergent, and naphthalene, as well as *in vitro* analyses (61, 76–78), suggest that a subset of basal cells serve a role as either tissue stem cells or facultative progenitor cells of the upper airway in mice. Furthermore, similar conclusions have been derived using human cells in *ex vivo* or *in vitro* culture systems (59–61). Overall, the data for this compartment, while still limited, provides the strongest evidence for a lung tissue–specific stem cell to date. However, the question remains as to whether the classical hierarchies defined for the intestine and hematopoietic system can be applied to the lung. If constrained to the classical model, all the data at present cannot yet be accounted for. It seems reasonable to define a model that works for the lung and consider the possibility that different compartments of the lung use distinct types of hierarchies. For instance, in the tracheobronchial region there are at least two facultative progenitor cell pools: the basal cells and the Clara-like cells. In the steady state, they take care of their own lineages. However, after injury progenitor-progeny relationships change. Incorporating these data into a revised model, and recognizing the fact that the data supporting a role for a lung tissue stem cell in repair comes from experimental systems in which extensive injury was induced, may provide a more cohesive explanation of lung tissue repair.

In mice, the predominant epithelial cell of the smaller airways is the nonciliated Clara cell, which exhibits characteristics of a facultative progenitor cell after injury to ciliated airway epithelial cells. However, unlike transit-amplifying cells in tissues with higher rates of epithelial turnover, such as intestine, Clara cells exhibit a low proliferative index in the steady state, are broadly distributed throughout the bronchiolar epithelium, and contribute to the specialized tissue function. Earlier pulse-chase studies identified the Clara cell as a progenitor for ciliated cells (72–75). More recently, these experiments were repeated using lineage tracing methods. The latter data set confirmed the injury/repair studies and provided the new observation that steady-state Clara cells self-renewed and that Clara cells functioned as a progenitor for distal airway ciliated cells during homeostasis (63). However, increasing data suggest fundamental differences between Clara-like and Clara secretory cells in proximal versus distal airways, respectively (60, 61), and thus the situation is more complicated than previously appreciated.

In another widely used approach, the Clara cell–specific toxin, naphthalene, has been used extensively to deplete the bronchiolar Clara cell pool. This toxin is metabolized by the cytochrome P450 isozyme found in Clara cells and parenteral treatment (i.e., intraperitoneal administration) results in dose-dependent depletion of the Clara cell population in mice. A population of naphthalene-resistant cells, termed variant Clara cells (vCE), was identified as a bronchiolar stem cell (56, 64, 65). vCE are located within discrete microenvironments that include the neuroepithelial body and bronchoalveolar duct junction (66, 67). In the bronchoalveolar duct junction, naphthalene-resistant Clara cells stain both for CCSP and for pro-Surfactant Protein C (SPC) (66). However, these cells were not shown to express the stem cell characteristic of label retention. Interestingly, the proSPC/CCSP dual positive cells increased in number after naphthalene injury and during compensatory lung growth following unilateral pneumonectomy in mice (66, 79). Further, when pro-SPC/CCSP dual positive cells were isolated using methods developed for enrichment of type 2 alveolar epithelial cells, some of the dual-labeled cells exhibited a unique cell surface phenotype, $Sca1^{pos}/CD34^{pos}/CD45^{neg}/CD31^{neg}$. These cells were found to proliferate in culture and give rise to progeny expressing CCSP, pro-SPC, or aquaporin 5 (66). As such, these cells were termed bronchioalveolar stem cells (BASCs). However, lineage tracing methods demonstrated that the bronchiolar and alveolar domains were distinct in the normal lung (80). Further, no contribution of lineage tagged Clara cells to alveolar epithelia was detected during steady state homeostasis or after hyperoxic injury (60, 63). These data suggest that cultured lung progenitor cells may assume a broader spectrum of cell phenotypes than their *in vivo* counterparts.

The complexity of the bronchiolar stem cell hierarchy is further confounded by the methods used to identify and evaluate the putative stem cell. Importantly, the presence of the abundant Clara cell facultative progenitor cell pool necessitates use of extreme levels of injury to activate the putative stem cell. Thus, the phenotype and function of the putative stem cell must be viewed in the context of epithelial injury and collateral damage to other tissue types and systemic effects. Further, the limited number of differentiated cell types found in the bronchiolar region, Clara, ciliated, and PNEC, has made it difficult to distinguish the putative stem cell from the facultative progenitor cell (29). A further complication is that the putative tissue-specific stem cell is quiescent during the response to some injuries such as ozone depletion of the ciliated cell pool (81) and the fact that bronchiolar stem cells did not play any greater role in normal airway epithelial homeostasis and turnover than did the abundant pool of facultative progenitor Clara cells (65). This study suggests that the neuroepithelial body–associated vCE likely function as a reserve population that can function in either normal maintenance or more relevantly following depletion of the facultative progenitor pool of vCE. Nonetheless, additional studies are needed to further confirm and clarify this hypothesis both in mouse as well as in human lungs.

Overall, it is possible that various cells thought to have stem or progenitor cell properties in the lung represent phenotypic variants of the same cell population(s). The diversity of interpretations highlight the widely recognized need for markers that are specific for the functionally distinct cell populations, more precise tools for lineage tracing, and further underscoring the importance of the *in vivo* microenvironment on cell behavior (82). For example, stem cell antigen (Sca-1), originally described as a marker of murine hematopoietic stem cells, has now been described as a marker for putative bronchiolar stem

cells and fibroblastic progenitor cells in the lung (67, 83, 84). Notably, two recent studies used flow cytometric purification methods for enrichment of putative bronchiolar stem cells from mouse lung. The first of these suggested that naphthalene-resistant bronchiolar progenitor cells (the putative bronchiolar stem cell) have a $Sca1^+/CD34^-$ cell surface phenotype and had low autofluorescence. However, this study did not use *in vitro* or *in vivo* functional analyses, thus limiting its interpretation (67). The second study suggested that clonogenic bronchiolar progenitor cells expressed EPcam and that co-culture of this cell with $Sca1^+$ mesenchymal cells promoted differentiation to $Muc1^+$ cells. The significance of Muc1 expression is unknown, as this marker is not typically used in *in vivo* analyses (83). Thus, although progress is being made in clarifying the identity and role of bronchiolar progenitor cells in mice, the role(s) of these cell populations in both normal homeostasis and in response to more severe injuries remains unclear. Moreover, little corresponding data as yet exists in other animal models or in human lungs.

In parallel with the identification of lung stem and progenitor populations, recent investigations have explored cell signaling and other regulatory mechanisms that manage putative airway progenitor populations in mice. For example, manipulations of Kras, p27, MAPK, p18, protein kinase C iota, or P*ten* have been shown to induce an expansion of bronchiolar progenitor and Bronchoalveolar Stem Cell (BASC) numbers and also to enhance lung tumorigenesis (85–94). Other cell signaling pathways such as Wnt/β-catenin, Hedgehog, and Notch cell are implicated in stem cell function in the lung and other tissues (80, 95–97). However, whereas stimulation of Wnt/β-catenin cell signaling appears to promote airway submucosal gland development, it inhibits differentiation of bronchiolar stem cells in the lung and does not appear to play a key role in maintenance or repair of the bronchiolar epithelium (80, 96, 97). The precise role of these and other pathways in endogenous lung stem and progenitor cells remains to be determined. The possibility remains that other endogenous stem or progenitor populations exist, and there is much room for additional information on regulatory mechanisms and pathways as have been elucidated in other epithelial progenitor cell populations (98).

Although it is attractive to speculate that lung diseases may in part be a consequence of endogenous airway stem cell failure, more studies are needed to draw direct connections. In particular, little is known of progenitor cell function in chronic diseases such as emphysema. More suggestive information is available for the genetic lung disease, cystic fibrosis (CF). The airway epithelium in patients with CF contains cuboidal cells that express primitive cell markers, including thyroid transcription factor and cytokeratin 7 (99). Neuroepithelial cells also express the CF transmembrane conductance regulator protein (CFTR), the defective protein in patients with CF that appears to play a role in neuropeptide secretion (100, 101). $CFTR^{-/-}$ mice contain fewer pulmonary neuroendocrine cells during embryonic development but increased numbers of these cells after birth (102). These observations suggest that endogenous airway progenitor cell pathways in CF lungs may be altered but this has not been extensively investigated or further clarified.

The question of an alveolar tissue-specific stem cell remains topical but less well explored than the airway. This deficiency is due in part to the vast reparative potential of this alveolar compartment and the critical role of gas exchange for organism viability. Alveolar epithelial reparative potential is centered on the alveolar type II cell (AECII) and the long held concept that AECII cells are precursors for AECI cells (74, 103, 104). The lack of an AECII-specific toxic agent is a further limitation to analysis of alveolar stem cells. These issues are being addressed through the use of stem cell markers and development of cell type cytotoxic genetic strategies (103, 105). In neonatal mice, a population of putative progenitor cells that expresses CCSP, stem cell antigen (SCA-1), stage-specific embryonic antigen 1 (SSEA-1), and the embryonic stem cell marker Oct-4 have been identified (106, 107). These cells were able to form epithelial colonies and differentiate into both type 1 and type 2 alveolar epithelial cells. However, the lineage relationship between alveolar type 2 and 1 cells has been challenged, and alveolar type 1 cells are a mitotic cell type *in vitro* (104). As indicated above, additional studies are needed to resolve these controversies.

Several studies suggest that tissue stem cells may be targets for environmental agents including pneumotrophic pathogens. Airway stem or progenitor-like cells were susceptible to infection with the severe acute respiratory syndrome (SARS) virus, raising the possibility that endogenous lung progenitor cells may be specific disease targets (106). Comparably, the basal epithelial cells of the trachea and upper airways appear more susceptible to infection with the common cold rhinovirus (108). However, it is likely that viruses such as SARS and rhinovirus target a wide range of respiratory epithelial cells in addition to progenitor cells. Endogenous progenitor cells may also be attractive candidates for targeting with gene transfer vectors that provide sustained expression. For example, intratracheally administered recombinant adeno-associated vectors may preferentially target vCE in adult mice whereas recombinant lentivirus vectors administered into the amniotic fluid may preferentially target airway progenitors in fetal mouse lungs (39, 109, 110).

Less information is available regarding the progenitor cell populations that maintain other cell populations such as interstitial, smooth muscle, or endothelial cells in the lung (24). Recently, several groups identified what appear to be resident mesenchymal stromal (stem) cells in mouse and sheep lung as well as in human nasal mucosa and in neonatal and adult human lungs (111–121). What role, if any, these cells might play in the repair of lung tissue, or in immune surveillance and immunomodulation, is unclear (115, 117, 120).

In addition to the role of endogenous lung stem and progenitor cells in repair from lung injury, increasing information suggests that mature differentiated lung cells may change their phenotype in response to environmental challenge and/or injury (122–124). This has been best described with alterations in the phenotype of AECII to AECI cells and vice versa (104). Epithelial–mesenchymal transition is a recognized phenomenon during development, however, its physiologic or pathophysiologic role in the adult lung remains unclear and controversial except perhaps for lung cancer and the development of metastases (125–129).

Overall, major challenges remain in this field. The obvious issues are development of adequate cell-specific markers and lineage tracing tools. Existing cell type–specific markers in particular are in need of refinement as increasing knowledge is obtained about the inherent plasticity of lung cell types and as previously identified lineages are deconstructed. These issues have been the focus of recommendations to the NIH and other funding agencies from previous conferences and continue to be at the forefront (Table 2). Also, problems remain with the terminology and methodologies used in different laboratories. For example, disagreement or lack of consistent interpretation and application of seemingly straightforward terminology, such as "differentiated versus undifferentiated" and "specialized versus unspecialized," has continued to impede progress. As mentioned above, a list of suggested terminology is illustrated in Table 1, but even this is likely to need revision in the near future.

Lung Cancer Stem Cells. There is intense interest in the connections between endogenous stem or progenitor cells and cancer stem cells. Cancer stem cells have been defined in

transplantation assays as the cell subset that is capable of propagating disease. These cells are frequently termed tumor initiating cells and are hypothesized to be the cells that maintain tumor progression and disease resistance (130–132). Cancer stem cells are best described in leukemias, breast cancer, and brain cancer, but increasing evidence suggests lung cancers may contain rare populations of cancer stem cells (7, 131, 133, 134). These studies indicate that the different types of lung cancer are initiated from distinct cell types and that the lung tumor–initiating cell may or may not have the same identity as the cancer stem cells that maintain established tumors. Given the diversity of lung cancer subtypes, this may not be surprising. Purification and characterization of the tumor-initiating cell and/or the cancer stem cell is an important aspect of studies designed to test the cancer stem cell hypothesis (135, 136). CD45 negative side population cells have been identified in several human lung cancer cell lines and exhibit tumorigenic properties when subcutaneously implanted into immunotolerant mice (137). Side population cells have also been identified in clinical lung cancer specimens (137). Dual positive pro-SPC/CCSP positive cells, the bronchioalveolar stem cells (BASCs) discussed in the above section, have also been suggested as tumor-initiating cells (66) A number of recent reports implicate $CD133^+$ cells as conferring resistance to chemotherapy and having tumor initiating properties (138–143). Recent studies have begun elucidating cell signaling and gene expression pathways including Pten, protein kinase C (iota), Wnt, hedgehog, c-kit, Akt, and others that may play roles in transformation of endogenous progenitor cells into lung cancer cells (85–93, 144). However, despite growing data, more work is needed to clarify the connections between endogenous lung progenitor cells, their potential roles as lung cancer stem cells, and most importantly, their potential role as therapeutic targets.

Bone marrow–derived or circulating MSCs, EPCs, and fibrocytes may contribute to development of primary and metastatic lung carcinoma and other malignancies in mouse models. These cells function, in part, by providing a supportive stroma for the cancers and/or by participating in tumor vascularization (145–159). In contrast, MSCs and EPCs have been demonstrated to home to areas of tumor development, and engineered EPCs and MSCs, as well as Hematopoietic Stem Cells (HSCs), have been used to suppress tumor growth in mouse tumor models of primary lung cancers, metastatic lung cancers, and of other cancers metastatic to the lung (148, 160–179). Cell based treatment may thus be useful in lung cancer therapeutics.

Structural Engraftment and Functional Effects of Circulating or Exogenously Administered Stem or Progenitor Cells

Structural engraftment. A number of publications over the past approximately 10 years initially suggested that a variety of bone marrow–derived cells including hematopoietic stem cells (HSCs), mesenchymal stromal (stem) cells (MSCs), multipotent adult progenitor cells (MAPCs), and other populations, as well as stem and progenitor cells isolated from other tissues such as adipose, placenta, cord, blood, and others, could structurally engraft as mature differentiated airway and alveolar epithelial cells or as pulmonary vascular or interstitial cells. This literature was predominantly based on studies in mice using techniques that evaluated histologic demonstration of donor-derived marrow cells in recipient lungs after systemic administration of marked donor cells (green flourescent protein [GFP]-labeled cells, male cells to female recipients, and other approaches), usually, but not always, after myeloablation of the recipient mouse bone marrow (reviewed in References 1, 2). Previous lung injury was usually necessary to observe engraftment, although lung injury did not always result in an increase of apparent engraftment (180, 181). Furthermore, the myeloablative regimen used, usually total body irradiation, was also felt to contribute to lung injury and be required for evident engraftment (182, 183). A smaller body of literature in clinical bone marrow and lung transplantation also demonstrated varying degrees of apparent chimerism in lungs of the transplant recipients (reviewed in Ref. 1). However, whether epithelial engraftment does in fact occur to any significant degree remains controversial (20, 21, 184, 185). Several technical issues contributed to misinterpretation of results in the initial reports including inadequate microscopic techniques in which donor-derived cells superimposed on resident airway or alveolar epithelial cells were not effectively discriminated. Exquisite care and sophisticated microscopic approaches, including confocal and deconvolution techniques, must be used to effectively demonstrate potential engraftment (1, 184, 185). Furthermore, a variety of leukocytes, notably airway and alveolar macrophages, reside in the lung. Many of the early reports did not use antibodies directed against CD45 or other leukocyte markers to exclude the possibility that cells of donor origin detected in airway or alveolar epithelium were donor-derived leukocytes rather than epithelial cells. Other tools, such as the use of GFP as a marker of donor-derived marrow cells obtained from transgenic GFP mice in recipient mouse lungs can be subject to error in the presence of autofluorescent cells commonly found in the lung (186).

Nonetheless, with a better understanding of, and better approaches to, the possible confounding factors discussed above, some reports suggest that engraftment of donor-derived airway and/or alveolar epithelium can occur at low levels after perturbation of airway or alveolar epithelium in models of lung injury. This has been observed with MSCs of bone marrow or cord blood origin (187), side population cells (188, 189), plastic adherent marrow stromal cells (189–193), or full marrow transplantation after a myeloablative regimen (189, 194, 195). These studies have tended to use more sophisticated microscopic and other analytical techniques. Nonetheless, epithelial engraftment in general is rare except under conditions discussed below. In parallel, recent studies also continue to demonstrate rare apparent engraftment of pulmonary interstitium and vasculature after total marrow transplant in a variety of injury models (196–198).

These reports suggest that engraftment of lung tissues with circulating or donor-derived cells can occur under certain conditions, usually following previous perturbation through induction of lung injury. However, there are many variables yet to be explored that may increase epithelial, interstitial, or pulmonary vascular engraftment with circulating or donor-derived cells. More vigorous injury regimens, such as serial naphthalene administration to deplete airway epithelial cells, coupled with busulfan to suppress endogenous bone marrow, appear to have increased engraftment of exogenously administered bone marrow cells (199). Comparably, several reports suggest that chronic or progressive lung injury may result in more substantial engraftment of AECII cells and of interstitial and pulmonary vascular cells with donor-derived cells in mouse or rat models (191, 196). However, not all chronic lung injury models result in more substantial engraftment (200). The effect of age of either donor cells or of recipients is also less well-explored; although transplantation of whole marrow into 1-day-old mouse pups, using a variety of conditioning regimens, did not increase the number of bone marrow-derived cells over those observed after administration of total marrow to adult mice (195). The route of administration of donor-derived cells is also less well-characterized, as most studies have investigated engraftment after systemic administration of donor cells. Direct

intratracheal administration of MSCs or other marrow-derived cells appears to result in apparent epithelial engraftment. However, levels of apparent engraftment are variable, and when initial engraftment is observed, it is not sustained (201, 202).

The types of stem/progenitor cells or fully differentiated nonpulmonary cells that might engraft as lung epithelium, interstitium, or pulmonary vasculature, remain to be fully explored. In addition to existing studies of HSCs, MSCs (of bone marrow, cord blood, and placental tissues origin), EPCs, and fibrocytes, the possibility remains that there may be other cell populations that could be recruited to the lung or that localize to the lung after systemic or other route of administration. For example, two different populations of cells isolated from human amniotic tissue—human amniotic epithelial cells (hAECs) and a population of multipotent cells termed human amniotic fluid stem cells (hAFSCs)—have both been described to apparently engraft in limited amounts in mouse lungs (203, 204). The hAFSCs localized to distal airway where they expressed thyroid transcription factor 1 (TTF1) and surfactant protein C (SPC), whereas the hAECs localized to both distal airway and to areas of lung injury where they expressed a range of surfactant proteins. Notably, administration of hAECs to bleomycin-injured immunocompetent mice resulted in abrogation of lung injury without apparent host response to the cells (203). Comparably, hAECs administered to rats with experimentally induced myocardial infarction were able to reduce the extent of infarction and also apparently acquired phenotypic characteristics of cardiomyocytes (205). This suggests that these cells may be used in other xenogeneic models of lung injury and may also exhibit paracrine effects that modulate lung injury, a topic discussed further in the section on mesenchymal stromal cells below. A population of circulating bone marrow–derived $CD45^+/CXCR4^+/cytokeratin^+$ cells has been described to participate in re-epithelialization of denuded tracheal xenografts (206). A recent report has also described a population of CCSP-expressing adult marrow cells that appear to more robustly lodge and engraft in lung after either systemic or intratracheal administration (Figure 2) (201). Other sources of stem or progenitor cells, such as adipose tissues, also have not been extensively characterized for their ability to engraft as lung tissue as have bone marrow and cord blood origin cells (187, 207). However, the ability to structurally engraft in adult lung may not solely be a property of stem or progenitor cells. Intratracheal administration of neonatal mouse lung fibroblasts resulted in apparent alveolar and interstitial engraftment, and engraftment was higher in areas of elastase-induced lung injury (208). Intratacheal administration of fibroblasts, transduced to express angiopoietin-1, mitigated acute lung injury and inflammation in mice (209). Comparably, intratracheal administration of AECII cells results in rare engraftment in areas of injured lung after administering bleomycin to rats (210). Notably, bleomycin-injured rats that received the AECII cells had less histologic injury and decreased hydroxyproline content. Most recently, AECII cells, derived *in vitro* from human embryonic stem cells, were able to both engraft and to mitigate bleomycin-induced lung injury in mice (211). These results suggest that lung injuries might be amenable to a variety of cell therapy approaches.

For those studies in which more robust evidence of engraftment has been suggested, the potential role of fusion is still not fully elucidated. Bone marrow-derived cells can be induced to fuse with lung epithelial cells *in vitro*, but *in vivo* investigations suggest that fusion is a rare occurrence (212, 213). One study suggests that fusion of donor-derived marrow cells with AECII cells can occur in mouse lungs but that the Y chromosome may be lost from the resulting heterokaryon cells (214). Nonetheless, fusion of exogenously administered cells with resident lung cells is thought to be a rare occurrence of uncertain physiologic significance.

Mechanisms by which circulating or systemically administered stem or progenitor cells might be recruited to the lung remain poorly understood. A number of studies demonstrate that after systemic administration, cells initially localize in the lung, and that lung injury results in increased localization and/or retention of marrow-derived cells in the lung (215–217). Whether this represents formation of cell emboli in the lung or a specific adherence to pulmonary vascular adhesion or other molecules remains unclear. In another study, embolization of systemically administered MSCs in lung was felt to result in secretion of an anti-inflammatory protein, TSG-6 (218). The timing of cell administration after lung injury can also influence recruitment and phenotypic conversion. Systemic administration of MSCs 4 hours after lung irradiation resulted in apparent engraftment of cells as epithelial and vascular endothelial cells (219). However, MSCs administered at later time points appeared to engraft as interstitial cells and participate in the development of fibrosis (215, 219). Recipient immune responses also play significant yet poorly characterized roles in retention of cells in lung (220). Commonly used approaches of sex-mismatched transplantation or cell administration may also result in clearance of cells (214). The range and identity of chemotactic soluble mediators released by injured lung cells and the role of up-regulation of adhesion molecules with which circulating cells might interact remains poorly understood (reviewed in 1 and 2, 215, 221–227). As with engraftment, a number of factors including age of donor or recipient, type of cell administered, route of administration, and so forth, might affect recruitment of cells to the lung.

Comparably, the mechanisms by which stem or progenitor cells isolated from adult tissues might be induced to acquire the phenotype of lung epithelial, interstitial, or vascular endothelial cells, remain poorly understood. *In vitro* studies demonstrate that soluble factors released from lung epithelial cells or from injured lung homogenates can induce the expression of lung epithelial markers in several types of marrow-derived cells, possibly through the activation of β-catenin and other cell-signaling pathways (228–230). Comparably, coculture of embryonic stem cells with fetal pulmonary mesenchyme can promote the development of cells expressing phenotypic markers of lung epithelial cells (231). One novel mechanism of inducing phenotypic change might involve the release of membrane-derived microvesicles, a recently appreciated means of intercellular communication that involves horizontal transfer of mRNA and proteins between cells (232, 233). Nonetheless, despite continuing interest in the possibilities of engraftment of exogenous cells in the lung, emphasis has moved to other areas, notably immumomodulatory effects of administered cells and *ex vivo* tissue engineering.

Endothelial progenitor cells. In the past decade, circulating bone marrow–derived cells putatively similar to embryonal angioblasts have been identified (234, 235). Termed "endothelial progenitor cells, or EPCs," these cells were reported to exhibit the potential to proliferate and differentiate into mature endothelial cells. Increasing evidence demonstrates that EPCs play a role in pathogenesis of a wide variety of lung diseases including pulmonary hypertension, pulmonary fibrosis, airway diseases, including asthma, COPD, acute lung injury, lung cancer, and most recently bronchopulmonary dysplasia and obstructive sleep apnea in children (236–256). However, studies of EPCs in lung diseases have been hampered by a lack of consensus regarding identification of these cells (257). Early investigations relied almost exclusively on the use of flow

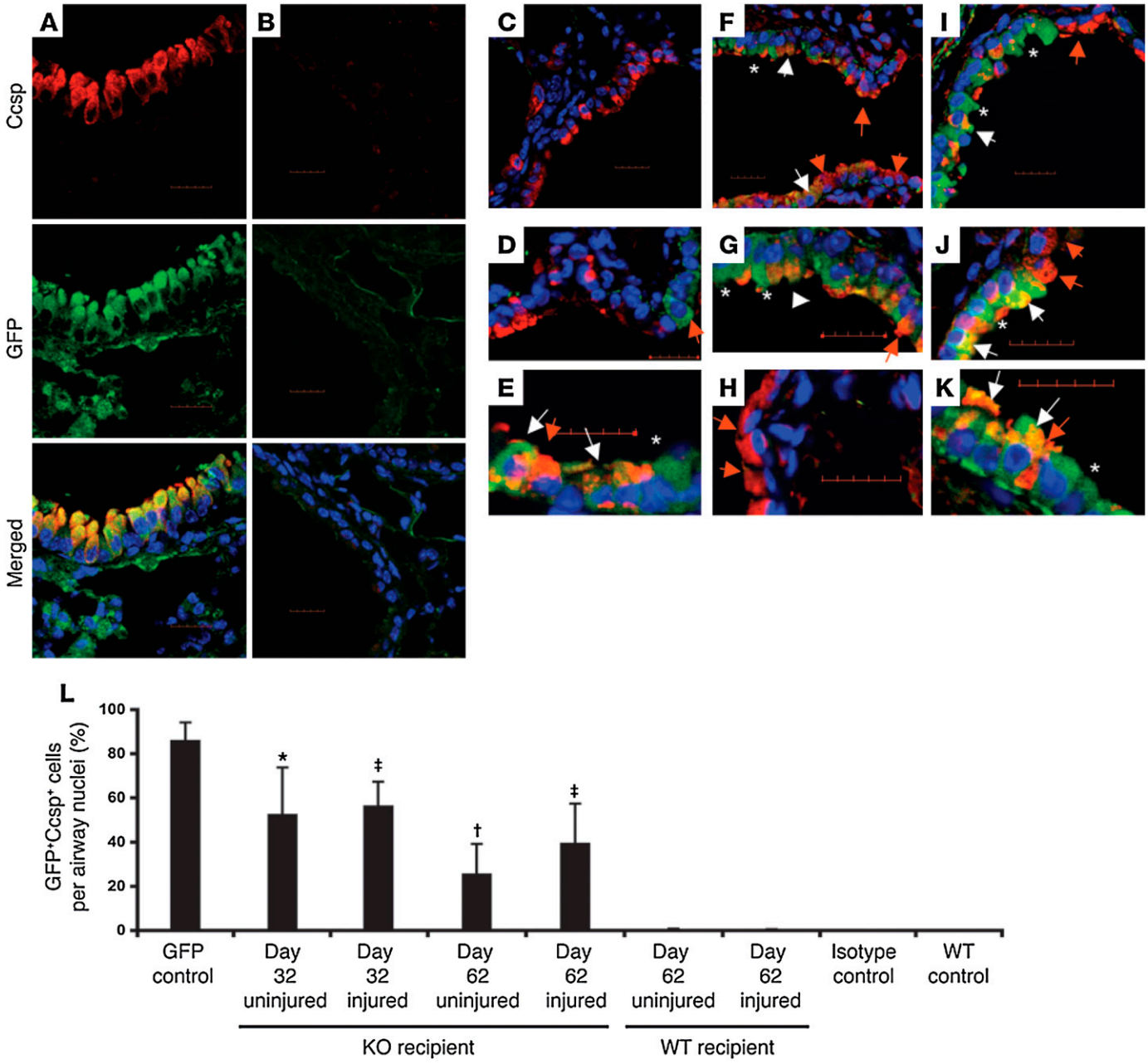

Figure 2. Endogenous bone marrow Ccsp+ cells can repopulate the airway epithelium. (*A–K*) Representative double immunofluorescence staining for GFP (*green*) and Ccsp (*red*) of lung sections from bone marrow transplant recipient mice that received Ccsp+Sca-1+ cells from GFP+ donors. *Asterisks* indicate a GFP+ cell that was Ccsp–. *White arrows* point to donor-derived Ccsp+ cells. *Red arrows* point to Ccsp+ cells that were not donor derived. (*A*) GFP+ (positive control) lung. (*B*) Isotype staining of lung from bone marrow transplant recipient. (*C–E*) Bone marrow transplant recipients had a wild-type background. (*C*) Low-power image. (*D* and *E*) High-power images. (*F–H*) Bone marrow transplant recipients were *Ccsp–/–* 60 days following bone marrow transplant without naphthalene. (*F*) Low-power image. (*G* and *H*) High-power image. (*I* and *K*) Thirty days after bone marrow transplant with naphthalene injury. (*I*) Low-power image. (*J* and *K*) High-power image. *Scale bars*: 20 μm. *Original magnification*: ×60 (*A, B, C, F,* and *I*); ×90 (*D, H,* and *J*); ×120 (*E, G, K*). Reprinted with permission from Reference 201.

cytometry in conjunction with immunostaining to identify and enumerate these cells in bone marrow as well as in the circulation. Furthermore, when similar techniques were used in different studies, a different group of markers were used. The lack of a unique cell surface maker to identify an EPC continues to complicate comparative assessments for similar disease processes. As a result, a growing consensus in the field encourages the use of functional assays both *in vitro* and *in vivo* in conjunction with the use of flow cytometry and immunohistochemistry (IHC) to not only enumerate EPCs, but to better characterize their true ability to form functional endothelium. A critical area for future study remains to develop a consensus-based approach to definition and use of EPCs with particular emphasis on functional capabilities of these cells.

At present, there appear to be two major types of EPCs that can be isolated from human peripheral blood by differential culture, an approach that avoids having to have prior certainty of surface selection markers for EPCs (257, 259). The first,

termed "early EPCs" are characterized by early growth *in vitro*, CD34/CD31/CD14 positivity, the inability to form tubes in a matrigel tube forming assay, and high levels of cytokine secretion. These cells are now known to be derivatives of the hematopoietic lineage differentiating along the myeloid lineage in response to certain cytokines and chemokines. The other type of EPC, termed "late outgrowth EPCs" or "outgrowth endothelial cells (OECs)" or "endothelial colony forming cells (ECFC)" is characterized by CD31, CD144, CD146, and CD105 positivity, lack of CD45, CD14, or CD115 expression, and the unique ability to spontaneously form human blood vessels when implanted in a gel into immunodeficient mice that inosculate with murine vessels to become a part of the systemic circulation. Each of these cell types may have a unique potential in lung microvascular repair, with early EPCs having a role as paracrine cells, and late EPCs functioning more specifically in restoring vascular structures of the lung. Intravenous infusion of each type of cell in an animal model has demonstrated their ability to preferentially localize to lung (259). The two EPC types may have a synergistic role in restoring vascular structure and function when infused together (258). Further characterization of these two EPC types, including methods to enhance their numbers *ex vivo* could have implications for the development of therapy specific to the phenotypic abnormalities of a given lung disease. In addition, the source of EPCs, for example adult peripheral blood versus umbilical cord blood, may also influence the differentiation potential of EPCs (260, 261). A schematic and summary of current classification and characterization of human EPCs is depicted in Figure 3.

The number of circulating EPCs has been correlated with a variety of clinical variables in several lung disease states, demonstrating the potential utility of EPCs as biomarkers. Although increased circulating EPC numbers correlate with survival in and acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and are associated with less residual lung damage in patients with pneumonia (238, 239), increased numbers do not necessarily correlate with better outcomes or more normal physiology in all lung diseases. For example, an decrease in the number of circulating EPCs in patients with COPD was associated with more abnormal spirometry (241), although a different study showed that levels of circulating EPCs were inversely correlated with COPD disease severity (240). Increased numbers of circulating EPCs also portended worse survival among those with non–small cell lung cancer (146, 253, 254). In asthma, numbers of circulating EPCs were increased compared with nonasthmatic control subjects, but this did not correlate with clinical outcomes (237).

Several clinical factors have been implicated in the mobilization of EPCs, and mechanisms for their effects have begun to be elucidated (220). Hypoxia appears to be a stimulus for EPC mobilization and recruitment, whereas hyperoxia is correlated with decreased circulating EPCs, particularly in preterm EPCs (222, 260, 262). These features may play a role in bronchopulmonary dysplasia in premature infants and neonates exposed to high oxygen levels (255, 262). They also suggest that EPCs could contribute to lung repair after acute lung injury. A recent study demonstrated that systemic administration of a population of bone marrow–derived angiogenic cells improved lung alveolar architecture after neonatal hyperoxia exposure in rats (263). Some degree of engraftment was observed, but it is unclear if structural contribution of the engrafted cells, or rather paracrine or other growth stimulating effects, were responsible. Defective lung development or defective lung repair in the setting of protracted inflammation and injury may result in part from an inadequate contribution of local or circulating EPCs. Age has been previously reported to be inversely correlated both with EPC number and also in the ability of EPCs to home to ischemic tissues based on age (264, 265). This may be mediated through the inability of aged tissues to normally activate the hypoxia-inducible factor-1 α-mediated hypoxia response (264). Use of HMG-CoA reductase inhibitors has been demonstrated to have a beneficial effect on the mobilization of EPCs (266). This may be related to the effect of this class of drugs in the prevention of EPC apoptosis in response to noxious stimuli, including the effects of TNF-α and IL-1β, thereby enhancing EPC survival and differentiation (267). Other pathways recently implicated in mobilization of EPCs include circulating vascular endothelial growth factor (VEGF) and CXCL12 (268, 269). Hypoxia-induced release of insulin-like growth factor 2 may also play a prominent role in EPC homing (270).

Goals of increasing numbers of EPCs, or developing methods to enhance their mobilization may not be appropriate for all diseases that affect the lung, particularly for lung cancers (253, 254). Although levels of circulating EPCs may serve as biomarkers for disease progression or severity (253, 254, 271), EPCs may have an effect on the development of lung tumor vasculature and homing to sites of lung metastases as well as in other cancers (146, 149, 150, 155, 157, 272). Because neovascularization involves the recruitment of EPCs from the bone marrow, these cells are a logical target for antiangiogenesis therapy. For example, an investigational drug, TK 1–2 (the kringle domain of tissue-type plasminogen activator), was demonstrated to be useful in blocking adhesion, differentiation, and migration of *ex vivo* human EPCs *in vitro* and also in decreasing tumor growth and vascularity in a SCID mouse tumor model (156). These findings suggest that blocking EPCs could be an important therapy in the prevention of cancer progression. Additionally, after systemic injection, EPCs localize to the lung and appear to home to metastatic tumors in the lung through as yet poorly understood mechanisms (160, 162). This suggests that modification of EPCs to express suicide genes or other therapeutic molecules could be potentially used in cell-based therapy approaches for lung cancer (160, 162). Mechanisms controlling mobilization and homing of EPCs to the lung remain poorly understood and are the subject for more intense investigation.

A number of studies in mice and dogs have demonstrated a role for exogenously administered EPCs in vasculogenesis and vascular repair in experimental models of pulmonary hypertension (44, 197, 246, 247, 251, 252, 273–279). Furthermore, EPCs can be transduced to express proangiogenic factors such as endothelial nitric oxide synthetase (eNOS) or inhibitors of smooth muscle cell proliferation such as calcitonin gene-related peptide and appear to home to sites of endothelial damage and lung injury (276). EPCs can also preferentially localize to areas of injured lung after systemic administration and may also have paracrine effects to decrease inflammation (216, 277, 278). As such, two pilot trials of autologous EPC administration for primary pulmonary hypertension conducted at Zhejiang University in Hangzhou, China in adult and pediatric patients demonstrated increased 6-minute walk capacity and improved hemodynamic variables, including mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output, 12 weeks after systemic administration of autologous EPCs with conventional therapy when compared with patients receiving conventional therapy alone (280, 281). Importantly, no adverse effects of EPC administration were noted, although long-term follow-up is pending. A therapeutic trial of administration of autologous early outgrowth EPCs transduced to express eNOS for patients with pulmonary hypertension, the Pulmonary Hypertension and Cell Therapy (PHaCeT) trial, has been

initiated at St. Michael's Hospital in Toronto and the Jewish General Hospital in Montreal. As of March 2010, the PHACeT trial has completed enrollment of the first two dose-panels with three patients receiving a total of 7 million early growth EPCs transfected to overexpress human eNOS in panel 1 and three more patients receiving 23 million cells in panel 2 (information courtesy of Duncan Stewart, M.D., F.R.C.P.C., University of Ottawa). The cell delivery procedure was well tolerated and there were no safety concerns. Notably, the first six patients showed a remarkable reduction in total pulmonary vascular resistance (PVR) over the course of the 3-day delivery period, which might represent the effect of increased NO release by the engineered EPCs within the pulmonary microcirculation. The trial's Data Safety Monitoring Board has approved moving to panel 3, which calls for a total of 50 million cells, in three divided doses over 3 days. Completion of the third dose panel will be followed by enrollment of an additional three patients at the highest tolerated cell dose, which should provide sufficient support to move forward with the design of a randomized controlled trial that can assess potential efficacy of this cell therapy approach in pulmonary arterial hypertension (PAH).

Going forward, clarification of the specific cell types involved in the process of neoangiogenesis will result in less confusion surrounding the term EPC. It is apparent that the early outgrowth cells are hematopoietic derivatives that can be identified by their classic cell surface markers, morphology, and *in vitro* hematopoietic colony-forming activity or *in vivo* reconstitution of the blood lineages in immunodeficient mice. In contrast, the ECFC or OEC are rare endothelial cells that display clonal proliferative capacity and *in vivo* vessel forming ability that can be discriminated by several cell surface antigens from the hematopoietic subsets. Roles for B lymphocytes, macrophages, dendritic cells, and red blood cells and/or their products in the neoangiogenesis process have also been described. Thus, going forward, rather than use the nondescript term "EPC," the field may wish to consider use of the terminology that best describes the cell population under investigation to be more specific about which cell, in which patients, at which points in time, are involved in a particular aspect of the neoangiogenic process (19, 40, 41, 282).

Circulating fibrocytes. Circulating fibrocytes were first described as a subset of circulating leukocytes that produced collagen and homed to sites of inflammation (283, 284). The identity and phenotypic characterization of circulating fibrocytes is more firmly established and these cells are described by the cell surface markers CD34, CD45, CD13, and MHC II and also express type 1 collagen and fibronectin. Circulating fibrocytes have been implicated in the pathogenesis of several lung diseases including both mouse and clinical models of pulmonary fibrosis, pulmonary hypertension, the subepithelial fibrosis that can develop in severe asthma, and in clinical bronchiolitis obliterans in patients with lung and bone marrow transplants (4, 8, 285–292). Further levels of circulating fibrocytes may be an indicator of worse prognosis in IPF (287, 293). Interestingly, numbers of circulating fibrocytes were highest in patients experiencing an acute exacerbation and the numbers returned to baseline with recovery (293). Several chemokines including stromal derived factor-1 (SDF-1) and the CCR2 and CCR5 axes have been implicated in recruitment of circulating fibrocytes to fibrotic lungs but overall mechanisms of fibrocyte recruitment to lung are poorly understood (286, 294–296). Matrix metalloproteinase expression may also be involved in recruiting fibrocytes to injured or post-transplant lungs (297). Similarly the mechanisms by which fibrocytes are induced to undergo phenotypic transformation into fibroblasts and myofibroblasts and contribute to fibrogenesis in lung are poorly understood although both haptoglobin and cysteinyl leukotrienes have been implicated (298, 299). Hypoxia is a potent stimulus for release of a variety of factors by pulmonary vascular endothelium that both serve to recruit fibrocytes as well as induce phenotypic conversion to fibroblasts or myofibroblasts (288). Notably, depletion of circulating fibrocytes abrogated hypoxia-induced perivascular remodeling in rats (288). Circulating fibroblasts may also be important in lung cancer development or metastasis. Circulating fibrocyte precursors found in blood of lung cancer patients contributed to tumor development when systemically administered to NOD SCID mice engrafted with human lung cancer xenografts (300). Bone marrow-derived cells may also contribute to fibroblasts and myofibroblasts in tumor stromal tissue (145). These results suggest that specific inhibition of fibrocytes or their use as drug delivery vehicles may also be important therapeutic targets in pulmonary vascular disease.

Mesenchymal Stromal (Stem) Cells. MSCs were first described in 1,968 as an adherent, clonogenic, non-phagocytic, and fibroblastic-like population of bone marrow cells (301). The nomenclature has changed over the years as MSCs were initially termed fibroblastic colony forming units (302), subsequently as marrow stromal cells, then as mesenchymal stem cells (303), and most recently as multipotent mesenchymal stromal cells or MSCs (303). MSCs have now been isolated from a wide variety of tissues including umbilical cord blood, Wharton's jelly, placenta, and adipose tissue (304–311). More recently cells with characteristics suggesting identity as MSCs have been isolated from adult mouse lungs (113–116), human nasal mucosa (117) and from lungs of both human neonates and human lung transplant recipients (118–121). Human lung MSCs appear to have some immunomodulatory capabilities similar to those of bone marrow-derived MSCs (120) whereas ovalbumin sensitization and challenge increase the number of lung MSCs in mice (115). This suggests that the lung MSCs may be involved in regulation of local inflammatory immune responses. However, the exact identity and physiologic role of putative lung MSCs is not yet clear. Other multipotent progenitor populations have been described in lungs from other species but the exact identify and roles of these cells is also not well understood (111, 112, 312).

However, definition and investigation of MSCs continues to be confounded by several issues. MSCs isolated from different sources generally express comparable cell surface markers and differentiate along recognized lineage pathways. However, differences in gene expression, lineage tendencies, and other properties have been described among MSCs isolated from different sources (313–322). As with EPCs, many of the published studies have used different definitions and characterizations of MSCs. This has complicated comparative assessments of published studies. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has defined minimal criteria for defining (human) MSCs (303) (Table 3). It is hoped that rigorous adherence to these criteria will help to focus comparative investigations of their potential use in lung diseases. Furthermore, fibroblasts have also been described as capable of exhibiting properties consistent with MSCs, as have adult mouse lung–side population cells (113, 116, 323). This includes both differentiation ability as well as immunomodulatory activities, discussed below.

The field of MSCs is complicated by a number of other factors. One is significant differences among MSCs from different species. Mouse MSCs from bone marrow have been particularly difficult. Early cultures grow slowly and are heavily contaminated by hematopoietic cells that usually require immunoselection to remove (reviewed in Refs. 1, 2, 14, 36). With

expansion, the cells pass through crisis, after which the cultures propagate rapidly as the few surviving cells are transformed and can become tumorigenic. In addition, MSCs from different mouse strains have different requirements of medium for optimal growth and different surface epitopes. These features present a significant obstacle for many potentially important experiments in mouse models for diseases and in transgenic mice. These features of murine MSCs were frequently not accounted for in publications in which cells termed "mesenchymal stem cells" or "MSCs" were used. As a result there are many important observations in a long list of publications that are difficult to interpret within the context of MSC biology and therapeutics. MSCs from rat bone marrow also present unusual features including strain differences, rapid initial growth, and a tendency of colonies to shed cells that generate new colonies (14, 36). Also, rat and mouse MSCs have a tendency to develop genomic instability with extensive expansion. At present, human MSCs from bone marrow are the best characterized MSCs but also present some challenging features. Early passage cells plated at low densities are enriched for small, rapidly replicating cells that are highly clonogenic and have a high potential for differentiation. However, the colonies formed by the MSCs have several surprising features. The cells within clonal, single-derived colonies become heterogeneous as the colonies expand with cells in inner regions expressing a different profile of genes than cells in the outer regions. The colonies formed on reclonning cells from a single colony generate new colonies that are heterogeneous in size and differentiation potential. Also, the cells within clonal, single-derived colonies become heterogeneous as the colonies expand with cells in inner regions expressing a different profile of genes than cells in the outer regions. Human MSCs that are extensively expanded at high density retain some of the properties of low density cultures but are enriched for large, slowly propagating cells with diminished clonogenicity and differential potential. A further complication is that parallel preparations of MSCs from bone marrow aspirates isolated from the same normal donors in the same session can differ in features such as rate of propagation and potential to differentiate.

TABLE 3. THE MESENCHYMAL AND TISSUE STEM CELL COMMITTEE OF THE INTERNATIONAL SOCIETY FOR CELLULAR THERAPY (ISCT) MINIMAL CRITERIA FOR DEFINING MESENCHYMAL STEM CELLS

1. Plastic adherence in standard tissue culture conditions
2. Expression of CD73, CD90, and CD105
3. No expression of CD 11b, CD14, CD19, CD34, CD45, CD79α, or of HLA-DR
4. Differentiation *in vitro* to osteoblasts, adipocytes, and chondroblasts

Adapted with permission from Reference 303.

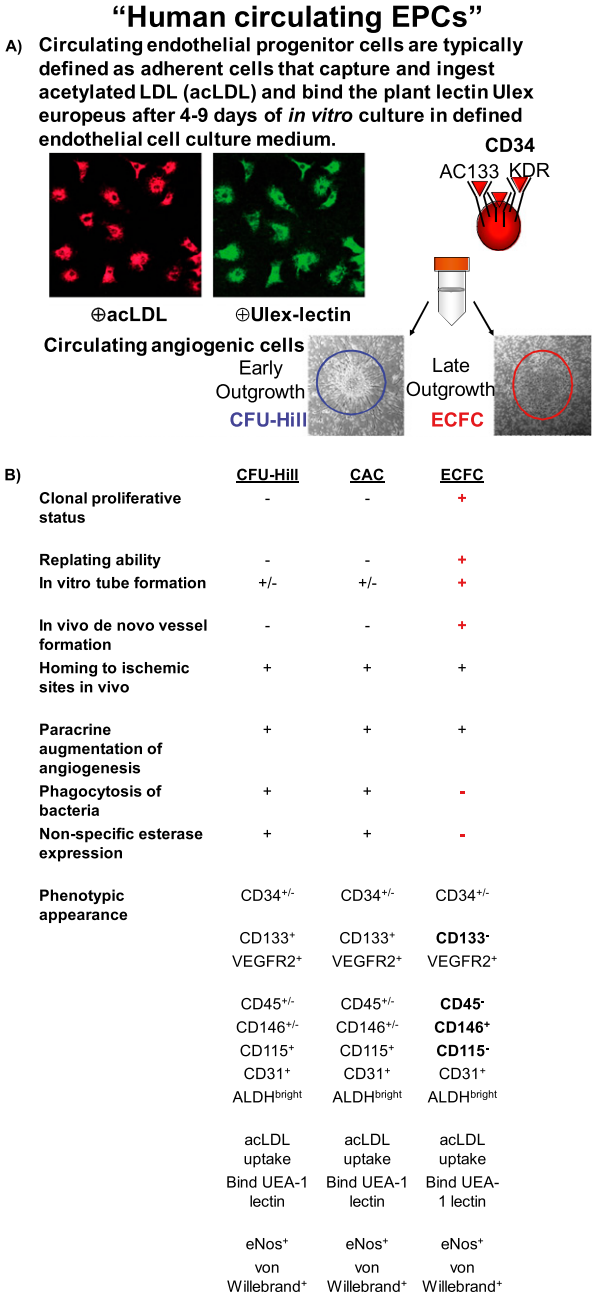

Figure 3. Schematics depicting current thinking in isolation and identification of endothelial progenitor cells. Reprinted with permission from Reference 19.

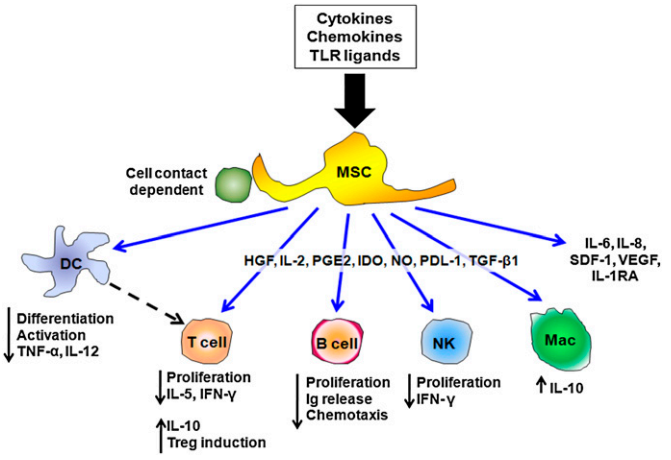

Figure 4. Schematic illustrating the range of *in vitro* immune-modulating effects described for mesenchymal stem cells (MSCs). DC = dendritic cell; HGF = hepatocyte growth factor; IDO = indoleamine 2,3-dioxygenase; IFN-γ = interferon γ; Ig = Immunoglobulin; IL = Interleukin; IL-1RA = Interleukin-1 receptor antagonist; Mac = Macrophage; NK = natural killer; PGE2 = prostaglandin E-2; SDF-1 = stem-cell derived factor 1; TNF-α = tumor necrosis factor-α; TGF-β1 = transforming growth factor- β1; TLR = Toll-like receptor; VEGF = vascular endothelial growth factor. Reprinted with permission from Reference 412.

In addition, culture variables, including culture surface composition and stiffness (324–328), oxygen environment (329–335), mechanical forces (336–339), temperature (340), and other factors such as culture density can profoundly influence phenotype and behavior of MSCs. This includes culturing in three- versus two-dimensional scaffolds as discussed below in the section on lung bioengineering. As discussed below, research on MSCs is confused by the marked differences in MSCs from mice and human MSCs in terms of properties such as cell surface epitopes, ease of expansion, and genomic stability. It is also becoming increasingly apparent that different inflammatory environments can profoundly influence MSC behavior (341–343). Furthermore, there is growing evidence that MSCs are heterogeneous and that different MSC subtypes exist (344–346). To address some of the variations in properties of cultured MSCs, an NCRR/NIH-sponsored center offers standardized preparations of murine and human MSCs that investigators can use as benchmarks for preclinical experiments (http://medicine.tamhsc.edu/irm/msc-distribution.html).

Nonetheless, despite these issues, substantial progress continues to be made with MSCs in lung injury and repair. Initial emphasis was on the potential ability of MSCs to acquire epithelial phenotype and engraft as structural lung cells. However, despite the ability to induce expression of markers expressed by either airway or alveolar epithelial cells in *in vitro* culture systems, engraftment with MSCs, as with most other cell types investigated so far, is a rare event of uncertain physiologic significance in lung (1, 2, 14, 15, 36,). As such, emphasis has increasingly shifted toward the profound immunomodulatory, anti-inflammatory, and non-immunogenic properties of MSCs. MSCs have ability to regulate hematopoietic cells and to secrete multiple regulatory molecules such as growth factors and anti-inflammatory cytokines which can modulate immune and inflammatory responses (reviewed in 347–353). MSCs also inhibit the proliferation and function of a broad range of immune cells, including T cells, B cells, natural killer (NK) cells and dendritic cells (DCs) in *in vitro* models systems (Figure 4), (354–378). Notably, MSCs inhibit T lymphocyte proliferation, activation, and cytokine release in response to either alloantigens or to mitogenic stimuli through a dose-dependent direct suppressive effect on proliferation. The mechanisms of MSC inhibition of immune effector cell proliferation and function *in vitro* are only partly understood and both direct cell–cell contact as well as release of soluble mediators has been proposed (Figure 4) (354–378). MSCs have also been described to have capacity to act as antigen-presenting cells in *in vitro* model systems, a property influenced by exposure to IFNγ and to TGFβ (379). Furthermore, MSCs may also affect actions of cells involved in innate immune responses, notably macrophages (380–382).

It is also becoming increasingly apparent that different inflammatory environments can profoundly influence MSC behavior (341–343). MSCs express a wide variety of chemokine and cytokine receptors, including those for TNFα, IL-4, IL-17, and IFNγ. The IL-17_1 receptor in particular is expressed in high abundance and IL-17 has been described as a proliferative stimulus for MSCs (383). Expression of MHC and costimulatory molecules can be altered by exposure to inflammatory mediators commonly found *in vivo* such as IFNγ and TGFβ (376–379). Chemotaxis and migration toward a wide variety of mediators including SDF-1, TNFα, macrophage migration inhibitory factor (MIF), TGFβ, hepatocyte growth factor (HGF), and others has been described (223, 225, 226). Expression of specific cell surface molecules including CD44 and MARCKS (224, 227) have been implicated in directed MSC chemotaxis. MSCs also express toll-like receptors including the endotoxin receptor TLR4 (384–387). Activation of the TLRs in MSCs can produce a wide variety of effects, many of which are still being elucidated. Furthermore, expression of TRLs by MSCs can be influenced by a number of factors including exposure to bacterial toxins and to a variety of inflammatory mediators. Hypoxia, characteristic of many lung injuries and diseases, can also alter MSC expression of cell surface molecules and secretion of soluble mediators by MSCs (331, 335). Overall, this illustrates a rapidly growing body of evidence demonstrating that MSCs are malleable and can be significantly influenced by local inflammatory environments, including those found in lung injuries.

MSCs also seem to be relatively nonimmunogenic due to their low constitutive expression of major histocompatibility complex (MHC) type 1 and lack of constitutive expression of MHC type 2 and the costimulatory molecules CD80, CD86, and CD40 (reviewed in Refs. 347–353) This allows administration of allogeneic MSCs without significant host responses. Although the frequency of MSCs in the adult bone marrow is low (less than 0.1%), once isolated from bone marrow or from other tissues, MSCs can be expanded *ex vivo*, which makes it possible to manufacture these cells for potential therapeutic purposes. MSCs can also be relatively easily transduced or genetically manipulated to deliver or to secrete selected disease-modifying molecules (148, 161–179, 388–390). Overall, these properties of MSCs make them an attractive potential therapeutic tool as vectors for delivery of disease-specific treatment substances or as immunomodulatory agents. As such, MSCs have now been used in clinical trials for inflammatory and autoimmune diseases such as graft versus host and Crohn's disease as well as in acute myocardial infarction (391–396). One of the important findings to come out of the published data is that administration of MSCs appears to be safe and well tolerated, even in severely ill patients. Although the long-term effects of MSC administration are unknown, there appear to be no significant short-term effects.

In this context, a steadily increasing number of articles demonstrate efficacy of either systemic or intratracheal MSC administration in a growing spectrum of lung injury models in mice (31, 35, 42, 397–399). This includes mouse models of acute lung injury and fibrosis (230, 400–412), sepsis (413–417), pulmonary hypertension (418–420), ischemia re-perfusion injury (421, 422) bronchopulmonary dysplasia (423–425), bronchiolitis obliterans (426), asthma (427–433), COPD (434–442), and other pulmonary diseases (198, 200, 443, 444) (summarized in Table 4). MSCs have also been demonstrated to have efficacy in models of primary and metastatic lung cancer (164, 167–171, 177). Recent studies have also demonstrated efficacy of MSC administration in endotoxin-injured human lung explants (445). Although the mechanisms of the MSC effects are not completely understood, soluble mediators released by the MSCs appear to play important roles in amelioration of acute and fibrotic injuries in the different models. Some of those implicated in the different model systems include IL-1 receptor antagonist, IL-10, keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), angiopoietin 1, and TGFβ. Transduction of the MSCs to express either angiopoietin-1 or KGF further decreases endotoxin-mediated lung injury presumably through abrogation of endotoxin-mediated endothelial injury (403, 445). MSCs appear also to act in part by decreasing the increased endothelial permeability found in acute lung injury and also by secreting antibacterial peptides (400, 445–447). MSCs may also exert effects on lung inflammation and injury through primary interactions with the immune system rather than through direct actions in lung. For example, a growing body of evidence suggests that MSCs ameliorate allergic airways inflammation in mice by promoting a Th1 phenotype

in vivo in antigen-specific CD4 T cells and in circulating antigen-specific immunoglobulins as a means of abrogating Th2-mediated lung injury (427, 428, 432). As such, MSCs appear to be capable of a spectrum of effects in different lung injuries.

However, as noted earlier in the discussion on MSCs, many of the studies discussed above used different preparations of MSCs. As detailed in Table 4, these ranged from populations of heterogenous plastic adherent adipose stromal cells to purified well-characterized bone marrow–derived MSCs obtained from core facilities such as the NCRR/NIH- sponsored Texas (formerly Tulane) Center for Preparation and Distribution of Adult Stem Cells (MSCs) (http://medicine.tamhsc.edu/irm/msc-distribution.html). Furthermore, differences between syngeneic, allogeneic, and xenogeneic MSC administration have been less well explored in lung injury models. As such, whereas the overall consistency of studies demonstrating ability of MSCs to ameliorate different types of lung injuries is encouraging, further rigor must be applied to understand the mechanisms of MSC effects when comparing MSCs isolated by different protocols and obtained from different tissue sources. The specificity of MSC effects compared with potential anti-inflammatory or immunomodulatory effects of other stromal cells such as fibroblasts must be carefully considered (323, 448) (Table 4). For example, systemic administration of primary dermal fibroblasts similarly decreased ragweed pollen or ovalbumin-induced allergic airways inflammation as did MSCs (429, 432). Fibroblasts transduced to express angiopoietin-1 decreased acute endotoxin-induced lung injury (209). However, intratracheal administration of 3T3 fibroblasts did not mimic effects of MSCs in ameliorating endotoxin-induced acute lung injury (400). These results suggest that MSCs and stromal cells such as fibroblasts may share similar anti-inflammatory mechanisms. However, fibroblasts are also heterogeneous depending in part on tissue source, and thus potential anti-inflammatory effects may differ depending on their origin. Further, fibroblasts are less likely to have the same degree of low immunogenicity as do MSCs and can provoke lung inflammation (432). Nonetheless, comparison of MSCs with fibroblasts in different injury models will likely provide important insights as to the anti-inflammatory mechanisms of MSCs and it is important to include appropriate cell controls such as fibroblasts when assessing MSC effects in different lung injury models.

MSCs are also increasingly described as vehicles for delivery of therapeutic genes and proteins (148, 161–179, 388–390). Notably, MSCs can home to tumors, through unclear mechanisms, and serve as vehicles for delivery of chemotherapeutic and other antitumor agents (148, 161–179, 388). This has recently been described in mouse lung tumor models and may provide a viable therapy for lung cancers (164, 167–171, 177, 449, 450). Notably, human bone marrow–derived MSCs engineered to express tumor necrosis factor–related apoptosis inducing ligand (TRAIL) were found to significantly clear tumor metastases in a xenograft model of metastatic human lung cancer in mice (171). Comparably human umbilical cord blood MSCs engineered to express IFNβ were found to significantly attenuate human bronchioalveolar carcinoma xenografts in mice (177). In contrast, MSCs may also contribute to tumor stroma and influence behavior of cancer cells (145, 148, 151, 154). A recent report demonstrated that human MSC administration could promote growth and pulmonary metastases in a mouse model of primary human osteosarcoma (174). Whether marrow-derived cells contribute to development of epithelial cancers remains an active area of investigation (147, 153).

Additional cautions with regard to systemic administration of MSCs have been raised in previous conference reports and are repeated here (1, 2, 16). Most culture strategies use fetal or bovine calf serum. Despite washing of the cells prior to systemic administration, some bovine antigens may remain adherent to cell surfaces and trigger immune reactions as well as decrease potential engraftment in recipient mice or patients (451). Culture of MSCs in medium with lower calf serum content, use of heterologous species–specific serum, or alternative serum substitutes such as platelet lysate, and removal of calf serum antigens before administration are proposed strategies to decrease these potential adverse effects (452–455). Additionally, after intravenous administration, MSCs initially lodge in the lung vasculature before moving through the pulmonary capillary system and on to other organs. However, depending on the preparative regimens used, MSCs can clump and potentially lodge as emboli in lung capillaries (456, 457). Pretreatment of mice with the vasodilator sodium nitroprusside has been proposed as a mechanism of decreasing MSC cell trapping in pulmonary capillaries (458).

Whether MSCs may undergo malignant transformation remains a topic of considerable debate and concern (16, 459–461). Murine MSCs that were extensively expanded in culture through many passages developed chromosomal instability and produced lung sarcomas in mice (456, 457). The results emphasize the greater propensity of mouse MSCs to acquire chromosomal abnormalities with serial passages in culture (456, 462–464). However, extensive culture of almost any mammalian cell in culture can lead to crisis, followed by immortalization and then transformation to tumorigenic cells, as has been well documented for mouse fibroblasts (465). Concerns about the potential tumorigenicity of human MSCs were raised by three reports that described escape from senescence and generation of malignant cells as the MSCs were expanded in culture (466–468). The reports were unexpected insofar as emergence from senescence had not been observed in laboratories that had studied the cells for over a decade. The discrepancies were largely explained by reports from two of the laboratories indicating that their cultures of human MSCs had been cross contaminated with human fibrosarcoma or osteosarcoma cell lines (469). This further highlights the need to develop better markers for defining MSCs and the important need to verify the identity of cells even if received from established sources. It is anticipated that additional strategies to maximize therapeutic use of MSCs while decreasing chance of any adverse effects will develop over the next several years.

The pros and cons of moving toward clinical trials with MSCs and other cell types (EPCs) have been vigorously debated at all three conferences to date. It is anticipated that this debate will continue. Nonetheless, clinical data is accumulating. In a Phase I, double-blind, placebo-controlled trial of PROCHYMAL (*ex vivo* cultured adult human mesenchymal stem cells) conducted by Osiris Therapeutics Inc. (Columbia, MD) in patients with acute myocardial infarction, an improvement in both FEV_1 and FVC was noted in treated patients (396). Although the mechanisms of improvement in pulmonary function in this patient population are not yet understood, these observations stimulated a multicenter, double-blind, placebo-controlled Phase II trial of PROCHYMAL (Osiris Therapeutics Inc., Columbia, MD) for patients with moderate to severe COPD ($FEV1/FVC < 0.70$; $30\% \leqslant FEV1 \leqslant 70\%$). The primary goal of the trial, which was initiated in May 2008, is to determine safety of MSC infusions in patients with lung disease. The secondary goal is initial estimation of the potential efficacy of MSCs for decreasing the chronic inflammation associated with COPD thus improving both pulmonary function and quality of life. The trial has recruited 62 patients in six participating U.S. sites. In the 6-month interim data analysis, no infusional toxicities or significant adverse events were reported (470). Notably, a significant decrease in the circulating

TABLE 4. MESENCHYMAL STEM CELL TABLE

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Acute Lung Injury							
Gupta 2007 (400)	Mouse intranasal LPS Intratracheal MSCs 4 h after LPS	Mouse BM Plastic Adherent	No	Syn	• Improved survival • Improved histologic inflammation and edema • Decreased BALF TNF-α, MIP-2 • Increased BALF and serum IL-10	None specified (soluble mediators)	Apoptotic MSC, 3T3 fibroblasts • Did not mimic effects on survival or inflammation
Xu 2007 (230)	Mouse intraperitoneal LPS Intravenous MSCs 1 h after LPS	Mouse BM Plastic Adherent CD11b,CD45-depleted	No	Syn	• Decreased histologic injury and lung edema • Decreased BALF neutrophilia but no change in BALF cytlokines • Decreased systemic inflammation (serum IL-1β, IFN-γ, IL-6, MIP-1α, KC)	None specified (soluble mediators and cell-cell contact)	Primary lung fibroblasts • Did not mimic effects on histology or edema
McCarter 2007 (209)	Rat intratracheal LPS Intravenous cells 24 hr prior to LPS	Primary skin fibroblasts	Plasmid transfection to express Ang1 or PFLAG	Syn	• Decreased histologic injury and edema • Decreased BALF inflammation • Increased Ang1 levels in lung	Ang1 secretion	Pflag-transduced fibroblasts Did not mimic effects on histology, underedema, or inflammation
Mei 2007 (403)	Mouse intratracheal LPS Intravenous MSCs 30 min after LPS	Mouse BM Plastic Adherent Texas (Tulane) MSC Core	Plasmid transfection to express Ang1 or PFLAG	Syn	• Decreased histologic injury • Decreased BALF total cell count • Decreased BALF cytokines and chemokines • Decreased LPS-induced protein leak • Decreased apoptosis • In general, MSC-Ang1 more effective than MSCs	Ang 1 secretion (other soluble mediators)	No
Xu 2008 (404)	Mouse nebulized LPS for 7 successive days Intravenous MSCs 2 h after 1st LPS dose	Mouse BM Plastic Adherent from Ficoll gradient	Lentivirus-transduced to express Ang1	Syn	• Decreased BALF total protein, neutrophils, TNFα at 7 but not 3 d • Decreased lung edema and MPO activity at 7 but not 3 d • Decreased histologic injury • Effects only seen with MSC-Ang1. Control MSCs had no effects.	None specified (soluble mediators)	No
Lee 2009 (445)	Explant human lung intratracheal LPS Intratracheal MSCs or conditioned media 1 h after LPS	Human BM Plastic adherent Texas (Tulane) MSC Core	No	Allo	• Decreased histologic injury • Decreased lung permeability/ improved alveolar fluid clearance	KGF secretion	No

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Iyer 2010 (408)	Mouse intraperitoneal LPS Intravenous MSCs 1 h after LPS	Mouse BM Plastic Adherent CD11b, CD45-depleted	No	Syn	• Decreased histologic injury and lung edema • Prevent oxidative stress induced by systemic LPS • Decreased systemic inflammation (serum IL-1β, IFN-γ, IL-6, MIP-1α, KC	None specified (soluble mediators and cell-cell contact)	Primary lung fibroblasts • Did not mimic effects on histology or edema
Asthma							
Cho 2008 (427)	Mouse ovalbumin-induced allergic rhinits Intravenous MSCs on Days 18–20 (during antigen challenge)	Mouse adipose Plastic adherent	No	Syn	• Decreased nasal inflammation • Decreased ova-specific IgE, IgG1 • Increased ova-specific IgG2a • Decreased IL4, IL-5, increased IFNγ in cultured splenocytes	None specified (soluble mediators)	No
Goodwin 2011 (432)	Mouse ovalbumin-induced allergic airways inflammation Intravenous MSCs on Days 1 and 7 (during antigen sensitization)	Mouse BM Texas (Tulane) MSC Core	No	Syn and Allo	• Decreased airways hyperresponsiveness • Decreased eosinophilic Th2 lung inflammation • Skewing of ova-specific CD4 T lymphocytes from Th2 to Th1 • Decreased ova-specific IgE and IgG1, increased IgG2a	None specified (soluble mediators)	EDCI-fixed MSCs and primary dermal fibrolasts • partially mimic MSC effects
Park 2010 (428)	Mouse ovalbumin-induced allergic airways inflammation Intravenous MSCs on Days 18–20 (during antigen challenge)	Mouse adipose Plastic adherent	No	Syn	• Decreased airways hyper-responsiveness • Decreased eosinophilic Th2 lung inflammation • Skewing of OVA-specific CD4 T lymphocytes from Th2 to Th1 • Decreased OVA-specific IgE and IgG1, increased IgG2a	None specified (soluble mediators)	No
Nemeth 2010 (429)	Mouse ragweed pollen-induced allergic airways inflammation Intravenous MSCs on Day 14 (at onset of antigen challenge)	Mouse BM Plastic adherent CD11b, 45 depleted	No	Syn and Allo	• Decreased histologic injury and mucus metaplasia • Decreased BALF inflammation (total cell counts, eosinophils, IL-4, IL-13) • Decreased serum IgE and IgG1 • Allo had same effect as syn MSCs	TGF-β1secretion	Primary dermal fibroblasts • partially mimic MSC effects
Bonfield 2010 (430)	Mouse ovalbumin-induced chronic allergic airways inflammation Intravenous MSCs during last week of 4-wk challenge	Human BM Plastic adherent	No	Xenogeneic	• No obvious lung inflammatory effects of xenogeneic MSCs in immunocompetent mice • Improved histologic injury • Decreased BALF inflammation (total cells, eosinophils, IL-5, IL-13, IFN-γ) • Decreased serum IgE • Decreased BALF macrophage iNOS mRNA	None specified (soluble mediators)	Syngeneic mouse bone marrow-derived macrophages • Do not mimic effects on histology, BALF total cell counts, serum IgE, body weight • Other endpoints not assessed

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Bronchiolits obliterans							
Grove 2010 (426)	Mouse subcutaneous trachea allografts Intravenous MSCs at time of transplant	Mouse BM Plastic Adherent Texas (Tulane) MSC Core	No	Syn and Allo	• Decreased allograft fibrosis and occlusion • Decreased allograft TGFβ and G-CSF • Increased allograft IL-10	None specified (soluble mediators)	• No
Bronchopulmonary dysplasia							
Aslam 2009 (424)	Neonatal mouse hyperoxia (75% FiO2) Intravenous MSCs 4 d after initiation of hyperoxia	Mouse BM Plastic adherent or conditioned media Neg selected for: CD11b, 14, 19, 31, 34, 45, 79α Pos selected for: CD73, 90, 105, cKit, Sca-1	No	Syn	• Decreased histologic injury • Decreased BALF inflammation • Decreased RV hypertrophy, pulmonary arteriole muscularization • MSC-conditioned media produced same effects	Heat labile secreted molecule(s) suggested for conditioned media	Pulmonary artery smooth muscle cells or mouse lung fibroblasts • PAMSC, PASMC-CM, or MLF did not mimic effects on RV hypertrophy or BALF inflammation • PAMSC or PAMSC-CM did not mimic effects on histologic injury or vascular remodeling
Chang 2009 (425)	Neonatal rat hyperoxia (95% FiO2) Intravenous or intraperitoneal MSCs 5 d after initiation of hyperoxia	Human umbilical cord blood Plastic adherent	No	Xenogeneic	• Decreased histologic injury (IP and IT) • Decreased apoptosis (IP and IT) • Decreased myeloperoxidase and IL-6 (IP and IT) • Decreased collagen, α-SMA, TNF-α, TGF-β (IT only)	None specified (soluble mediators)	Primary human foreskin fibroblast (IT only) • Did not mimic effects on histology • Other endpoints not assessed
van Haaften 2009 (423)	Neonatal rat hyperoxia (95% FiO2) Intratracheal MSCs 4 or 14 d after initiation of hyperoxia	Rat BM plastic adherent	No	Syn	• Decreased histologic injury • Improved capillary density • Improved RV hypertrophy and hemodynamics	MSC-conditioned media had protective effects in several in vitro assays	Pulmonary artery smooth muscle cell • Did not mimic effects on histology, capillary density, RV hypertrophy, hemodynamics
COPD							
Shigemura 2006a (435)	Rat elastase following lung volume reduction surgery Direct topical application of ASC- seeded polyglycolic acid at time of surgery	Rat adipose Plastic adherent	No	Syn	• Improved histologic repair	HGF secretion	No
Shigemura 2006b (436)	Rat elastase Intravenous ASCs 1 wk after elastase	Rat adipose Plastic adherent	No	Syn	• Decreased apoptosis, improved histologic repair • Improved gas exchange and exercise tolerance	HGF secretion	No

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Yuhgetsu 2006 (437)	Rabbit elastase Intratracheal MSCs 24 hr after elastase	Rat BM mononuclear cells from Ficoll gradient of total BM	No	Syn	• Improved histology and lung function • Decreased BALF fluid inflammation • Decreased tissue MMP2 and MMP9 expression	None specified (soluble mediators)	No
Zhen 2008 (439)	Rat papain ± 7.5 Gy TBI Intravenous MSCs after papain (timing not specified)	Rat BM Plastic adherent mononucelar cells from Percoll gradient	No	Syn	• Decreased histologic injury • Decreased alveolar cell apoptosis	None specified	No
Schweitzer 2010 (440)	Mouse cigarette smoke for 4 mo Intravenous ASCs every other week for 3rd and 4th months of CS exposure	Human or mouse adipose plastic adherent	No	Syn or Xenogeneic	• Decreased histologic injury and caspase activation • Decreased BALF inflammation • Decreased CS-induced MAPK signal transuction • Decreased weight loss • Decreased BM suppression	None specified	No
	NOD-SCID mouse (for xenogeneic) human ASCs 3 d after VEGFR blockade	Human or mouse adipose plastic adherent	No	Syn or Xenogeneic	• Human ASCs decreased histologic injury and caspase 3 activation following VEGFR blockade • Emboli if used $> 5 \times 105$ MSCs or if > passage 3	None specified	No
Zhen 2010 (442)	Rat papain Intravenous MSCs 2 h after papain	Rat BM Plastic adherent mononucelar cells from Percoll gradient	No	Syn	• Decreased histologic injury • Partial restoration of VEGF expression in lung homogenates	TNF-α–stimulated VEGF secretion by MSCs	No
Katsha 2010 (441)	Mouse intratracheal elastase Intratracheal MSCs 14 d after elastase	Mouse BM Plastic Adherent	No	Syn	• Decreased histologic injury • Decreased IL-1β • Transient increase in lung EGF, HGF, and secretory leukocyte protein inhibitor	None clarified (soluble mediators)	No
Fibrosis							
Ortiz 2003 (401)	Mouse bleomycin Intravenous MSCs immediately or 7 d after bleomycin	Mouse BM Plastic adherent CD11b, 34, 45-depleted	No	Syn	• Decreased histologic and inflammatory injury • Decreased hydroxyproline, MMP2, MMP9 content	None specified (soluble mediators)	No

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Rojas 2005 (568)	Mouse bleomycin Intravenous MSCs immediately after bleomycin	Mouse BM Plastic Adherent CD11b, CD45-depleted	No	Syn	• Increased osteopontin • Decreased histologic injury and lung fibrosis • Decreased inflammatory cytokines	None specified (soluble mediators)	No
Ortiz 2007 (402)	Mouse bleomycin Intravenous MSCs immediately after bleomycin	Mouse BM Plastic Adherent CD11b, 34, 45-depleted	No	Syn	• Decreased systemic inflammation (serum IL-1β, IFN-γ, IL-6, MIP-1α, KC)	None specified	No
Zhao 2008 (405)	Rat bleomycin Intravenous MSCs 12 h after bleomycin	Rat BM plastic adherent mononuclear cells isolated from Percoll gradient of total BM	No	Syn	• Decreased histologic injury • Decreased hydorxyproline, laminin, hyaluronan • Decreased TGF-β, PDFG-A, PDGF-B, IGF mRNA	None specified (soluble mediators)	No
Aguilar 2009 (408)	Mouse bleomycin Intravenous MSCs 8 h and 3 d after bleomycin	Mouse BM Texas (Tulane) MSC Core Mouse HSCs	Lentivirus transduced to express KGF	Syn	• MSCs: Decreased lung collagen mRNA and protein, no change in histologic injury • HSCs: decreased collagen, histo injury, aSMA, TNFa, CCL-2, CCL-9 • HSCs: ncreased type 2 cell proliferation	KGF secretion	No
Kumamoto 2009 (409)	Mouse bleomycin Intravenous MSCs 3 d after bleomycin	Mouse BM Plastic adherent for 2 hours or 9 days CD11b, 31, 45-depleted	No	Syn	• Decreased histologic injury and hydroxyproline • Decreased inflammatory cells	None specified (soluble mediators)	No
Moodley 2009 (406)	SCID mouse bleomycin Intravenous MSCs 24 h after bleomycin	Human umbilical cord (Wharton's jelly) Plastic adherent	No	Xenogeneic	• Decreased histologic injury • Decreased hydroxyproline, type 1 collagen mRNA • Increased MMP2, decreased TIMP-2	None specified (soluble mediators)	Primary human lung fibroblasts • No effect on bleomycin induced collagen mRNA or MMP-2 expression • other endpoints not clarified
Lee 2010 (411)	Rat bleomycin Intravenous MSCs 4 d after bleomycin	Rat BM Plastic Adherent	No	Syn	• Decreased neutrophilic lung inflammation • Decreased BALF proinflammatory cytokines, nitrite, nitrate • Decreased collagen accumulation	None specified (soluble mediators)	No
Cargnoni 2010 (410)	Mouse bleomycin Intravenous, intraperitoneal, or intratracheal cells 15 min after bleomycin	BALB/c mouse placenta	No	Allo and Xenogeneic	• Improved body weight (allo and xeno, IP and IT) • Decreased histologic fibrosis at D14 but not D9 (allo IV, IP, IT)	None specified (soluble mediators)	Allogeneic Mouse BCF fetal membrane-derived cells • No effects on lung inflammation at 9 or 14 days (IP or IT)

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
		Human term placenta amnion and chorion-derived stromal cells human term placenta-derived epithelial cells used as a mix of 50% hAMCS + hCMSCs and 50% hAECs	No	Allo and Xenogeneic	• No change in lung inflammation at D9 or 14 (allo IV) • Decreased tissue neutrophils on D14 (allo IP, IT) • No change in lung inflammation D3,7,9,14 (Xeno IP,IT) • Decreased severity of histologic injury at D14 (Xeno IP, IT) • Decreased tissue neutrophils on D14 (Xeno IP but not IT) • Increased lung inflammation at D14 in control mice (Xeno IP, IT)	None specified (soluble mediators)	• Decreased lung fibrosis at D14 (IP or IT) • Stimulated inflammation in PBS control mice at D9 IIP and IT) and D14 (IT)
Ischemia-reperfusion injury							
Manning 2010 (421)	Rat left lung ischemia for 2 h Intravenous MSCs 2 h after injury	Rat BM Plastic adherent	Retrovirus-transduced to secrete IL-10	Syn	• Improved oxygenation • Decreased histologic injury and apoptosis • Improved microvascular permeability • MSC-IL10 generally better than MSC-vector control	IL-10 secretion other paracrine effects-soluble mediators	No
Obstructive Sleep Apnea							
Carreras 2010 (443, 444)	Induced obstructive apneas rat	Rat BM Texas (Tulane) MSC Core	No	Syn	• Increased MSC chemotaxis and adhesion to endothelial cells in presence of apneic serum	None specified (soluble mediators)	No
Pulmonary Hypertension							
Baber 2007 (426)	Rat monocrotaline Intratracheal MSCs 2 wk after injury	Rat BM Plastic adherent	No	Syn	• Improved cardiopulmonary physiology	None specified (soluble mediators)	No
Sepsis							
Gonzalez-Rey 2009 (414)	Mouse cecal ligation and puncture or intraperitoneal LPS Intraperitoneal MSCs 2 d after injury	Human adipose	No	Xenogeneic	• Decreased mortality, PLF inflammatory cells and bacterial CFU • Decreased serum/lung/liver/intestine inflammatory cytokines and MPO activity	None specified	No
Nemeth 2009 (413)	Mouse cecal ligation and puncture Intravenous MSCs 1 h prior, concomittant, or 24 h after surgery	Mouse BM Plastic adherent CD11b, 45 depleted	No	Syn and Allo	• Improved survival and organ function • Decreased circulating TNF-α, IL-6 • Increased circulating IL-10	LPS and TNFα-stimulated MSCs stimulated macrophages to produce IL-10 through cell-cell contact and iNOS-dependent release of PGE2	Whole bone marrow, heat-killed MSCs, skin fibroblasts • No effects on survival • Other endpoints not assessed

(*Continued*)

TABLE 4. (CONTINUED)

Injury Model	Experimental Model, Route, and Timing of Treatment	MSC Source	MSCs Modified	Syn or Allo	Outcome Compared to Injury Effects	Potential Mechanisms of MSC Actions	Cell Controls?
Mei 2010 (415)	Mouse cecal ligation and puncture Intravenous MSCs 6 h after surgery	Mouse BM Plastic Adherent Texas (Tulane) MSC Core	Yes	Syn	• Improved survival and organ function • Decreased pulmonary and systemic inflammation • Decreased BALF IL-1β, IL-6, JE, KC, CCL5 • Enhanced bacterial killing by host immune cells • Decreased inflammatory gene expression by microarray • Increased phagocytic gene expression by microarray	None specified (soluble mediators)	No
Yagi 2010 (416)	Rat intraperitoneal LPS or 30% burn Intramuscular MSCs immediately after LPS or burn	Human MSC Texas (Tulane) MSC Core	No	Xenogeneic	• Improved tissue injury (lung/liver/kidney)	None specified (soluble mediators)	No
Yagi 2010 (417)	Rat intraperitoneal LPS Intramuscular MSCs immediately after LPS	Human MSC Texas (Tulane) MSC Core	No	Xenogeneic	• Improved tissue injury (lung/liver/kidney) • Decreased circulating TNF-α, IFN-γ, IL-6	soluble TNFR1 secretion	No

Definiton of abbreviations: Allo = allogenic; BALF = bronchoalveolar lavage fluid; BM = bone marrow; COPD = chronic obstructive pulmonary disease; KC = keratinocyte chemoattractant; KGF = keratinocyte growth factor; LPS = lipopolysaccharide; MIP = macrophage inhibitory protein; MPO = myeloperoxidase; MSCs = mesenchymal stem cells; SCID = severe combined immunodeficiency; Syn = syngeneic; TIMP = tissue inhibitor of metalloproteinase.

inflammatory marker C-reactive protein, commonly elevated in patients with COPD, was noted in treated patients as was a trend toward improvement in quality of life indicators. The trial ends in the fall of 2010 and further results will be forthcoming.

Lung tissue bioengineering. One rapidly growing area of investigation is that using three-dimensional (3D) matrices or other artificial scaffolding for growth of functional lung tissue from stem cells *ex vivo* and *in vivo*. These approaches have been increasingly successful in regenerating other tissues including skin, vasculature, cartilage, bone, and more recently, heart and liver (471, 472). Given the complex 3D architecture and structure–function relationships of the lung, this is a daunting task, Nonetheless, there has been significant progress in several areas. Notably, MSCs isolated from amniotic fluid, umbilical cord blood, or bone marrow can be seeded on biodegradable polyglycolic acid or other biosynthetic scaffolds and also onto de-cellularized cadaveric scaffolds for generation of tracheal cartilage for use in repair of congenital tracheal defects and also tendon tissue for use in congenital diaphragmatic defects (321, 328, 473–483). This has recently culminated in successful clinical use of a bioengineered bronchus and more extensive use of bioengineered trachea and bronchi is expected (484).

Three-dimensional culture systems have also been used as matrices for *ex vivo* lung parenchymal development and for study of growth factors and mechanical forces on lung remodeling (111, 312, 485–492). For example, culture of fetal rat lung suspensions in a 3D glycosaminoglycan scaffold resulted in formation of alveolar-like structures in the scaffold (489). Fetal mouse cells cultured in 3D hydrogels and in synthetic polymer scaffolds resulted in the generation of alveolar-like units (485). Stimulation of the fetal mouse cells in polymer scaffolds with different isoforms of fibroblast growth factor stimulated different patterns of development (486). Fetal rat lung cells cultured in a biosynthetic gelatin matrix and subsequently injected into normal rat lungs induced formation of branching, sacculated epithelial structures reminiscent of lung parenchymal architecture (492). Comparable scaffold approaches have been used to study creation of pulmonary vascular networks and to investigate the effects of vascular endothelial cells on the development of airway and alveolar epithelial tissues (493, 494).

These studies demonstrate the power of 3D culture systems to evaluate lung development and repair. However, many of these studies were not designed to demonstrate functionality of the engineered lung tissue, such as the potential for ventilation and gas exchange. It is also not clear if artificial scaffolds provide the best framework for generating functional lung tissues. Artificial scaffolds neither fully replicate the complexity of the lung architecture nor do they contain all the matrix components essential for normal lung development and function. As such, recent work has focused on the use of more natural models including nasal septa and decellularized whole lung as a more physiologic scaffold in which the native structure of the lung and relevant extracellular matrix components, including collagens, elastin, and laminin, are left intact after removal of cellular material with detergents and other agents (45, 495–500) (Figure 5). In particular, the use of decellularized lungs for growing alveolar cells in culture was first described 25 years ago (496) and has been rediscovered for testing in *in vivo* applications. The decellularized lungs can be both mechanically ventilated and undergo vascular perfusion to provide more physiologic study of *ex vivo* lung generation. Two recent studies demonstrated that inoculating decellularized rat lungs with different mixes of fetal rat lung homogenates, endothelial cells, and A549 carcinoma cells resulted in recellularization of the decellularized scaffolds (499, 500). Although the resulting cellular architecture did not fully resemble that of native lung, the recellularized lungs were able to be surgically implanted in rats

that had previously undergone unilateral pneumonectomy with short-term survival of the rats and some degree of gas exchange and vascular perfusion achieved (499,500; Figure 5). These are important proof of concept studies that are expected to stimulate a number of comparable studies utilizing de-cellularized lungs, including human lungs.

However, there are few studies as yet evaluating whether stem or progenitor cells isolated from adult bone marrow, cord blood, or other sources, including the lung itself, can also comparably form airway or alveolar-like structures when cultivated in 3D matrices, including decellularized whole lungs, or other scaffolding material, or further whether stem or progenitor cells cultured in a such fashion that can be used for functional lung regeneration *in vivo*. Populations of cells described as adult lung somatic progenitor cells isolated from adult sheep lungs,and cultured in synthetic polymer constructs resulted in the expression of airway and alveolar epithelial markers by the cells (111). Structures resembling lung airways and parenchyma developed when impregnated constructs were implanted subcutaneously in nude mice or inserted into the wound cavity after wedge lung resection in sheep. Implantation of adult sheep lung mesenchymal stem cells cultured in synthetic fibronectin-hydrogel scaffolds into lungs of sheep with experimentally induced emphysema resulted in improved tissue mass and lung perfusion (312, 501). However, it is not clear whether the MSCs differentiated or had paracrine effects in this model. Adipose-derived MSCs, cultured *ex vivo* in sheets of polyglycolic acid and then applied to wound edges after lung volume reduction surgery in rats, accelerated alveolar and vascular regeneration (436). Data presented in abstract form at this meeting and also at the 2009 and 2010 ATS meetings demonstrate that intratracheal inoculation of murine MSCs into decellularized whole mouse lungs results in growth of the cells along both airways and in parenchymal lung regions with some of the cells demonstrating expression of pro-SPC (502). Intratracheal inoculation of decellularized rat lungs with murine ESCs resulted in apparent differentiation into several epithelial and vascular structures (498). Lung tissue bioengineering with stem cells is projected to be an area of intense investigation.

In addition to genetic regulatory programs and culture approaches, including use of 3-dimensional scaffolds, mechanical forces also play a role in how differentiating tissues respond to gene instructions (503–505). During breathing, lung cells are subjected to complex mechanical loading that includes shearing due to air movement, compression due to pressurization, and stretch due to the expansion of the lung tissue during inhalation (490). *In utero*, the mechanical forces are, in part, generated by fetal breathing-like movements produced by rhythmic contractions of the respiratory muscles with varying frequency and amplitude (506–509). The normal movement is essential in the development of fetal lung, differentiation of AECII cells, and synthesis of surfactant protein (510–513). Several *in vitro* studies have demonstrated that application of cyclic mechanical stretch to cultures of mixed fetal rodent lung cells increases SPC mRNA expression compared with cells cultured under static conditions (510–514). The influence of stretch induced by mechanical ventilation in clinical and experimentally induced lung injuries has been an area of growing investigation (515–

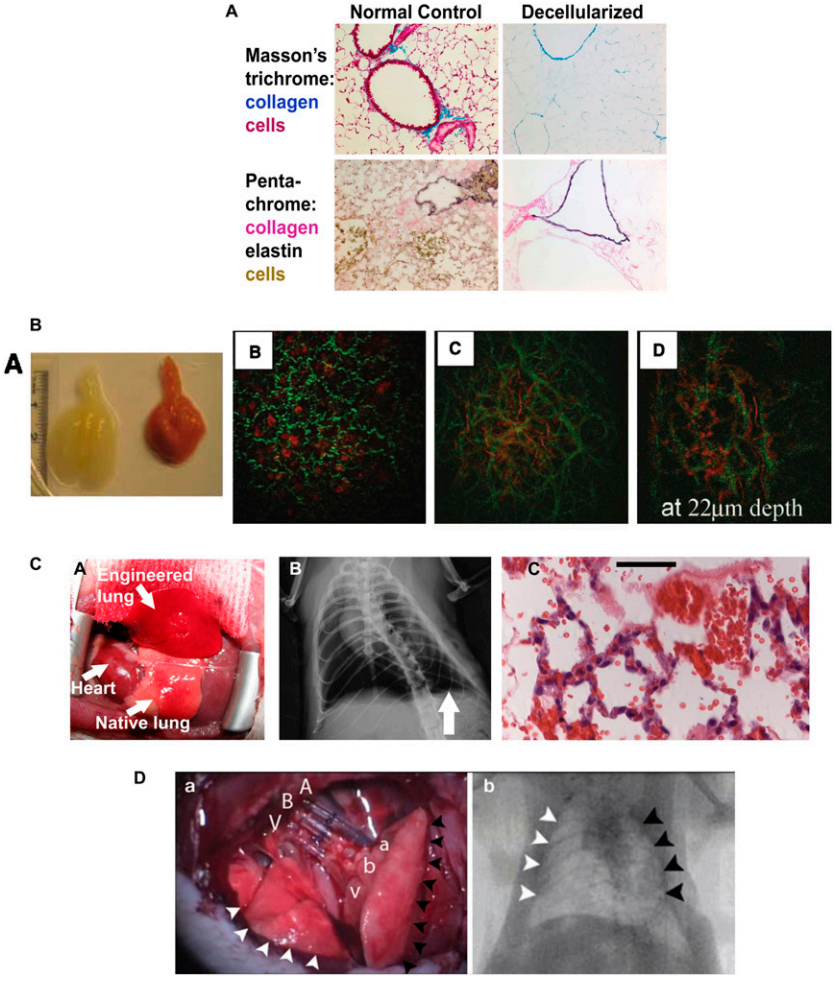

Figure 5. (*A*) Massome's trichrome and pentachrome staining of extracellular matrix proteins collagen and elastin in native and de-cellularized mouse lung. Reprinted with permission from Reference 497. (*B, A*) Gross image of AC rat lung (*left*) next to mESC-recellularized lung (*right*) after culture for 14 days showing contraction of the ECM. (*B–D*) Two-photon imaging: three-dimensional reconstructions of (*B*) normal fresh rat lung tissue, (*C*) AC lung, and (*D*) recellularized rat lung tissue. *Green color* corresponds to SHG showing collagen and red to autofluorescence of cells, elastin, and other ECM. (*D*) Recellularized lung tissue imaged at a depth of 22 mm. Reprinted with permission from Reference 498. (*C, A*) Tissue-engineered left lung was implanted into adult Fischer 344 rat recipient and photographed approximately 30 minutes later. (*B*) X-ray image of rat showing the implanted engineered left lung (*white arrow*) and the right native lung. (*C*) Hematoxylin and eosin stain of explanted lung. Red blood cells perfusing septa are evident, and some red blood cells are present in airspaces. *Scale bar* = 50 μm. Reprinted with permission from Reference 499. (*D, a*) Photograph of left rat chest after left anterior thoracotomy, left pneumonectomy, and orthotopic transplantation of a regenerated left lung construct. Recipient's left pulmonary artery (A), left main bronchus (B), and left pulmonary vein (V) are connected to regenerated left lung pulmonary artery (a), bronchus (b), and pulmonary vein (v). *White arrowheads*, the recipient's right lung (infracardiac and right lower lobe); *black arrowheads*, the regenerated left lung construct. (*D, b*) Radiograph of rat chest after left pneumonectomy and orthotopic transplantation of a regenerated left lung construct. *White arrowheads*, recipient's right lung; *black arrowheads*, regenerated left lung construct. Reprinted with permission from Reference 500.

517). Stretch and other mechanical influences such as fluid shear have been intensely investigated for influence on development of tissues such as cartilage, bone, and vascular structures from MSCs and EPCs as well as from ESCs (336, 518–520). However, there are few studies evaluating effects of physical forces on development of lung epithelial cells or on development of 3D lung structures from endogenous lung progenitor cells or from either embryonic or adult stem cells. Preliminary data presented at this meeting demonstrates that cyclic bi-axial mechanical stretch of MSCs can induce generation of both airway and alveolar epithelial proteins (521).

Further investigation of the effects and mechanisms of mechanical forces on development of lung from stem and progenitor cells is expected to be an area of intense investigation. The obvious challenges in developing complex 3D functional lung tissues *ex vivo* will be in recapitulating the normal dynamic integrated 3D network of epithelial cells, endothelial cells, fibroblasts, neuronal cells, and inflammatory cells in the appropriate environment and architecture with the correct effector molecules and mechanical forces, all of which are vital for proper function. A thorough an understanding of the synergistic interactions between cells in physiologically relevant conditions is critical for the development of tissues that recapitulate the structure and function of the parental tissue *in vivo.* Use of bioreactor systems may provide significant inroads into this area (522–524). Thus far, relatively limited data is available for modeling the lung. Several recent studies have demonstrated the power of rotating bioreactor systems to assess interactions of pathogenic bacteria such as *P. aeruginosa* with lung epithelial cells (525, 526). Comparably, growth of lung epithelial cells, dendritic cells, and macrophages on porous filters has been shown to mimic some of the innate immune pathways of lung airways (527). A more recent study demonstrated that human lung cells and human blood capillaries were able to be coated onto porous polydimethylsiloxane chips mimicking the alveoli of the lungs (528). This "lung-on-a-chip" device was further used to evaluate how nanoparticles and bacteria enter the lungs and may also be useful for high throughput screening of drugs. Clearly, the field is ripe for further comparable technologic advances in bioengineering approaches for lung regeneration (Figure 8).

Embryonic Stem Cells and Induced Pluripotent Stem Cells

Progress using embryonic stem cells for lung regeneration or repair has been slower to develop (27, 47). In studies over the past approximately 5 years, several laboratories have demonstrated that both mouse and human ESCs can be induced in culture to acquire phenotypic markers of type 2 alveolar epithelial cells, including expression of surfactant proteins and lamellar bodies, and even form pseudoglandular structures (27, 231, 529–534). However, in general, this occurred at a low level unless the ESCs were transduced to select for surfactant protein-expressing cells (535). More recent protocols and manipulation of cell signaling pathways guiding embryologic lung development and development of definitive endoderm have yielded more robust *in vitro* derivation of cells with phenotypic characteristics of AECII cells from murine ESC (Figure 6) (536, 537). Derivation of airway epithelial cells from ESCs has proven even more elusive, although development of cells with phenotypic markers of airway epithelial cells has been demonstrated after culturing ESCs under air–liquid interface conditions (538, 539). More recently, effect of matrix proteins, 3D scaffolds, and mechanical forces on ESC differentiation to lung epithelium has been investigated (498, 519, 523, 540–542). This is projected to be an area of increased investigation.

Moreover, there are still only limited available studies of effects of ESC administration to lung *in vivo*. Endotracheal administration of mESC-derived AECII cells to fetal mice resulted in survival of the cells in the lungs and maintenance of pro-SPC expression over a 24-hour period (27). However, whether the cells truly engrafted or had functional significance was not determined. More recently, it has been demonstrated that intratracheal administration of the AECII cells derived from hESCs (hES-ATII cells) (535) 1 or 2 days after induction of acute lung injury resulting from intratracheal bleomycin administration to immunocompromised SCID mice resulted in a substantial number of hES-ATII cells appearing to have engrafted in lung with persistent SPC expression up to 9 days later (211). Up to 20% of the total SPC-expressing cells appeared to be of hES-ATII origin. A small number of hES-ATII cells also appeared to have differentiated into type 1 cells *in vivo*. No apparent engraftment was observed if hES-ATII cells were administered to naïve uninjured mice or if a control monocyte population was administered. Notably, bleomycin-induced lung injury was significantly reduced in mice receiving hES-ATII cells but not monocytes or vehicle control. Reduced injury was measured by both qualitative and quantitative measures, including, importantly, functional measurements of tidal volumes and arterial oxygen saturations (211).

This is the first published investigation demonstrating amelioration of lung injury by ESC administration. Notably, these studies reflect the importance of assessing functional physiologic outcomes in parallel with changes in lung injury endpoints such as histologic inflammation and hydroxyproline content. Whether the observed amelioration of lung injury resulted from structural engraftment of the administered cells or reflected a previously unsuspected paracrine effect of the hES-derived cells is not yet clear. Nonetheless, these results have a number of ramifications, including the study and potential use of ESCs in genetic lung diseases, as human deltaF508 embryonic stem cell lines have been established (543–545). These cells exhibit normal morphology and protein expression compared with other hES cell lines but have not been studied in detail.

The American Thoracic Society issued a statement in 2006 calling for expanded human embryonic stem cell research and a follow-up statement in 2010 after the temporary injunction on the use of human stem cells in the United States (3, 546). It is hoped that human embryonic stem cell research will continue to expand in the U.S. and that there will be further rapid advances in the study of ESCs for lung injury and repair.

Rapid advances in techniques for generating, and studying induced pluripotent stem cells have raised hope that these cells will also be useful tools for generating lung epithelial and other lung cells *ex vivo* (547–551). An exciting recent advance was the generation of disease-specific human-induced pluripotent stem cells (iPS) cell lines from patients with both genetic and acquired lung diseases including cystic fibrosis, α 1 anti-trypsin disease, sickle cell, and scleroderma (552) (Figure 7). However, while iPS cells can be readily differentiated into cells with characteristics of primitive endoderm, generation of cells expressing phenotypic markers of lung epithelial cells has thus far proved elusive. Also, there is no strategy yet developed to ensure that cultures of extensively differentiated iPS or ESCs do not contain a remnant of undifferentiated tumorigenic cells. Nonetheless, it is expected that rapid advances in this field will occur over the next few years.

SCIENTIFIC PRESENTATIONS

Session 1: Endogenous Lung Progenitor Cells and Lung Cancer Stem Cells

This session focused on recent developments in the characterization, organization, and regulation of regenerative cells in the adult lung in the steady state and in lung disease, as well as on

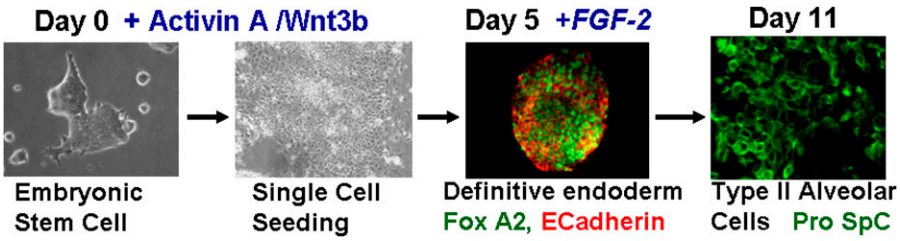

Figure 6. Schematic of protocol used to differentiate esc into cells with phenotypic characteristics of type 2 alveolar epithelial cells (537). Embryonic stem cells (ESCs) can be effectively manipulated *in vitro* to differentiate into type 2 alveolar epithelial cells using lung development cell signaling pathway to guide ESCs differentiation. FGF-2 = Fibroblast growth factors-2, Pro Spc = Pro-surfactant protein C. Adapted with permission from Reference 537.

the relationship of normal endogenous lung stem cells to lung cancer stem cells.

Paul Simmons (University of Texas) presented an overview of models of stem cell organization, comparing and contrasting the functional properties of stem and progenitor cell hierarchies in rapidly renewing tissues with those in complex solid organs such as the lung where tissue maintenance and repair involves the coordinated interaction of diverse cell types of differing proliferative and differentiative potentials. He pointed out that although stem and progenitor cell fate in all organ systems is specified by their complex interaction with the extracellular matrix proteins, soluble and insoluble factors and stromal cell elements that comprise their niche (553), their organization, behavior, and functional properties may not conform to a classical stem cell model. He then discussed the fidelity, or lack thereof, of biomarkers in classifying different cell types in the lung. For example, although lung mesenchymal cells are characterized by the expression of the Sca-1 antigen, and can be resolved from lung epithelial cells on the basis of their differential expression of Sca-1 (84), work in his laboratory has identified Sca-1^{neg} lung fibroblastic perivascular cells in the distal lung, which are defined by their expression of PDGFα receptor.

Susan Reynolds (National Jewish Health, Denver) described experiments analyzing the organization of tracheobronchial stem and progenitor cell compartments in the normal steady-state and in naphthalene treated mice. These studies have

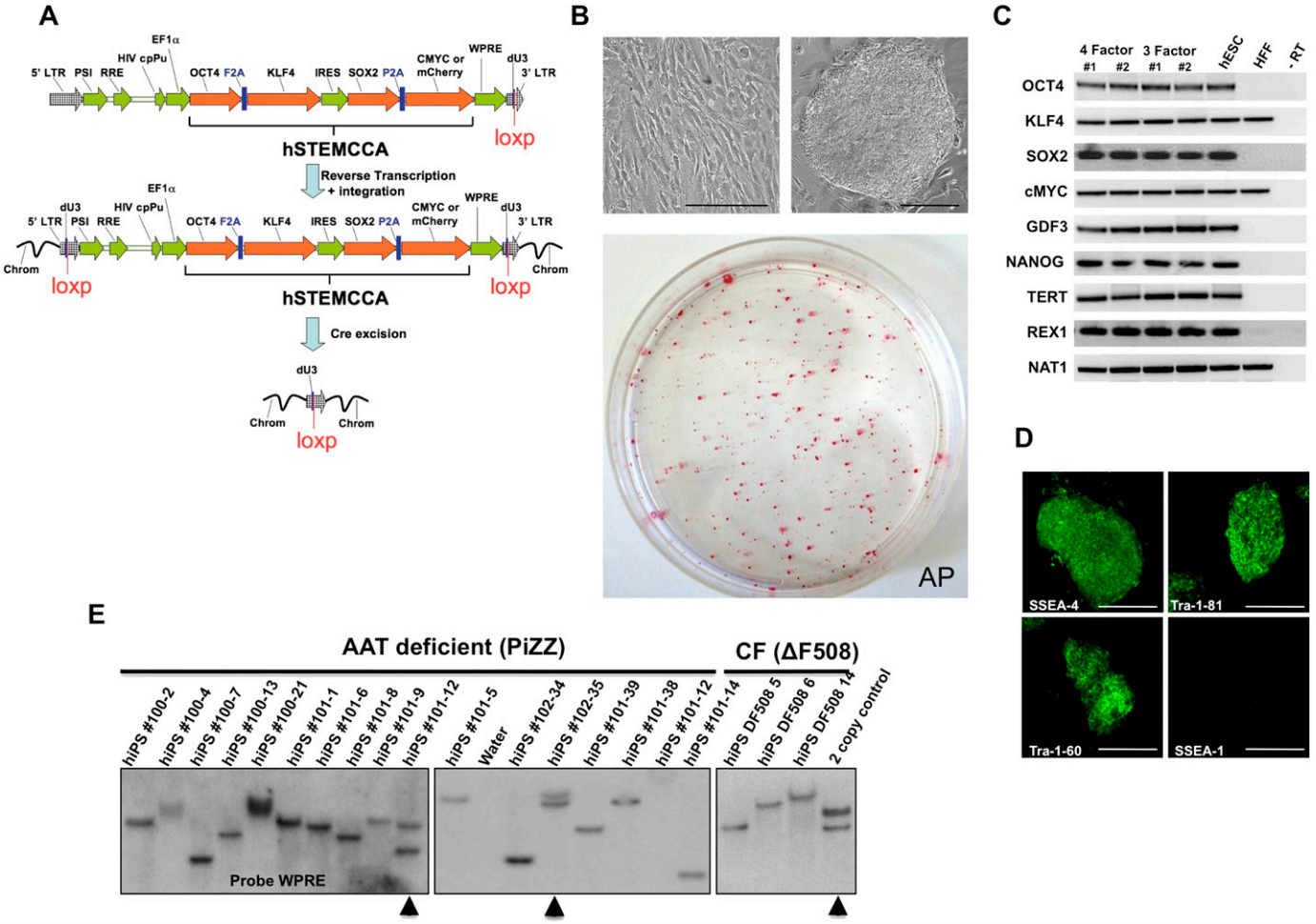

Figure 7. Derivation of transgene-free iPS cell line from tail tip of a CFTR knockout (KO) mouse. (*A*) The human floxed STEMCCA reprogramming vector. (*B–D*) generation of iPS cells by reprogramming human dermal fibroblasts, and their characterization by RT-PCR and immunostaining. (*E*) Southern blot of iPS cell clones generated from dermal fibroblasts from CF (DF508 CFTR) and α_1-antitrypsin deficient (PiZZ) volunteers. The Southern blot shows high frequency of single copy STEMCCA integrants in the resulting clones prior to Cre-mediated excision of this vector to generate human transgene-free iPS cells. Adapted with permission from References 551 and 552.

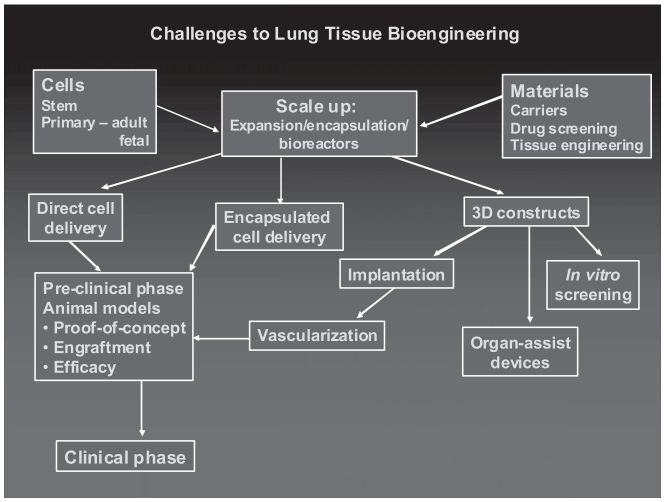

Figure 8. Schematic of challenges and issue for lung bioengineering. Slide courtesy of Dame Julia Polak DBE, MD, DSc.

revealed the existence of three related cytokeratin 14^{pos} tracheobronchial epithelial stem/progenitor cells of differing potentiality that are defined by the differential expression of cytokeratins 5 and 15 (64). Her data suggests that tracheobronchial Clara and ciliated cell lineages are maintained by unipotent cytokeratin 14^{pos} cells in the steady state but by multipotent cytokeratin $14^{neg}5^{pos}15^{pos}$ cells, which up-regulate cytokeratin 14 after naphthalene-mediated ablation of tracheobronchiolar epithelial cells. This work highlights the power of perturbation models in revealing the organization and regenerative potential of adult lung stem and progenitor cells and how they are recruited to repair the lung after injury.

Ed Morrisey (University of Pennsylvania) spoke about the critical role of the Wnt signaling pathways: not only in lung endodermal progenitor cell fate specification during development, but also in regulating and maintaining the integrity of epithelial and mesenchymal cell lineages in the adult lung in the steady state and in response to injury or disease. In particular, he highlighted the role of Wnt7b in promoting and regulating smooth muscle cell differentiation by transcriptional activation of the extracellular matrix protein, Tenascin C, which in turn up-regulates PDGFα and β receptor expression (554). Significantly, loss of Wnt7b results in defective lung vascular and airway smooth muscle cell development, whereas up-regulated Wnt7b-Tenascin C-PDGFR signaling characterizes allergen-induced asthma in mice and human pulmonary hypertension. The presentations that followed analyzed the response of bronchoalveolar stem and progenitor cell pools in response to injury or insult, and their potential role as candidate lung cancer stem cells when mutated or transformed.

Barry Stripp (Duke University) described studies in chimeric and transgenic reporter mouse models that combined lineage tracing, clonal analysis, and halogenated thymidine analog labeling of proliferating cells to measure the cycling and turnover of lung stem and progenitor cell pools in the steady state, and after naphthalene-induced airway injury, or perturbation, of stem cell pool size and differentiation potential by manipulation of canonical Wnt signaling (65, 80, 96). These studies confirm that epithelial stem and progenitor cell pools that maintain the integrity of airway epithelium in the steady state are functionally distinct from those that are recruited to repair the lung after injury: bronchiolar epithelial stem cells with the highest regenerative potential being relatively quiescent, less susceptible to cytotoxic insult, and only recruited in response to severe injury.

Majd Mouded (University of Pittsburgh) described the analysis of the behavior of candidate bronchioalveolar stem cells (BASC) in the course of elastase or cigarette smoke–induced emphysema using an airway lineage (CC-10) tagged ROSA26 reporter mouse model. Preliminary experiments show increased proliferative activity in the BASC compartment after injury together with augmented alveolar Type 2 cell numbers near the bronchioalveolar duct junction consistent with the participation of BASC or a related precursor in alveolar repair. Although promising, he identified a need for better and more informative animal reporter models and definitive biomarkers for the delineation and characterization of cells participating in alveolar repair in lung disease.

Kerstin Sinkevicius (Boston Children's Hospital) shared her data on the analysis of cancer propagating cells in lung tumors established by orthotopic transplantation of primary adenocarcinomas from Kras and Kras-p53-deficient mice, which recapitulate key features of human non–small cell lung cancers. These experiments compared the immunophenotypic staining pattern of candidate lung cancer–initiating and propagating cells with that of their normal counterparts and tested the hypothesis that they share a common cell surface phenotype. This work (555) demonstrates the fidelity of the orthotopic transplant model in recapitulating the properties of primary lung tumors from which they are derived, but also reveals that a difference in genotype at a single locus significantly influences the marker repertoire that defines cancer propagating cells in different tumors. Briefly, whereas cancer-propagating cells derived from Kras-p53-deficient tumors were highly enriched in the Sca-1^{pos} cell fraction, this was not the case for Kras tumors where Sca-1^{pos} and Sca-1^{neg} cells were equally tumorigenic. On the other hand, analysis of only Sca-1^{neg} cells are able to propagate tumors in EGFR mutant tumors. Although the study demonstrates the significance of confounding factors in determining the nature and properties of aberrant cells in disease pathophysiology, it also highlights the limitations of phenotypic analysis in the absence of robust functional assays in assigning stemness.

The panel discussion that followed raised questions about the nature and properties of stem cell niches in the lung and the role of the niche in specifying stem cell fate and regulating regenerative potential. A discussion of the prerequisite functional characteristics that must be met to assign stemness in the steady-state and after perturbation or injury was also debated, questioning if regenerative and reparative cells differ in different contexts. The concept of the temporal niche was also raised. The fact that the properties of the stem cell niche alter during disease progression poses a challenge in the development of strategies to harness the potential of endogenous regenerative cells, or to deliver exogenous reparative cells. Consequently, the repair or normalization of the niche is a critical consideration in developing successful stem cell–based or stem cell–directed therapies for lung repair. In summary, cell lineage tracing studies, the interrogation of gain-of-function, loss-of-function, and the temporal analysis of lung perturbation models have significantly enhanced our understanding of the organization and regulation of regenerative cells in the lung. However, there remains a pressing need for the identification and validation of biomarkers and biomarker repertoires for classifying lung stem and progenitor cells and the stromal elements that comprise their niches, as well as robust functional assays predictive of their regenerative potential.

Session 2: Embryonic Stem Cells, iPS, and Lung Regeneration

The theme of this session focused on the regenerative potential for lung cell lineages and tissue. Various types of stem cells were

discussed, including ESCs and iPS cells as well as the importance of devising and providing the correct functional niche.

Konrad Hochedlinger of the Harvard Stem Cell Institute pointed out that although the properties of ESC and iPS are similar, the efficiency of reprogramming iPS is low. Key bottlenecks in this field are that actual reprogramming of individual stem cells is a rare event, that viral infection is inefficient, that there is a risk of insertional mutagenesis, and that reprogramming is a stochastic process requiring several events to occur in the correct sequence (556). Furthermore, Hochedlinger pointed out that most differentiated cells have limited replication potential in culture and ultimately undergo senescence, which may be a critical barrier to scalable reprogramming. Additionally, iPS cells may actually be derived from adult progenitor cells as opposed to mature cells, as had been previously assumed. As a corollary of that, cellular immortalization may therefore eliminate a key roadblock during cellular reprogramming. Thus, he suggested INK4a/ARF and p53 as key putative molecular targets, the deletion or inhibition of which may remove major roadblocks to cellular reprogramming.

Darrell Kotton from Boston University focused the discussion of ESC and iPS technology on driving differentiation of endoderm and of lung epithelium from endoderm. He pointed out the caveat that iPS cells still have many issues to be worked out, including the necessity for multiple integrations, as well as the far-from-negligible potential for oncogenesis of iPS cells *in vivo*, not least because of the requirement for integration of oncogenes such as Myc. Nevertheless, he showed that postnatal mouse fibroblasts can be induced into an iPS phenotype using a lentiviral "stem cell cassette" (STEMCCA), which expresses Oct4, Sox2, Kif4, and cMyc simultaneously. These cells were validated in puripotency assays including the formation of teratomas *in vivo*, as well as chimera derivation from mouse blastocysts, including the derivation of lung endoderm, as shown by the expression of Sox2-GFP in lung epithelium. He then showed elegant proof of principle that subsequent Cre-mediated excision of the STEMCCA does not cause reversion of the iPS phenotype previously induced by STECCA expression (550, 551). Taking a lesson from endoderm derivation during gastrulation, which is driven by spatial information imparted by BMP4, Wnt, and Nodal signaling, among other factors, including FGF, he then showed that lung-specific endoderm TTF1-GFP reporter gene expression can be driven within STECCA iPS-derived endoderm using a cocktail of fibroblast growth factor (FGF) and Nodal.

Carolyn Lutkzo from the Saban Research Institute at Children's Hospital in Los Angeles shared her protocol for differentiating definitive endoderm from hESC, which follows from developmental principles. She finds that she is able to sequentially induce expression of mesoderm, definitive endoderm, and primitive gut tube markers including *Gata6*, *Sox17*, *Foxa2* and *Ttf1* over the course of 7days from cultures of hESC using sequential exposure to Activin A/Wnt3a, Activin A/0.2% serum, and KGF/2% serum. Then the introduction of FGF10 to the mix induces *Ttf1*, *CC10*, and cytokeratin 18, but not *SpC*. This system requires further optimization, but it shows distinct promise for the feasibility of deriving respiratory endoderm from hESC *in vitro*.

Rick Wetsel from the University of Texas Health Science Center in Houston described a genetic strategy using an SPC promoter-driven neomycin resistance cassette–based selection system to drive purification of AEC2 derived from hESC to greater than 90% purity (535). He now finds that not only are his hESC-derived AEC2 capable of acting as progenitors for AEC1, but that they also ameliorate bleomycin-induced lung injury after *in vivo* engraftment, which results in a complete rescue of lethality in mice (211).

Lastly, Peter Lelkes from Drexel University pointed out the importance of the 3D tissue niche in directing differentiation of murine ESC into alveolar-like constructs. He demonstrated examples of organotypic recombination of fetal mouse lung cells within matrigel/collagen gels. Furthermore, he showed the presence of vascular endothelial vascular structures within these constructs and indicated that FGF and VEGF signaling, as well as Shh and tenascin, play important roles in this process. He then demonstrated the potential for synthetic electrospun collagen matrix (versus elastin matrixes that mimic decellularized human lung) to some extent support morphogenesis of fetal lung cell-derived organotypic epithelial and vascular structures.

Taken together, these presentations demonstrate significant progress not only in the derivation of definitive endoderm as well as respiratory endoderm from both mouse and human ESC, but also in the potential for the derived cell lineages to contribute to regenerative medical applications in the lung *in vivo*. Moreover, some excitement was generated about the potential for matrix-driven tissue engineering using organotypic recombination of stem/progenitor cells in matrix. Nevertheless it is evident that much remains to be accomplished, particularly in terms of scalability, toxicity, tolerance, and connectivity in translating these undeniably exciting potential approaches to human disease applications.

Session 3: Bioengineering Approaches to Lung Regeneration

The goal of this session was to review the progress in tissue engineering, encompassing biologic scaffolds and tissue culture systems as they apply to the lung. The session began with Dame Julia Polak, Imperial College, London (by videoconference), who provided a general overview of lung tissue engineering and highlighting the many challenges currently facing the field. It is not yet clear which progenitor-cell sources will be optimal for generating lung tissue. Candidates include primary cells (adult and fetal), embryonic stem cells (ESCs), adult stem cells from bone marrow and cord blood, circulating mesenchymal cells, niche-specific cells, and adipose-derived cells. Much effort is currently spent on how to enhance lineage-specific differentiation using chemical factors and factors of the microenvironment. There has been some success in driving murine ESCs to express Type II pneumocyte proteins (e.g., SP-C) via direct and indirect coculture methods. An early report showed that removal of retinoic acid from culture increases differentiation of ES cells, but the process was long (33 days) and the yield was low (2.8%) (557). Nevertheless, it is a step in defining factors that inhibit or stimulate the differentiation process. The use of pneumocyte cell extracts may decrease the culture time required and increase yield (558, 559). Wang and colleagues did show that the use of gene modulation by transfection with an SPC-promoter-neomycin transgene (for selection) resulted in an almost pure population of cells with lamellar bodies, SP-A, SP-B, SP-C, and other Type II pneumocyte markers (535). Recently, several laboratories have begun studies in an effort to engineer lung tissue *ex vivo* using 3D constructs. The material used to generate such constructs should be able to stimulate cell growth and differentiation, cell attachment, and deposition of extracellular matrix, as well as act as a template for tissue vascularization growth in 3D, be resorbable for implantation, and be insoluble for *in vitro* screening and toxicity studies. Another major challenge is that of scale up. For expansion, static two-dimensional cultures have many drawbacks, including lack of mixing and control options, and they require constant feeding, which typically results in low yield and poor quality of cells. In contrast, the advantages of 3D cultures is their simplicity, applicability to process integration, and automation,

which leads to enhanced proliferation, thus providing higher yields in a shorter time. 3D cultivation methods better approximate the *in vivo* environment of cell to cell and cell to matrix interactions. The use of a bioreactor permits nutrient penetration, and integrated monitoring systems can assess viability and specific cell needs. Alginate beads (from brown seaweed), that are biocompatible and FDA approved, have been used for cell expansion and bone formation. Cell encapsulation has been used for mass production of embryoid bodies, maintenance of pluripotency of human ES cells, and engineering of bone tissues (560–563). The key features of stem cell bioprocessing are control, reproducibility, automation, validation, and safety. Preclinical testing requires animal models, demonstration of safety, engraftment, efficacy, growth control, and immunocompatibility. Demonstration of correction of a defect *in vivo* by a cell construct would be required so that clinical quality production can proceed toward clinical applications. Alternatively, it is possible that the cells could be used in organ-support devices to increase efficiency such as in an interventional lung assist device. A multidisciplinary approach is required to engineer grafts to replace part of a diseased organ, as was recently demonstrated by Macchiarini (484). Denuded trachea was used as the biologic scaffold onto which cultured tracheal epithelial cells and cartilage from bone marrow stem cells was seeded and placed in a custom bioreactor. With this engineered construct, 5 cm of the left bronchus was replaced with restoration of normal function up to 4 months. Current challenges that are a barrier to the translation of stem cell therapy for lung diseases are the poor understanding of lineage specific differentiation, low functionality of differentiated cells, the need for protocols with defined reagents and inefficient 3D growth in culture. Other issues to be considered if a product is developed include the cost and reimbursement, industrial scale production, transport and logistics such as storage and tracking of tissues. Stem cell cultures require a reliable method for cell expansion and differentiation to reduce variability and heterogeneity and to increase the affordability of the reagents. Regulatory protocols need to be implemented to address any donor-associated risks, contamination during bioprocessing, final product purity (including any genetic instability), efficacy, safety, and survival *in vitro*. Other regulatory challenges may include deciding on which *in vivo* models are appropriate to assess cell dose, microenvironment, tumor formation, and immunological issues. Dr. Polak discussed a proposal that was approved in May of 2007 for a single European Union-wide integrated and tailored regulatory framework for advanced therapies. With pharmaceutical companies and industry now starting to be involved in cell therapies, the basic question that needs to be addressed is whether the health care system is ready to incorporate cell-based therapies for lung disease and whether it will be limited to selected patients. The session ended with the conclusion that stem cell therapies for lung disease are reaching maturity, but many challenges have yet to be met, as outlined above.

Christine Finck, Connecticut Children's Medical Center, discussed work aimed at using stem cells to treat pulmonary hypoplasia of preterm infants. Treatments to date consist of mechanical ventilation, surfactant therapy, inhaled NO, extracorporeal membrane oxygenation (ECMO) ECMO, and VEGF administration with variable success. A hypoplastic lung has reduced lung weight, fewer generations of airways resulting in fewer alveoli, poor epithelial cell differentiation and surfactant deficiency, along with hypoplasia of the corresponding pulmonary arteries. Hence, this represents an ideal environment to introduce stem cell therapy, albeit with the advantages and challenges as described by Dr. Polak. Dr. Finck summarized what is known of how the lung is derived from definitive endoderm and how attempts to devise a protocol to recapitulate the critical induction events of the early embryo (e.g., Wnt, TGFb, Activin-A) have been used to induce pluripotent cells to become definitive endoderm (530, 564). In a comparison of seeding methods, it was shown that if ESCs are first allowed to become embryoid bodies (EBs) before seeding onto collagen IV coated plates, only cells that migrated away from the EBs expressed Gata4 whereas cells within the EBs retained expression of Oct4 (486). In contrast, ESCs plated as disaggregated cells on collagen-IV coated plates expressed Gata4 uniformly and no Oct4 (i.e., more directed differentiation). To further direct differentiation of derived endoderm, the Finck lab developed and optimized a 2-step protocol (537) in which ESCs are first incubated in media containing Activin-A and Wnt3a (6 days, single-cell suspension on collage-IV coated plates) as was similarly shown for derivation of hepatic endoderm (564, 565). Importantly for the lung, using 50 ng/ml Activin A with 1 ng/ml Wnt3a, which is important in migration of epiblast cells though the primitive streak in embryogenesis, gave rise to the highest number of cells expressing the definitive endoderm markers Sox17 and FoxA2 and lowered the expression of the visceral endoderm marker HNF4 (qRT-PCR). Increasing the dose of Activin above 50 ng/ml resulted in more cells but not a greater proportion of $FoxA2^+$ cells. Cells expressing E-cadherin and cytokeratin were seen by immunofluorescent staining after a 5-day induction with optimal concentrations of Activin A and Wnt3a. Step 2 of the protocol involves the addition of FGF2, which is important in patterning the foregut endoderm (566). After 5 days in as little as 5 ng/ml FGF2, the cells began to express surfactant proteins A, B, and C, as well as CC10 (Clara cell secretory protein). Increasing FGF2 to 50 ng/ml resulted in increased FoxA2 and SPs and decreased expression of the pancreatic marker PDX-1. In these conditions, approximately 12% of the cells expressed SP-C, 48% expressed vimentin (i.e., mesenchymal), 37% expressed Sox17 (i.e., endodermal), and 1% expressed Pax6 (ectodermal). Lamellar body formation was also demonstrated. Future work will determine if the addition of FGF10 will enhance yield. Preliminary data was shown using the protocol in the context of 3D collagen and matrigel hydrogel with similar results. In other work to develop nonepithelial tissues of the lung, differentiating ESCs in hypoxic conditions (5% O_2) for 5 days decreased the expression of endoderm markers (FoxA2, Bry) and increased expression of the endothelial marker Flk-1 (binds VEGF, as does Flt1). After 11 days, vessel-like networks were observed as well as increased frequency of $PECAM^+$ cells. Using an explants model of pulmonary damage, premature mouse lungs (E18.5) were treated with Flt1 receptor to block VEGF resulting in a hypoplastic lung (decreased mean cord length and increased frequency of $TUNEL^+$ cells). Undifferentiated and differentiated endodermal cells were added to the bottom of a transwell system containing hypoplastic lung at the air–liquid interface. When compared with undifferentiated ES cells, differentiated endodermal and distal airway cells promoted alveologenesis (increased cord length and frequency of $CD31^+$ cells and decreased $TUNEL^+$ cells), likely through production of VEGF. Intratracheal delivery of ES-derived distal airway cells into adult mice resulted in increased levels of VEGF in the brochoalveolar lavage (BAL) fluid. In other experiments relevant to treatment of premature babies *in utero*, it was demonstrated that cells injected into the tail vein of the dam resulted in cells migrating to the lungs of the pups. Future work will investigate the derivation of pure populations of differentiated cells, the use of scaffolds and treatment of premature mice (via intratracheal, tail vein, and intra-amniotic injections).

A vascularized approach to tissue-engineered lung was described by David Hoganson (Massachusetts General Hospital). Because organ shortage is the most critical problem in transplantation,

lessons learned from stem-cell biology, tissue engineering, and regenerative medicine may be able to supply transplantable tissue or an organ replacement. At its basic level, successful engineering of tissue must result in delivery of oxygen and nutrients to the cells, which depends on surface area, concomitant with removal of waste and CO_2. For a 3D organ, tissue engineering must match surface area to volume for effective mass transfer (surface area increases as r^2 but volume increases as r^3). The challenge of vascularization by tissue engineering is to mimic the complexity and microfluidics of the vasculature. This will vary depending on the application, for example, making a fully developed tissue-engineered adult lung versus a neonatal lung versus an ambulatory lung assist device. In recapitulating the basic structure of an alveolus, Dr. Hoganson described the concept of a lung assist device that would be composed of an alveolar chamber separated from a microvascular network of channels, lined with endothelial cells, by a respiratory membrane that would be covered with pneumocytes. Such a device would be a bridge to transplant for end-stage lung disease. It would be implanted in the upper chest with blood flow from the pulmonary artery and returning to the left atrium. Oxygen tubing would be hooked up to and from the device (through the skin). A prototype multilayered (more surface area) cellular device has been developed with "vascular" and "alveolar" chambers separated by a silicone respiratory membrane that is highly permeable to O_2 and CO_2. While anticoagulated sheep blood was circulated through the device at 37°C, O_2 and CO_2 transfer was observed to increase with an increasing rate of blood flow. However, in extrapolating the data for use as an adult lung assist device, it appears that 357 layers of chambers separated by a silicone-coated membrane would be required for 0.64 m^2 of surface area at a flow rate of 4 ml/min per layer. It would remove 20% of CO_2 generated at rest and would handle 1.4 L/min blood flow, which is approximately 25% of cardiac output. This would increase P_{O_2} of that blood from 57 to 79 mm Hg. Further improvements to the design are needed to mimic a high density capillary system by maximizing the network while minimizing physiologic shear stress (otherwise platelets will get activated and obstruct the conduits). Biomimetic design principals were used, taking into account physiological vessel diameters and lengths (parent and daughter vessels) as well as bifurcation angles (that are smoothly rounded at the apex). This resulted in all channels having a 1:1 aspect ratio with the smallest channels having a diameter of 100 μm. Micro-machining using brass molds can be used to create exquisite 3D features (i.e., connections in 3 dimensions). *In vitro* testing with anticoagulated sheep blood demonstrated atrial pressures varying with flow rate almost identical to the computational fluid dynamics analysis. To achieve more physiological relevance, biomimetic vascular design must be translated to biomimetic scaffold material consisting of collagen-coated channels with diameters down to just 2 μm. To determine if gas exchange efficiently occurs across a collagen membrane, transwells were created with a thin collagen film as a respiratory membrane and pneumocytes and endothelial cells were juxtaposed to opposite sides of the membrane. Transwells were attached to gas exchange fixtures so that blood was allowed to flow on the endothelial side of the collagen membrane at a flow rate of 0.0625 ml/min while the pneumocyte side was exposed to 100% oxygen at a flow rate of 20 ml/min. The presence of pneumocytes and endothelial cells resulted in an increase in P_{O_2} in the blood from 20 mm Hg (no cells, or endothelial cells only) to 40 mm Hg. No effect was seen on P_{CO_2} using cells on the collagen membrane. When cultured for 14 days in the presence of pneumocytes and endothelial cells, the thickness of the collagen film decreased from approximately 45 μm to 31 μm. With further incubation, collagen thickness decreased to 8.6 μm, thus beginning to approximate physiological measurements. It is hoped that the collagen would be digested over time and replaced with natural extracellular matrix. Although these studies were conducted using cell lines, human endothelial cells have successfully been seeded onto collagen membranes but it is unknown whether specialized endothelial cells are required.

Ed Ingenito (Brigham and Women's Hospital) spoke about biologic scaffolds for stem and progenitor cell engraftment in the lung. Emphysema was chosen as a target disease, but the principles learned may be applicable to any structural lung disease. One must understand the progenitor cells to be used as well as the cells to be repaired. Other elements to incorporate are scaffold design, impedance matching, mechanical considerations, cell attachment and spreading, cell kinetics, and *in vivo* testing. Phenotypic modulation could be accomplished in several ways including use of growth factors and small molecules (pharmacologic), modulation of extra- cellular matrix (epigenetic), and use of RNAi or gene therapy (genetic). In an ovine model of elastase-induced bullous emphysema, autologous adult progenitor cells and endobronchial delivery were selected over other cell types and intravenous delivery to facilitate cell attachment, spreading, migration, and survival. At 4 weeks after site-specific injection of cells using a fibrin-based engraftment scaffold, the size of the emphysematous lesion was reduced by 40%. Progenitor cells isolated from biopsies of sheep lung demonstrated the presence of epithelial cells (pancytokeratin$^+$, collagen IV$^-$, and fibrillin$^-$) and mesenchymal cells (vimentin$^+$, collagen IV$^+$, and fibrillin$^+$). In culture (at passage 5), mesenchymal lung progenitors (MLPCs) express αSMA, vimentin, S100A4, and PDGFRα. They make extracellular matrix proteins such as collagen I and collagen IV, the adhesive glycoproteins fibronectin, vitronectin and laminin, as well as elastin components such as fibrillin-1 and tropoelastin. They also express growth factors such as VEGF-A, FGF2 and FGF10, important for lung growth and development. These cells have a doubling time of 36–48 hours and demonstrate stable genetics and phenotype. MLPCs express the integrins β1, β5, αV, and α5. Integrin characterization defines the extracellular matrix (ECM) attachment and spreading patterns important in both cell kinetics and cell phenotype. This needs to be taken into consideration when designing scaffolds with attachment ligands to maximize attachment and spreading. If there is a barrier to attachment, the injected cells will apoptose. The 3-part strategy to promote engraftment of progenitors is to *1*) prime the target tissue (precondtioning), *2*) develop an engraftment system, and *3*) modulate the desired effect. The design specifications of preconditioning should be focal, easy to control, result in cell removal, be biocompatible, and be conducive to provisional matrix deposition. Various enzymatic systems were tested and tryspin was selected as it was found to be safe, simple to control, it stimulates cell cycling, is Good Manufacturing Practice (GMP) available, and inexpensive. Its omission was found to reduce engraftment *in vivo*. Other approaches that use detergents and thermal shock have not been developed. Several potential scaffold components with relevant properties were tested alone and in combination for their capacity to promote cell attachment and spreading *in vitro* in different medium conditions. It was found that col IV, fibronectin, and poly-L-lysine (PLL), each alone, promoted good attachment at 20 μg/ml. However, expression of the attachment ligands varied with growth conditions and cell cycle phase. For promotion of spreading (*in vitro* assay), combinations containing 20 μg/ml fibronectin, or col I or col IV worked best, although data was shown that these components alone worked almost as well (as did laminin). PLL alone did not promote spreading but did not adversely affect it in combination with the spread-promoting components (i.e., does not inhibit formation of focal adhesion complexes by integrins). As with attachment, spreading varied with growth and cell cycle conditions. There are also mechanical consider-

ations that need to be considered in scaffold design. Hydrogels with mechanical properties different from lung tissue can cause damage and prevent engraftment. For example, 1.2% alginate is a physical hydrogel that supports cell growth but is very rigid and brittle, whereas 2% fibrinogen is chemical hydrogel that supports cell growth, and is soft and elastic with stress–strain properties similar to lung (at low physiologic stress). Substrates also influence the ability of progenitor cells to enter cell cycle after delivery. They also affect cell survival/apoptosis after degradation of the scaffold, which has implications for engraftment potential. For example, fibrin-mediated apoptosis can be blunted by integrin binding. *In vivo* testing of the role of site conditioning and scaffold composition in healthy sheep showed that sites that received an autologous MLPC graft ($10–50 \times 10^6$ cells in each segmental site) delivered in fibrin-fibronectin-PLL hydrogel had no radiographic evidence of parenchymal inflammation or pleural or mediastinal pathology at 3 weeks after treatment, and the lungs appeared grossly normal. Histological examination of the injected sites demonstrated that cell retention (defined as number of fields with labeled cells) was substantially greater and was maintained longer after grafting was performed with hydrogel scaffold compared with grafting of cells in aqueous suspension. Transplanted cells were shown to express both collagen IV and fibrillin-1 at 3 weeks after injection, showing maintenance of the phenotype. *In vivo* testing in sheep with experimental elastase-induced emphysema showed that treatment with MLPCs in FFV hydrogel scaffold was associated with a reduction in bulla size and improved perfusion when compared with sham treated with scaffold alone. Furthermore, the MLPC treatment was associated with a retention of the infused cells at the treatment sites, increased tissue cellularity, increased extracellular matrix,, and increased tissue elastin at 4 weeks. Dr. Ingenito did say that there is an angiogenic response but it is not known if this is due to the cells infused or due to the scaffold. It was also stated that lung regeneration relies on host epithelial cells to re-epithelialize the basement membrane, but it is not known if mesenchymal to epithelial transition (MET) is occurring in this system. Future areas of research will include the role of MMPs, ADAMs, and s100A4 in cell migration; prevention of apoptosis; role of matricellular proteins (TN-C and TSP-1) and pre-incubation strategies to augment scaffold binding or obviate the need for scaffolds.

Viranuj Sueblinvong (Emory University) presented work on lung tissue engineering from MSCs. *In vitro* experiments that induce mechanical stretch in a uniaxial stretching system (1 Hz with 5–10% elongation) in basal medium promoted AT2 cell differentiation after 2 to 5 days. The stretch does not cause significant morphological changes of the MSCs after 2 or 5 days, but it does induce MSC proliferation at 2 days and expression of CCSP and CFTR mRNA and surface protein. SPC and Aquaporin-5 expression were not induced. Notably, stretch attenuated the expression of collagen type I and α-smooth muscle action (SMA) messenger (m)RNA. Using small airways growth medium (SAGM) in place of basal medium, increased levels of AQP-5, CCSP, CFTR and SP-C mRNA (5–15 cycles) and protein were achieved with stretch, and this effect was synergistic. Small airways growth media (SAGM) also augmented the effects of stretch on reducing collagen type I and α-SMA mRNA expression. Dr. Sueblinvong concluded that MSCs are an excellent source of cells for *ex vivo* lung tissue regeneration due to their differentiation potential and their ease of supply. Furthermore, mechanical stretch can be used to augment lung differentiation potential. Consideration must be given to the composition of the matrix/scaffold because ECM regulates lung development and repair and ECM has been shown to regulate MSC differentiation *in vitro* (324–328). When MSCs are cultured on fibronectin (10 μg/ml), they show more fibroblastic morphology and increased α-SMA and α-integrin receptors than those cultured on laminin (10 μg/ml). The importance of applying force to promote cell growth was also demonstrated in a report of the clinical transplantation of a tissue-engineered airway using the recipient's cells seeded onto donor bronchi (484). Future work is required to understand how mechanical stretch and surface matrices affect stem cell phenotypic aquisition of receptors that enable interaction with matrix components and other mechanoreceptors such as CFTR and ion channels.

The session ended with Charles Vacanti's (Harvard, Brigham and Women's) talk on the use of adult stem cells for the engineering of new pulmonary tissue. The two main components in tissue engineering are the cell delivery system, or the scaffold, and the appropriate cell type. The limitation of biologic material, such as decellularized lungs, is the batch-to-batch variability. In contrast, hydrogels provide consistency, are biodegradable, and result in a good distribution of cells when seeded. Reverse thermally sensitive polymers such as pluronics are liquid at room temperature (and can be injected *in vivo* in this form), and they gel upon reaching body temperature and can used to generate specific shapes. Cells suspended in this gel remain viable after implantation. The best type of cell to use for engineered transplantable tissue is likely one that avoids rejection. Therefore, adult stem cells, very-small-embryonic-like (vsel) cells, and pluripotent stem cells are good candidates. Dr. Vacanti's lab has described an adult stem cell named "SPORE-like cells" (i.e., sub population of retained embryonic-like stem cells). They are small cells (less than 7 microns in diameter) and are present in all tissues of the body. It is unknown if they are seeded from the bone marrow. After isolation from tissue, they initially express many embryonic stem cell markers (e.g., Oct4 and Sox9) and can be induced to differentiate into cells of all three germ layers in appropriate culture conditions. Initial sphere formation begins with a small aggregation of clusters. If not driven toward any particular cell type, the cells typically prefer to revert to the cell type of the tissue from which they were isolated. Dr. Vacanti described that the SPORE-like cells from the lung were procured predominantly from distal lung tissue, minced, exposed to enzyme, passed through a 40 μm filter followed by a long-drawn Pasteur pipette. Nonadherent cells form into aggregates and pneumospheres that differentiate into lung cells expressing SP-C, and CC10 when cultured in serum-containing medium. When put *in vivo* on hydrogel scaffolding, they appeared similar to distal airway tissue, but the cell patterning was atypical. Dr. Vacanti concluded that, in the short term, using decellularized lung may be a better scaffold to guide pneumospheres. Pneumospheres can also be driven to other cell types if cultured in appropriate conditions. It is unclear if SPORE-like cells are stem cells or if they exist/function *in vivo* (or are culture phenomena).

The discussion panel consisted of Drs. Jason Bates (UVM), Peter Lelkes (Drexel), and Thomas Gilbert (Pitt). The overall consensus was that there has been "too much engineering and not enough tissue." Investigators have been relying on having a design and hoping that the cells being used follow the design principle and self-organize (after a little prodding to direct them into the right differentiation path). It was agreed that using 3D matrices is better than 2D culture dishes and that the normoxic microenvironments typically used are wrong because, in fact, for the embryonic environment, when the lung is developing, normoxia is hyperoxia (fetal hemoglobin conducts oxygen to tissues at much lower oxygen tension than postnatal). The discussion then moved to a question of "what is the research question?" From the Ph.D. perspective, the question is "what is the language of the cells?" In other words, it is necessary to understand the rules of how cells behave (which requires the

interdisciplinary fields of math and computing). From the M.D. perspective, the question is "can pulmonary function be improved in patients with deteriorating lung function?" Ultimately, we may not need cells, because if we understand the factors involved in development and repair, then perhaps administering the appropriate factors or extracellular matrix material may suffice. The discussion then evolved to questions of whether the cells are smart (i.e., do they know what to do to provide a correct environment?), and whether one really needs to know how lung engineering works or just that it works (case in point: not much is known about how anesthesia works). How does one define the endpoint of what one wants the cells to be? Should it be by function or by expression of certain markers, and if so, how many markers? The session ended with an expression of the continuing need for an *in vitro* assay system as well as *in vivo* functional assays to evaluate stem/progenitor cells. It may be possible to use intravital microscopy to evaluate the function of infused cells. Since the last workshop meeting, advances have been made in the use of 3D *in vitro* models but not in the translation of these models for *in vivo* functional assays.

Session 4: MSC Modulation of Immune and Inflammatory Responses

The goal of the session 4 was to review the different studies that have identified how MSCs interact with cells of the innate and adaptive immune systems. Several groups have documented the anti-inflammatory effects of exogenously administered MSC in animal models of injury, including lung injury, asthma, and sepsis (31, 35, 42, 397–399, 401–411, 413–444). Discussed in this session were preclinical data for the therapeutic use of MSC in humans and the promises and challenges associated with exploring potential clinical protocols to investigate the safety and efficacy of MSC therapy for lung diseases (445).

Armand Keating, (University of Toronto) presented key issues in MSC studies: novel type of bone marrow–derived progenitor cells and their role on immune modulation. The studies of MSCs in tissue regeneration and in immunology have progressed at a remarkably rapid pace over the past 5 years, and with potentially important new data appearing weekly in the scientific literature, there has been little opportunity to either fully assess their contribution or adequately evaluate the direction of the field. In this presentation, two key areas were explored in a first attempt to provide some context: the potential importance of novel progenitors in tissue repair and the role and significance of MSCs in mediating immune responses. Studies with murine and human MSCs from different sources were discussed as well as how these populations might provide insights into the investigation of tissue repair. Investigations of MSCs in the modulation of immune responses were placed into context and evaluated on the basis of the *in vitro* assays, preclinical models used, the source of cells, the species differences, and the immune cells affected. All of these key issues have relevance in future cell therapies for lung diseases.

Ryang Hwa Lee, (Texas AandM Health Science Center) presented a novel work demonstrating that in mice, intravenous administration of human MSCs (hMSC) reduce myocardial infarction damage. The protective effect apparently is by a paracrine effect on the secretion of anti-inflammatory proteins by MSCs trapped in the lung in the absence of significant engraftment (218). Furthermore, the hMSCs in the lung can up-regulate the expression of multiple genes with a large increase in the anti-inflammatory protein TSG-6. TSG-6 is a multifunctional 35-kD–secreted protein associated with inflammation. TSG-6 is not constitutively expressed in normal adult tissues but is rapidly induced in many different cell types by inflammatory mediators such as IL-1β, TNF-α, endotoxin, and prostaglandin E2 (PGE_2). Intravenous administration of hMSCs, but not hMSCs transduced with TSG-6 small interfering (si)RNA, decreased inflammatory responses, which in turn reduce infarct size and improve cardiac function. A similar effect in reducing inflammatory responses and infarct size is observed by IV administration of recombinant TSG-6. The results suggest improvements in animal models and patients after IV infusions of MSCs are at least in part explained by activation of MSCs to secrete TSG-6.

Conrad Liles, (University of Toronto/University Health Network; McLaughlin-Rotman Centre for Global Health, Toronto General Hospital) presented work on the use of MSC as a therapy for sepsis. Sepsis and its complications, including acute lung injury/acute respiratory distress syndrome and multiple organ dysfunction syndrome, are important causes of morbidity and mortality in critically ill patients, despite appropriate antimicrobial therapy. It has been previously demonstrated that administration of MSCs can ameliorate experimental LPS-induced pulmonary inflammation ALI in mice (230, 400–411). However, one of the main concerns regarding the use of MSC in patients with acute lung injury is if by down-regulating the immune response there is increased possibility of sepsis. Dr. Liles hypothesized that administration of MSCs would also reduce sepsis-associated inflammation and organ injury in a clinically relevant model of sepsis. To investigate this hypothesis, sepsis was induced in C57Bl/6J mice by cecal ligation and puncture (CLP), followed by an intravenous injection of MSCs or saline 6 hours later. MSC treatment significantly reduced systemic and pulmonary cytokine (IL-6, IL-1β, IL-10, KC, JE, and CCL5) production in mice with CLP-induced sepsis (415). Sepsis-related acute lung injury and organ dysfunction were prevented by MSC treatment. In addition, bacterial clearance was significantly improved by MSC treatment. Importantly, MSC treatment significantly reduced mortality in septic mice receiving appropriate antimicrobial therapy. Expression microarray analysis of the spleen revealed that MSC therapy affects a number of pathways implicated in sepsis-related morbidity and mortality. These findings, in conjunction with the results reported in a study by Nemeth and colleagues (413), demonstrate the feasibility and effectiveness of MSC administration for experimental sepsis and providing the basis for development of a potentially effective immunomodulatory strategy to decrease morbidity and mortality in clinical sepsis.

Michael A. Matthay, (University of California, San Francisco) presented stimulating work on the use of MSCs to reduce acute lung injury in mice and in the *ex vivo* perfused human lung. Matthay's research group has used *in vitro*, *in vivo* and *ex vivo* experimental models to test the therapeutic value of MSCs for the treatment of ALI (400, 445). A spirited discussion ensued as to whether MSCs should be considered for treatment of acute lung injury. According to Dr. Matthay, the evidence that supports the use of MSCs is founded on growing evidence suggesting profound immunonodulatory actions of MSCs in lung injury models. To demonstrate the efficacy of MSC in ALI, intratracheal delivery of allogeneic MSCs were used to improve the function of the alveolar epithelium, reduce inflammation, and improve survival in mouse models of ALI. In mice injured with intratracheal endotoxin (5 mg/kg), bone marrow–derived MSC (750,000 cells), given into the airspaces 4 hours after the endotoxin, reduced the severity of lung injury as measured by lung protein permeability, pulmonary edema, and lung histology. The therapeutic effect was associated with a decrease in proinflammatory cytokines and an increase in anti-inflammatory cytokines, including IL-10. Survival was also improved with MSC treatment (400). More recent work by this group has indicated that MSCs have therapeutic value in bacterial pneu-

monia in mice. By using a standard model of *Escherichia. coli* pneumonia in mice (instilled 10^6 bacteria) treated with MSC 4 hours after instilling live bacteria, bone marrow–derived MSCs reduce lung injury and improve survival. MSC treatment was associated with a reduction in the number of live bacteria in the lung compared with saline and fibroblast controls. In summary the results of MSC studies in mice demonstrated that treatment with MSC improves survival and reduces lung injury in the endotoxin model of ALI. This can be as a consequence of the down-regulation of proinflammatory response while increasing anti-inflammatory cytokines. At the same time, MSC reduces lung injury and improve survival following live *E. coli* lung injury, with an associated decrease in bacterial counts.

The most relevant results to translate these observations into clinic are derived from the *ex vivo* lung reperfusion model. Using a perfused (5% albumin in a physiologic solution plus fresh human blood) human lung preparation that was developed to study lung fluid balance under clinically relevant pathological conditions, the administration into the airspaces of either allogeneic human MSC or the medium from cultured human MSC reversed endotoxin-induced lung injury as measured by lung water, lung endothelial permeability, histology, and the rate of alveolar fluid clearance (445). Based on siRNA knockdown experiments, the beneficial effects were mediated in part by secretion of keratinocyte growth factor (KGF) by MSC, which reduced lung edema, normalized lung endothelial permeability, and restored the amiloride sensitive sodium and fluid transport capacity of the injured human alveolar epithelium. Mechanisms for how KGF may restore alveolar fluid clearance include up-regulation of sodium transporters ENaC and NaKATPase at a transcriptional level (446).

An important consideration is the development of more preclinical studies to determine mechanisms, efficacy, and safety of the use of MSC in ALI. It is also necessary to determine the most efficient route, dose, and timing of delivery, as well as to when and how many MSCs need to be inoculated. Finally, we should test MSC-conditioned medium or key products in the medium, such as IL-10 or KGF or the recently described TGS-6. All of these are important considerations that need to be evaluated before MSCs can be use in humans as cell therapy for any type of lung disease.

Daniel J. Weiss (University of Vermont College of Medicine) presented the use of MSCs in immunomodulation of allergic airways inflammation. This work is based on previous *in vitro* observations that MSCs can inhibit proliferation and activities of a wide range of immune cells, notably CD4 T lymphocytes. These properties have served as a basis for recent successful clinical trials of autologous and allogeneic mouse MSCs for immune and inflammatory diseases such as graft versus host disease, Crohn's disease, and most recently, chronic obstructive pulmonary disease. However, the mechanisms by which the MSCs suppress inflammatory-based diseases *in vivo* are poorly understood, and it is not clear whether effects observed *in vitro* parallel those occurring *in vivo*. Dr. Weiss reasoned that asthma, a CD4 T lymphocyte-mediated disease, might be an amenable target for therapeutic MSC administration and might also provide an opportunity to elucidate mechanisms of MSC actions *in vivo*. His group found that systemic administration of either syngeneic or allogeneic MSCs during antigen sensitization in a mouse model of ovalbumin-induced allergic airways inflammation inhibited both airways hyperreactivity as well as Th2-mediated eosinophilic lung inflammation through an IFNγ-dependent but T regulatory cell, Th17, and TRL9-independent mechanism (432). They further found that CMFDA Cell Tracker Dye–labeled MSCs migrate to mediastinal lymph nodes and spleen, sites of antigen-specific CD4 T lymphocyte activation in this model. Contrary to existing *in vitro* data, MSC administration did not affect antigen-specific CD4 proliferation but rather promoted a Th1 phenotype *in vivo* by increasing antigen-specific Th1 immunoglobulins in serum. Dr. Weiss and colleagues therefore speculated that the mechanism by which MSCs decrease allergic airways inflammation is, at least in part, by influencing the CD4 phenotype at sites of CD4 T lymphocyte activation in addition to direct actions in lung. In addition, MSC administration has no obvious effect on the generation of T regulatory cells. Dr. Weiss concluded that systemic MSC administration during antigen-sensitization does not inhibit antigen-specific CD4 T cell proliferation, interfere with antigen presentation by dendritic cells, effect T regulatory cell function to inhibit proliferation of clonal antigen-specific T cells, or have an obvious effect on Th17 CD4 T cells. What they do is shift CD4 T cell phenotype from Th2 to Th1, and IFNγ is necessary for the ameliorating effects of MSCs on allergic airways inflammation.

Session 5: EPCs and Clinical Trials in Lung Diseases

This session addressed the methods used to define endothelial progenitor populations and the current limitations of these approaches. Participants discussed the contributions of specific populations of endothelial, hematopoietic, and endogenous lung progenitor cells to postnatal vasculogenesis, and reviewed the potential therapeutic applications of specific cell types in lung diseases, including PAH, ALI, and ARDS.

The session began with Mervin Yoder (Indiana University) who defined different circulating populations of proangiogenic cells and discussed approaches to modulate the ability of endothelial colony forming cells (ECFCs) to generate vascular networks *in vivo*, focusing on modulation of vessel formation by 3D collagen matrices. He began with an overview of different types of circulating angiogenic cells, emphasizing the difficulty in identifying true EPCs and the need for complementary approaches to establish progenitor potential of whichever population is being assessed given that there is no specific surface marker with which to identify true EPCs (Figure 3) (19, 40, 41, 282). Circulating EPCs are typically defined as adherent cells that capture and ingest acetylated low-density lipoprotein (Ac-LDL) and bind the lectin Ulex europaeus I (UEA-1) after 4 to 9 days of *in vitro* culture in defined endothelial cell culture medium. However, uptake of Ac-LDL is not specific for endothelial cells and UEA-1 does not distinguish between endothelial cells (EC), progenitors, and hematopoietic cells, necessitating the use of additional properties to define specific cell types. The adherent population of mononuclear cells can be divided into early outgrowth cells (also known as CFU-Hill) and late outgrowth cells (known as ECFC) depending on the method used for culture. Dr. Yoder reviewed the evidence supporting a true progenitor role for a population of cells described as ECFC. ECFC are defined functionally by their ability to undergo clonal proliferation and replate into secondary endothelial colonies, to form tubes *in vitro* and new vessels *in vivo*, and are contained within the $CD34^+CD45^-$ population. ECFCs derived from different sources vary in growth potential, with cord blood–derived ECFC displaying greater proliferative potential than ECFC from adult peripheral blood. Similarly, rat pulmonary microvascular endothelial cells (PMVECs) display a higher proliferative potential following serum stimulation than do pulmonary arterial endothelial cells, suggesting enrichment for ECFC. *In vivo* transplantation experiments demonstrate that ECFC cultured in collagen/fibronectin gels for 24 hours and then implanted subcutaneously in NOD/SCID mice demonstrate vessel formation after 2 to 4 weeks with strong

CD31 staining. Biophysical properties of the gels are modulated by collagen concentration, which in turn dictates the morphology of ECFC-derived structures *in vivo*, with increased matrix collagen concentration resulting in increased average and total vessel area. Dr. Yoder then discussed newer strategies for isolation of ECFC from cord blood based on surface phenotype and suggested that modifications in approach to flow cytometry could allow identification of populations not previously detected. In particular, bi-exponential display may facilitate detection of previously undetected populations. This approach was used to characterize $CD34^+CD45^-$ cells in cord blood, which was shown to be highly enriched for ECFC, and this population also expresses CD31, CD146 and CD105. $CD34^+CD45^-$ ECFC form blood vessels *in vivo* in collagen gels. Women with breast cancer appear to mobilize live $CD34^+CD45^-$ ECFC and apoptotic $CD34^+CD45^-$ circulating endothelial cells in their peripheral bloodstream after treatment with sunitinib and paclitaxel.

In the second part of the talk, Dr. Yoder discussed the role of cells referred to as circulating progenitor cells (CPCs) that have been reported to serve as a biomarker for cancer progression. Analysis of CPCs with advanced flow cytometry techniques demonstrated that these cells have variable expression of AC133 and are able to undergo multilineage engraftment in NOD/SCID mice but are unable to give rise to tube-like structures *in vitro* and blood vessels in NOD/SCID mice *in vivo*. Tail vein injection of CPC increases melanoma growth. Women with breast cancer have a higher ratio of proangiogenic to nonangiogenic CPC and this ratio decreases with treatment. Thus, $CD31^+CD34^+CD45^+AC133^+$ CPC are enriched in hematopoetic stem/progenitor activity, are proangiogenic and promote tumor growth, and are enriched in the bloodstream of patients with breast cancer. In contrast to the $CD34^+CD45^-$ cells described above, these tumor promoting cells appear not to be vascular progenitors but to be capable of promoting vascular growth. Together, these findings suggest that there are circulating cells that can either promote vasculogenesis or can actually, themselves, participate in vasculogenesis. The former are likely of hematopoietic origin, whereas the latter are true ECFCs and are suggested to be derived from the endothelium.

The second topic in this session addressed the contribution of bone marrow–derived precursors and tissue-resident endothelial progenitors to vascular remodeling in PAH and was presented by Serpil Erzurum (Cleveland Clinic Foundation). Dr. Erzurum reviewed the histopathological features of PAH, noting abnormalities that indicate endothelial injury and dysfunction including the presence of thrombotic lesions, plexogenic lesions with aneurysm formation, platelet aggregates and fibrotic lesions, as well as increased endothelial cell proliferation reflected in an increase in Ki67 staining. In contrast to the ECFC described above by Dr. Yoder, the cell under consideration is a bone marrow–derived cell identified by the expression of CD34 and CD133. These cells are normally present in the circulation at low levels and give rise to daughter endothelial-like cells or colony forming units of endothelial cells (CFU-EC). Dr Erzurum addressed two central questions, namely, whether bone marrow–derived EPCs that are $CD45^+CD34^+CD133^+$ are increased in the circulation of patients with PAH, and whether these numbers are related to disease severity and remodeling. FACS data from her laboratory (567) demonstrate an increase in $CD133^+CD34^+$ progenitors in patients with idiopathic pulmonary arterial hypertension (IPAH), which correlates directly with increases in pulmonary artery pressure. Similar to their work, a study by Toshner and colleagues (245) demonstrated an increase in $CD133^+/CD34^+/VEGFR2^+$ cells in PAH and an increase in $CD133^+$ cells in PAH vascular lesions *in vivo* consistent with dysfunction of endothelial progenitors in PAH. Work from Dr Erzurum's laboratory also showed an increase in CFU-EC derived from circulating proangiogenic precursors in IPAH, which was related to the percentage of $CD133^+CD34^+$ cells. Dr. Erzurum then described the role of isolated primary lung endothelial cells in vessel formation in IPAH and demonstrated increased affinity of CFU-EC for mature endothelial cells in a tube formation assay. Following *in vivo* innoculation of PAH CFU-EC in Matrigel plugs subcutaneously into NOD/SCID mice, there was increased vessel formation with migration into the surrounding tissue. Dr. Erzurum described a xenograft model of human $CD133^+$ endothelial cells isolated from IPAH explant lungs. PAH $CD133^+$ cells have higher engraftment than controls, increased myelopoiesis, and a trend for higher megakaryopoieis and increased morbidity. In contrast, animals injected with $CD133^-$ cells had no unfavorable events. Strikingly, infusion of PAH cells led to cardiac and lung endothelial injury and *in situ* thrombosis. Plasma von Willebrand Factor and nitric oxide levels indicate endothelial cell activation/injury as well as greater vascular network formation in lungs of mice engrafted with PAH $CD133^+$ cells. These findings suggest an association between circulating human progenitors and lung vascular remodeling and that PAH $CD133^+$ cells foster pulmonary angiogenic remodeling in NOD/SCID mice associated with *in situ* thrombus formation. Increased von Willebrand Factor further suggests endothelial cell injury and platelet activation as underlying mechanisms and that dysfunctional proangiogenic cells promote vascular injury and remodeling in PAH. Questions that remain to be answered include: what determines whether pro-angiogenic cells are beneficial to recovery from vascular injury or are they contributory to remodeling? Is it chronicity that determines the response? Does underlying lung health select for the type of EPC produced in the bone marrow and direct their function? And, lastly, is there a benefit to therapy that inhibits EPC for regression of vascular remodeling/inflammation?

Asrar Malik (University of Illinois at Chicago) then discussed work from his laboratory on the derivation of mesodermal cells from human embryonic stem (ES) cells and their protective effect in a septic shock model, and also addressed the contribution of bone marrow–derived progenitor cells (BMPCs) in the regulation of endothelial barrier function in mice as defined by microvascular permeability alterations at the level of adherens junctions. The hypothesis underlying the first part of the talk was that coexpression of vascular endothelial growth factor receptor (VEGFR) and ACE defines emerging endothelial and vascular lineages during differentiation of human embryonic stem cells. For these studies, ES cells were differentiated to embryoid bodies (EB) in suspension cultures. FACS on Day 7 allowed identification of MSC (KDR^-, ACE^-), HSC (ACE^+, KDR^-), and EPC (KDR^+, ACE^+). Subculture of dissociated EB cells on Day 7 revealed morphologically identifiable hematopoietic colonies and also EPC colonies. The hematopoietic cells were $CD43^+$ and VE-cadherin$^-$, whereas ACE^+/KDR^+ cells were VE-cadherin$^+$. The KDR^+/ACE^+ cells formed tubes/lumens in Matrigel (BD Biosciences, Bedford, MA). Transplantation studies demonstrated improved survival following lipopolysaccharide (LPS) with administration of hES-derived EB cells (both KDR^+/ACE^+ and KDR^-/ACE^+), whereas there was no advantage after transplantation of hES-derived adherent cells or of conditioned medium from EBs. Lung IL-10 levels were greatest in LPS^+ Day 7 EB-derived cells. In the second part of the talk, Dr. Malik discussed effects of BMPC on accumulation of extravascular lung water after LPS. BMPC reduced extravascular lung leak after LPS. There

TABLE 5. CLINICAL INVESTIGATIONS AND TRIALS OF STEM/PROGENITOR CELLS IN LUNG DISEASES LISTED IN CLINICALTRIALS.GOV

Pulmonary Hypertension	
(*1*) Safety and Efficacy Study of Transplantation of EPCs to Treat Idiopathic Pulmonary Arterial Hypertension. (completed, Wang *et al.* Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J Am Coll Cardiol*. 2007;49:566-1571). NCT00257413. Completed.The First Affiliated Hospital, College of Medicine, Zhejiang University, China PI: Junzhu Chen, M.D.	*Objectives*: The goal of this study was to investigate the feasibility, safety, and initial clinical outcome of intravenous infusion of autologous endothelial progenitor cells (EPCs) in patients with idiopathic pulmonary arterial hypertension (IPAH). *Background*: Experimental data suggest that transplantation of EPCs attenuates monocrotaline-induced pulmonary hypertension in rats and dogs. In addition, clinical studies suggest that autologous progenitor cell transplantation is feasible and safe in patients with ischemic diseases. *Methods*: We conducted a prospective, randomized trial comparing the effects of EPC transplantation plus conventional therapy with those of conventional therapy alone in patients with IPAH. The primary end point was change in the 6-minute-walk distance using a standardized protocol. The secondary end points were changes in hemodynamic variables as assessed by right heart catheterization. *Results*: After 12 weeks of follow-up, the mean distance walked in 6 minutes increased by 48.2 m in the cell infusion group (from 263 $\pm$ 42 m to 312 $\pm$ 34 m), and an increase of 5.7 m occurred in the conventional therapy group (from 264 $\pm$ 42 m to 270 $\pm$ 44 m). The mean difference between the two groups was 42.5 m (95% confidence interval 28.7 to 56.3 m, $P < 0.001$). The patients in the cell infusion group also had significant improvement in mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output. There were no severe adverse events with cell infusion. *Conclusions*: This preliminary study showed that intravenous infusion of autologous EPCs seemed to be feasible and safe, and might have beneficial effects on exercise capacity and pulmonary hemodynamics in patients with IPAH.
(*2*) Pulmonary Hypertension: Assessment of Cell Therapy (PHACeT). NCT00469027. Recruiting.PIs: Michael Ward, M.D.. (St. Michael's Hospital, Toronto, ON, Canada); David Langleben, M.D., Sir Mortimer B. Davis (Jewish General Hospital, Montreal, PQ, Canada)	The primary objective is to establish the safety of autologous progenitor cell-based gene therapy of heNOS in patients with severe pulmonary arterial hypertension (PAH) refractory to conventional treatment. Northern Therapeutics. This is a two-center, phase I clinical trial. A total of 18 patients will be studied using an open-label, dose-escalating protocol; three patients will be entered into each of the five dosing panels. An additional three patients will be entered into the final dose panel to establish safety at the maximum tolerated dose.
(*3*) Endothelial Progenitor Cells and Pulmonary Idiopathic Arterial Hypertension. NCT00551408. Active, not recruiting. Unidad de Investigacion Clinica en Medicina SC, Monterrey, Mexico PI: Carlos J. Sanchez Diaz, M.D.	Current evidence established that endothelial progenitor cells (EPC) participate in several models of vascular disease as acute coronary syndromes, stroke, diabetes, peripheral artery disease, etc. However EPC in the setting of PAH is less well established. The target of this study is to demonstrate if the number of EPC is increased in a Mexican population of patients with PAH.
COPD	
(*1*) A Phase II, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety and Efficacy of PROCHYMAL (*Ex Vivo* Cultured Adult Human Mesenchymal Stem Cells Intravenous Infusion for the Treatment of Subjects With Moderate to Severe Chronic Obstructive Pulmonary Disease [COPD]).NCT00683722. Active, not recruiting.Osiris Therapeutics, Columbia, MD.	COPD is currently the fourth leading cause of death in the United States. It is clear that there is a significant unmet medical need for safe and effective therapies to treat moderate to severe COPD. This patient population has a high mortality rate and requires frequent hospitalizations due to disease-related exacerbations. Based on severity distribution estimates, approximately 70% of all current COPD patients have either moderate or severe COPD. COPD has no known cure, thus current therapeutic intervention is aimed at providing relief of symptoms. Oxygen therapy is the only treatment that has been shown to improve survival. Smoking cessation has been shown to slow the rate of FEV_1 decline and COPD progression. In general patients are treated with bronchodilators and inhaled corticosteroids, but again, these measures do not provide any significant benefit regarding disease progression or prognosis. The characteristics and biologic activity of PROCHYMAL, along with a good safety profile in human trials to date, suggest that PROCHYMAL may be a good candidate for addressing this unmet medical need.
(*2*) Unicentric Study Protocol of Cell Therapy in Chronic Obstructive Pulmonary Disease. NCT01110252. Active, not recruiting.Aboratório de Genética Humana e Terapia Celular, Unesp–Assis Instituto de Molestias Cardiovasculares, Sao Paulo, Brazil.PI: João T. Ribeiro-Paes, Ph.D., M.D.	The main feature of the pulmonary emphysema, included in range of the chronic obstructive pulmonary disease (COPD), is the airflow obstruction resulting from the destruction of the alveolar walls distal to the terminal bronchiole, without significant pulmonary fibrosis. The existing clinical approaches has contributed to the enlargement and amelioration of the emphysema patients life quality, although no effective or curative treatment has been achieved. The surgical treatment, on the other hand, involves complex procedures and, in the specific case of lung transplantation, a lack of donors. Considering these aspects, several experimental models have been proposed aiming to increase knowledge about the pathophysiological processes and enable new clinical approaches to the pulmonary emphysema. The cell therapy, briefly described as the use of cells in disease treatment, presents itself as a promising therapeutic approach with great potential applicability in degenerative pulmonary diseases. In this way, it is intended in this project, the proposition of a protocol to evaluate the safety of cell therapy with pool of mononuclear cells from bone marrow in patients with clinical and laboratory diagnosis of pulmonary emphysema in advanced stage (stage IV dyspnea).

(*Continued*)

TABLE 5. (CONTINUED)

Pulmonary Hypertension	
Lung Cancer	
(*1*) Microarray Analysis of Gene Expression and Identification of Progenitor Cells in Lung Carcinoma. NCT00568906. Recruiting. Stanford. PI: Glenn D. Rosen.	This study will investigate gene expression profiles in normal human lung tissue, lung carcinoma, and metastatic tumor to the lung. The expression of up to 20,000 genes in a given lung tissue sample will be examined by cDNA microarray analysis and compared to normal lung tissue. In addition, we hope to identify a particular subset of lung cancer cells with an enhanced capacity for proliferation and self-renewal, analogous to the stem cells recently identified for certain types of leukemia, breast cancer, and brain tumors.
(*2*) Detection of Circulating Endothelial Progenitor Cells (EPCs) in Peripheral Blood From Non-Small Cell Lung Cancer Patients. NCT00826683. Recruiting.University Hospital, Limoges, Belgium. PI: Boris Melloni, M.D.	The aim of this study is to study blood circulating levels bone-marrow-derived progenitor cells (EPCS). In a first phase, EPCs will be detected in healthy nonsmokers volunteers to validate flow cytometry method (n = 25). In addition, EPC will be characterized by primary cultures to analyze EPC-specific markers. In a second phase, EPCs will detect in peripheral blood from 50 patients with chronic obstructive pulmonary disease (COPD) and 50 patients with non-small cell lung cancers (NSCLC). Primary cultures will be made to confirm EPCS isolation.
Miscellaneous	
(*1*) Phase-1 Study of Autologous Bone Marrow Cell Intrabronchial Instillation for Patients with Silicosis. NCT01239862. Recruiting. Federal University of Rio de Janeiro, Brazil. PI: Marcelo Marcos Morales, M.D., Ph.D.	This study will perfume the safety (Phase I) study of 10 patients with silicosis treated with intrabronchial instillation of autologous bone marrow derived mononuclear cells (BMDMC, 2 × 107) through bronchoscopy. The inclusion criteria is: age between 18 and 50, chronic and accelerated silicosis, characterized with a fibrotic increase in the last two years, $FEV_1 < 60\%$ and $> 40\%$, FVC $> 60\%$ and SaO2 $> 90\%$, while the exclusion criteria were: smoking, active tuberculosis or other infections, cancer, auto-immune disorders, hematological, hepatic or cardiac diseases, and pregnancy. All patients will be subjected to clinical examination, answered questionnaires of quality of life (SGRQ and SF36) and dyspnea score (Borg), performed high resolution CT of thorax, pulmonary function tests with D_{LCO} and 6-minute-walk test and lung perfusion scintigraphy before and 7, 30, 60, 180, and 360 days after treatment.

appeared to be integration of progenitor cells, suggesting that leak was prevented by barrier re-annealing. Future studies will be directed at characterizing surface markers of mesoderm derived precursors, lineage tracing and characterization, evaluating effects of growth factor signals, and evaluating functional studies *in vitro* and *in vivo*. BMPCs characterized as $Sca1^+$, $CD133^+$, $CD31^-$, VE-cadherin low and $CD14^+$. BMPCs express TLR4, which is LPS sensing and taken up into the lung at 3 days post-LPS challenge. BMPCs induce activation of RhoGTPAses in endothelial cells involving Rac1 and Cdc42 and required paracrine release of sphingosine-1-phosphatase suggesting cellular cross-talk as the basis for progenitor cell mediated barrier protection.

Judith Shizuru (University of California, Los Angeles) next described an approach that led to the enrichment of a population of lung-derived cells that engraft and are able to generate alveolar endothelium. She first described the strategy traditionally used to isolate and characterize hematopoietic stem cells (HSC), which was based on their ability to repopulate the bone marrow of lethally irradiated mice. This approach was adapted to isolate and transplant a population of marked lung cells using irradiation injury to create niche space in the lung. To obtain candidate lung-derived progenitor cells, lungs from juvenile mice (5 wk of age) that constitutively express green fluorescent protein (GFP) were dissociated and sorted into distinct populations that were based on positive selection for surface markers expressed on stem cells in other tissues, namely, Sca-1, Thy-1, and Notch-1, and negative selection for markers of mature lung cells (e.g., CD45, T1α, and Maclura pomifera lectin). Marked lung subfractions were intravenously cotransplanted together with nonlabeled purified HSC into lethally irradiated adult recipient mice. Whole bone marrow was used as control. Of five fractions sorted on the basis of surface expression or lack of expression of CD45, Sca1, and Notch, only the population marked by $CD45^-$, $Sca1^+$, and Notch-$1^{-/lo}$ exhibited engraftment and expansion as GFP^+ clusters in recipient lungs. At 24 hours postinfusion, the $CD45^-$ fraction could be visualized in the lung and persisted up to 35 days. The greatest frequency of these cells was obtained from young mice 4 weeks of age. The clusters demonstrated the morphology and surface marker expression of capillary endothelial cells and showed increased expansion between 4 and 8 weeks. Compared with bone marrow–derived $CD45^-$ cells, the population was enriched for cells expressing markers of EPCs ($CD34^+$, $CD133^+$, and KDR^+). Thus, a candidate lung population of cells has been identified that express stem cell and EPC surface markers and are capable of expansion and engraftment as clusters of contiguous cells in radiation-injured recipient lungs *in vivo*. Further questions to be addressed include whether these cell clusters are clonal, whether the cells contribute meaningfully to repair after lung injury, whether there is a finite amount of niche space for these cells, where these cells reside in normal lung, and finally, what are the dynamics of the orchestrated regeneration of endothelial and epithelial components. In addition, whether the cell clusters are in continuity with capillaries will need to be determined.

In the final presentation of this session, Duncan Stewart (Ottawa Health Research Institute, University of Ottawa) reviewed preclinical studies assessing cell-based therapies for PAH and ALI/ARDS. The rationale for administration of bone marrow–derived EPCs is based on evidence that EC apoptosis is the initiating lesion in PAH that subsequently leads to degeneration or obliteration of precapillary arterioles and the possibility that administration of EPCs may contribute to repair and regeneration of pulmonary vessels. Studies of culture-derived EPCs in the monocrotaline (MCT) model of PAH demonstrated engraftment of these endothelial-like cells into distal precapillary arterioles 1 week post-MCT and prevention of development of pulmonary hypertension at 21 days when cells are delivered 3 days after MCT. Delaying cell therapy until 3 weeks postinjury still had an effect and prevented further progression. Delivery of EPCs that were genetically engineered to overexpress endothelial nitric oxide synthase (eNOS) reversed established PAH. Based on these preclinical findings, an early phase clinical trial (pulmonary hypertension and cell therapy trial, or PHACeT) has

been initiated to test efficacy and safety of eNOS transfected autologous EPCs in patients with refractory PAH. The study was designed as a safety study with up to 18 patients and the primary endpoint was tolerability of cell transplantation in patients with PAH refractory to all standard therapies. Autologous early growth EPCs transfected with eNOS were delivered by Swan Ganz catheter to the right ventricle. Dose ranging studies were performed over 3 days with cells given in divided doses in an overlapping protocol. Exclusion criteria included hemodynamic instability or hospitalization for worsening right heart failure in the previous 3 months. Preliminary data thus far indicate a trend toward lower total pulmonary vascular resistance at 3 days with at an intermediate dose. Seven more patients are needed to complete the trial, with an anticipated time line of 7 to 12 months until completion. The last part of the talk focused on MSC therapy for ARDS. Advantages of MSCs include ease of growth and transfection, immunomodulatory activity, and the potential for use of allogeneic cells. Preclinical studies were performed in an LPS model in mice using MSCs or MSCs transduced with angiopoietin-1 30 minutes after administration of LPS. There was a dramatic improvement in lung histology, release of interferon-γ, TNF-α, IL-6 and IL-1β. Similar studies were undertaken in a cecal ligation model. Cells were administered 6 hours after surgery with killing at 48 hours. There was a significant reduction in circulating cytokines, improvement in multiorgan dysfunction, and an improvement in survival. It is suggested that the MSCs may reprogram monocytes/macrophages to increase IL-10 through release of prostaglandin E_2. In ALI and ARDS, endothelial activation is one of the first events in the pathological chain leading to increased air–blood permeability, leukocyte adhesion, and alveolar filling.

TABLE 6. SUMMARY OF CONSIDERATIONS FOR CURRENT AND FUTURE CLINICAL TRIALS OF STEM/PROGENITOR CELLS IN LUNG DISEASES

Pro/Prospects

1. There is a considerable safety record with the clinical use of adult stem and progenitor cells. A long history of clinical trials with stem cells for hematopoietic cells has justified the risk versus benefit evaluations for many diseases. Approximately 80 clinical trials with MSCs and other cells from bone marrow are currently listed in www.clinicaltrials.gov and no significant adverse effects have been reported to date.
2. Promising results have been obtained with administration of MSCs for several non-pulmonary diseases, even though definitive data have not yet been published.
3. Promising results on both safety and efficacy have recently been published on use of endothelial precursor cells in patients with primary pulmonary hypertension. Most recently, interim results from the current trial of allogeneic MSCs for COPD demonstrates safety and suggests efficacy.
4. Numerous recent reports indicate that MSCs and similar cells can repair injury to lung and other tissues in animal models by being activated to secrete therapeutic factors and without long-term engraftment of the cells. Therefore, the probability of long-term adverse effects from engraftment of the cells is greatly decreased.
5. If the therapeutic factors can be identified, administration of the factors may provide an alternative strategy for the therapy of some lung diseases. However, MSCs and similar cells demonstrate a remarkable ability to engage in cross-talk with injured tissues and produce therapeutic benefits both by secretion of soluble factors and by cell-to-cell contacts that may not be duplicated by administration of soluble factors. Therefore, optimal therapy for lung diseases may involve administration of soluble factors for some conditions, cells for others, and a combination of both for still other conditions.
6. Impressive data have recently been obtained demonstrating that MSCs or other cells such as alveolar cells, or conditioned medium from the cells, are both safe and effective in animal models for lung disease including, acute lung injury, asthma, COPD, bronchopulmonary dysplasia, and others. Most recently, human MSCs have been demonstrated to have comparable effects in endotoxin-injured isolated human lung preparations. These data appear to provide a firm basis for developing clinical trials in patients.
7. Rapid progress continues to be made with a number of creative approaches in identifying progenitor and stem-like cells in the lung and in defining the hierarchy of differentiation of such cells both during embryonic development and in response to injury. New therapeutic strategies are likely to arise by further research on these topics that may identify factors for lung diseases that enhance propagation and differentiation of such stem-like cells.
8. Results in animal models have indicated that some cells from bone marrow and other sources can engraft and differentiate into to multiple types of cells in the lung. However, early reports were marred by artifacts and the degree of engraftment is usually low. Whether there are specific cells in bone marrow that engraft in lung is not well understood. Therefore, there is no obvious current clinical strategy for therapies requiring long-term engraftment in lung of cells from bone marrow.
9. In contrast, progress has recently been made in devising artificial lung constructs that may provide temporary lung assist devices for patients awaiting lung transplant much as left ventricular assist devises have proven useful for patients awaiting cardiac transplants.
10. In parallel, rapid progress is being made in devising *ex vivo* three-dimensional and other culture systems for generating functional lung tissue that might conceivably be implanted into diseased lungs. As such, reconstructing the damaged structure of the lung in patients with chronic lung diseases remains a specific but still distant goal for current clinical research.
11. Clinical trials with cell therapies in lung diseases have lagged behind similar therapies for other diseases in part because these diseases present difficult disease targets. However, representatives of several patient oriented respiratory disease foundations asked that we not allow "perfect to become the enemy of good."

Con/Precautions

1. Clinical trials of cell and related therapies in lung diseases must proceed with a keen awareness that every known therapeutic measure in patients has the potential to produce adverse effects. Any trial of a new therapy must therefore be based on careful evaluation of the potential benefits and potential risks to patients as far as these can be discerned.
2. Among the potential adverse effects of cell therapies currently under consideration is the creation of tumors and malignancies in patients. The risk is present with any cell type that can be propagated in culture. However, the risks vary greatly by the biological properties of the cells and the conditions under which they are cultured. On the basis of this evidence, there is clear difference in the probability of risks between administration of cells like human MSCs and similar cells that regularly undergo senescence as they are expanded, and those associated with administration of immortalized cells like ES and iPS cells that do not undergo senescence in culture.
3. Cell therapies probably pose other risks that are still undefined. Among the potential risks: After intravenous infusion, many cells are trapped in the lung and therefore pose the risk of lethal pulmonary emboli. Cells that repair tissues may enhance the growth of undetected cancers, as has been demonstrated for MSCs in some animal models. Local administration of a bolus of cells may prompt the cells to aggregate and differentiate into bone and other inappropriate structures.

Definiton of abbreviations: COPD = chronic obstructive pulmonary disease; ES = ; iPS = ; MSCs = mesenchymal stem cells.

A subsequent discussion on the pros and cons of moving toward further clinical trials was led by Drs. Zea Borok (University of Southern California), Kenneth Brigham (Emory University), Polly Parsons (University of Vermont), Stephen Rennard (University of Nebraska), and Steven Shapiro (University of Pittsburgh). There are currently two active clinical trials of stem/progenitor cells in lung diseases, one each for pulmonary hypertension and for COPD. There is also one clinical investigation of progenitor cells in lung cancer occurring in the United States and Canada. These are listed in clinicaltrials.gov (Table 5). The discussion began with the question of whether a single cell type will be the answer for lung regeneration. There was some discussion regarding which cell type is best and that it may be different for different diseases. It was suggested that perhaps multiple cell types and a more orchestrated approach will be needed. The question was raised as to whether we are ready for clinical trials, and considerable discussion focused on the need for empiricism in clinical medicine. It was felt that the field as a whole had advanced from initial discussions 4 years ago regarding whether cells had engrafted to now evaluating other effects of cell-based therapy, including immune effects. It was felt by some panel members that overall there was a need to proceed with human studies and that animal studies were insufficient. There was strong sentiment regarding the need for good databases and sample/tissue banks to generate as much information as possible from ongoing clinical trials. It was felt that a registry should be established to include small trials and that all trials should store blood and other samples for later analysis. There was concern that many commercially run trials are not collecting sufficient samples or patient information and are not making the data publicly available. The final discussion focused on the ultimate goal of cell-based therapy being to alter the structure of the lung with a need for good functional/physiologic correlates. There was no uniform agreement on the best end-point to evaluate structure. The session ended with the recognition that since clinical trials have started, there is a major need to define mechanistic and clinical end-points to learn as much as we can from ongoing trials. Overall, there was a consensus that lung diseases still present major challenges, but there is increasing, although not uniform, support to cautiously move ahead with additional carefully designed trials and investigations. This is a shift from previous conferences. Salient points from the pro-con discussion are summarized in Table 6.

Session 6: Recommendations—Summation, and Future Directions

This session included a presentation from Diane Krause (Yale) on the current status of potential engraftment of bone marrow–derived cells as lung epithelium. This concept remains controversial, although several papers suggest that less-explored or less-understood bone marrow–derived cell populations may indeed engraft, although still at low level, as airway or alveolar epithelium. A subsequent presentation from Jeffrey Kahn (University of Minnesota) further considered the ethical and policy issues regarding the use of stem cells and moving toward further clinical trials and cell therapy investigations in lung diseases. Following this, James Kiley PhD, Chief of the Lung Biology and Disease Branch of the NHLBI, presented the NHLBI perspective on stem cell and cell therapy approaches for lung diseases. Available resources from the NHLBI and the NIH were described including the NIH Production Assistance for Cellular Therapies (PACT) program, at http://www.pactgroup.net. Additional resources are listed in the conference recommendations. Also included in this session were presentations from Daniel Rose, M.D.; John Walsh, M.D. (University of Florida); and Amy Farber, Ph.D., CEOs, respectively, of the Pulmonary Fibrosis Foundation, Alpha One Foundation, and the LAM Treatment Alliance, on the importance of stem cell research to these particular patient populations and the role of nonprofit research organizations in supporting this and other novel areas of research. Zea Borok, M.D. (University of Southern California) gave a comparable presentation on behalf of the American Thoracic Society.

SUMMARY AND CONCLUSIONS

A continuing accumulation of data in both animal models and in 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 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 the mechanisms of repair of lung injury and a sound scientific basis for therapeutic use of stem and cell therapies in lung diseases.

Author Disclosure: D.J.W. was a consultant for GlaxoSmithKline, is on the Advisory Board for the ALA, and received clinical trials support from Osiris Therapeutics Inc. I.B. owns a patent through the Australian Stem Cell Centre. Z.B., C.K., A.P.-M., S.R., and M.R. do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.S. received lecture fees from AstraZeneca. D.W. and D.J.P. do not have a financial relationship with a commercial entity that has an interest in the conference.

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

Acknowledgments: The organizers are indebted to the staff 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 to Carla Kim, Bethany Moore, Susan Reynolds, Barry Stripp, Viranuj Sueblinvong, and Mervin Yoder for editing and conceptual support for the final conference report.

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