VERMONT STEM CELL CONFERENCE

Stem Cells and Cell Therapies in Lung Biology and Diseases: Conference Report

Daniel J. Weiss, Jason H. T. Bates, Thomas Gilbert, W. Conrad Liles, Carolyn Lutzko, Jay Rajagopal, and Darwin Prockop

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 (ATS), the LAM Treatment Alliance, and the Pulmonary Fibrosis Foundation, convened a workshop, “Stem Cells and Cell Therapies in Lung Biology and Lung Diseases,” held July 25 to 28, 2011 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 and *ex vivo* bioengineering approaches for lung diseases. These are rapidly expanding areas of study that both provide further insight into and challenge traditional views of mechanisms of lung repair after injury and pathogenesis of several lung diseases. The goals of the conference were to summarize the current state of the field, discuss and debate current controversies, and identify future research directions and opportunities for both basic and translational research in cell-based therapies for lung diseases.

This conference was a follow-up to three 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; “Stem Cells and Cell Therapies in Lung Biology and Diseases” sponsored by the NHLBI, Alpha-1 Foundation, ATS, Pulmonary Fibrosis Foundation, University of Vermont College of Medicine, and the Vermont Lung Center in 2007; and “Stem Cells and Cell Therapies in Lung Biology and Diseases” sponsored by the NHLBI, Alpha-1 Foundation, ATS, Emory Center for Respiratory Health, LAM Treatment Alliance, Pulmonary Fibrosis Foundation, University of Vermont College of Medicine, and the Vermont Lung Center in 2009. Those conferences have been instrumental in helping guide research and funding priorities (1–3).

Since the 2011 conference, investigations of stem cells and cell therapies in lung biology and diseases have continued to rapidly progress. The field has further expanded to include *ex vivo* lung bioengineering. A growing number of preclinical studies of immunomodulation and paracrine effects of adult mesenchymal stromal (stem) cells (MSCs) derived from bone marrow, adipose, and other tissues continue to provide evidence of safety and efficacy in animal models of acute lung injury (ALI), asthma, bronchopulmonary dysplasia, chronic obstructive pulmonary disease (COPD), sepsis, silicosis, ventilator-induced lung injury, and other lung diseases. In parallel, more sophisticated understanding of the mechanisms by which MSCs can act has provided growing

insight into their potential applicability for clinical lung diseases. Notably, a pioneering multicenter, double-blinded, randomized placebo-controlled trial of MSCs in patients with moderate to severe COPD has provided valuable safety data for MSC administration to patients with lung diseases and has also suggested potential mechanisms of MSC actions *in vivo* in patients with lung disease (4). Planned North American investigations of MSC administration in patients with acute respiratory distress syndrome (ARDS), sepsis, and idiopathic pulmonary fibrosis (IPF) are paralleled by an increasing number of clinical investigations of MSCs in lung diseases in other countries. Other cell types, including bone marrow-derived mononuclear cells and human amnion-derived stem cells, also appear to have efficacy in preclinical mouse models of lung diseases and may provide alternative approaches to parallel those using MSCs. Significant advances continue to be made in novel areas of investigation, particularly increasing exploration of three-dimensional culture systems and bioengineering approaches to generate functional lung tissue *ex vivo* and *in vivo*. Similarly, progress continues in studies of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), with recent data demonstrating more convincing evidence of derivation of cells with phenotypic characteristics of both airway and alveolar epithelial cells. Significant progress also continues

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Correspondence and requests for reprints should be addressed to Daniel J. Weiss, M.D., Ph.D., 226 Health Sciences Research Facility, University of Vermont College of Medicine, Burlington, VT 05405. E-mail: dweiss@uvm.edu

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to be made in investigations of local (endogenous) stem and progenitor cells resident in adult lungs. Advances in lineage tracing approaches and other techniques continue to provide important insights into understanding of the identity and lineage expansion properties of previously identified putative endogenous stem and progenitor populations and suggest an increasingly complex network of cellular repair after injury. Recent data have broadened this beyond consideration of epithelial progenitors to also include endogenous pulmonary vascular and interstitial progenitors. However, ongoing challenges are to better define, access, and manipulate the appropriate niches and to continue to devise more refined lineage tracing and other study mechanisms to define, characterize, and explore potential therapeutic and/or pathologic properties of endogenous lung progenitor cells. This includes studies of lung cancer stem cells, an area of increasing focus and high interest that remains incompletely understood. Another challenge is that most studies of endogenous progenitor cells continue to use mouse models. Correlative information in human lungs remains less well defined.

Stem and progenitor cell nomenclature remains a thorny issue, although some progress has been made. Despite suggested guidelines from previous conferences and from other sources, precise definitions and characterizations of specific cell populations, notably the putative endogenous cell populations in the lung as well as mesenchymal stem (stromal) cells and endothelial progenitor cells (EPCs), are not agreed on. In many respects this reflects more sophisticated knowledge about cell plasticity and a realization that phenotypic boundaries are not as rigid as previously believed. Nonetheless, the terms “stem cell” and “progenitor cell” are still used with varying degrees of clarity and precision by different investigators and in recent publications. This continues to complicate comparison of different investigative approaches. A suggested glossary of relevant working definitions applicable to lung, originally presented in the report of the 2007 conference, is again depicted in Table 1. This glossary does not necessarily reflect an overall consensus for the definition of each term and will undergo continuing revision

as overall understanding of the cell types and mechanisms involved in lung repair continue to be elucidated. Nonetheless, it remains a useful framework.

The conference was divided into five sessions, each featuring a plenary speaker, research talks presented by leading international investigators, and a panel-led debate and discussion. In the first session, “Endogenous Lung Progenitor Cells/Lung Cancer Stem Cells,” after an overview of the field by Wellington Cardoso (Boston University), respective presentations by Emma Rawlins (Cambridge University), Ivan Bertoncello (University of Melbourne), Carla Kim (Boston Children’s Hospital), Susan Reynolds (National Jewish Hospital), and Barry Stripp (Duke University) reviewed the current state of knowledge of endogenous progenitor cell populations, mechanisms regulating their behavior, and their potential to initiate or augment repair. Key points emphasized during this session, as in previous meetings, are that stem cells are operationally defined not solely by their intrinsic developmental potential but by their interaction with the microenvironments in which they reside. Furthermore, 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, remain increasingly valid and necessary. The session also included a presentation from Mark Magnuson (Vanderbilt University) describing the National Institute of Health’s Beta Cell Consortium and how this type of organizational approach could be applied to the lung endogenous progenitor field.

The second session on “Embryonic Stem Cells, iPSCs, and Lung Regeneration” included a featured talk on the current state of knowledge with respect to type 2 alveolar epithelial cells by Jeff Whitsett (Cincinnati Children’s Hospital) followed by presentations from Gustavo Mostoslavsky (Boston

University), Ali Samadikuchaksaraei (Imperial College, London), Darrell Kotton (Boston University), Amy Wong (University of Toronto), Hans-Willem Snoeck (Columbia University), and Brian Davis (University of Texas) highlighting developments in these areas. Notable advances in this area include the improved sophistication in directing both ESCs and iPSCs *in vitro* through stages involved in generation of definitive endoderm and subsequently into cells with some phenotypic characteristics of airway and alveolar epithelial cells. However, although showing promising progress, full phenotypic and functional characterization of putative lung cells derived from ESCs or iPSCs remains incomplete and is an area in which further exploration is required.

The third session, “Bioengineering Approaches to Lung Regeneration,” was expanded to a full day, reflecting the rapid advances in this overall area. The morning session was focused on structure and matrix for three-dimensional scaffolds to be potentially used for *ex vivo* lung regeneration. A featured overview talk by Dame Julia Polak (Imperial College, London) was followed by presentations from Paolo Macchiarini (Karolinska Institute), Joaquin Cortiella (University of Texas, Galveston), Christine Finck (University of Connecticut), Andrew Hoffman (Tufts University), Peter Lelkes (Temple University), Angela Panoskaltsis-Mortari (University of Minnesota), and Zachary Borg (University of Vermont) exploring advances in scaffold systems, particularly with respect to whole lung decellularization and recellularization. The afternoon session was focused on functional aspects of *ex vivo* lung bioengineering and included presentations by Doris Taylor (University of Minnesota), David Hoganson (Washington University), Harald Ott (Harvard University), Laura Niklason (Yale University), Daniel Huh (Harvard University), and Daniel Tschumperlin (Harvard University). A final presentation on imaging approaches for lung bioengineering was presented by Jason Woods (Washington University). The fifth session, “EPCs, MSCs, and Cell Therapy Approaches for Lung Diseases,” highlighted recent advances in preclinical and clinical cell therapy approaches

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 embryonic 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 (ESC): Cell lines developed from the inner cell mass of early developing blastocysts. ESCs 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, ESCs cannot form extraembryonic tissue, such as trophoblast.
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 (iPSC): Reprogrammed adult somatic cells that have undergone dedifferentiation after the expression of reprogramming transcription factors such as Oct 3/4, Sox2, c-Myc, and Klf4. iPSCs are similar to ESCs in morphology, proliferation, gene expression, and ability to form teratomas. <i>In vivo</i> implantation of iPSCs results in formation of tissues from all three embryonic germ layers. iPSCs have been generated from both mouse and human cells.
Progenitor cell: A 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 an 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 Club 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 (i.e., 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 after 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 cells, 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 whose progeny differentiate into all of the different blood cell lineages, including mature leukocytes, erythrocytes, and platelets.
Endothelial progenitor cell (EPC): 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 <i>in vitro</i> and <i>in vivo</i> . 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 (MSC): 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 published the minimal criteria for defining (human) MSCs. MSCs have been described to differentiate into a variety of mature cell types and may also have immunomodulatory properties.

(Continued)

Table 1. (CONTINUED)

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, and 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 Club cell secretory protein (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 after depletion of transit-amplifying (Club) 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 <i>in vivo</i> by dual labeling with CCSP and surfactant protein C (SP-C) 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-SP-C, or aquaporin 5, leading to speculation that a single cell type has the capacity to differentiate into both bronchiolar (Club cells) and alveolar (type 1 and 2 pneumocytes) lineages. At present, the relationship of the cells studied <i>in vitro</i> to those observed by dual labeling <i>in vivo</i> is unclear. Human correlates have not yet been identified.

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for lung diseases. After a featured presentation by Darwin Prockop (Texas A&M), talks given by Jacques Galipeau (Emory University), Donald Phinney (Scripps Institute, Florida), Michael Matthay (UCSF), Sam Janes (University College, London), Meagan Goodwin and Daniel Weiss (University of Vermont), Mervin Yoder (Indiana University), and Duncan Stewart (University of Ottawa) highlighted different areas of advances in EPC and MSC biology and further progress toward an expanded investigation of clinical cell therapy approaches for different lung diseases, including lung cancers.

The final session, "Summation and Direction," featured a review and open discussion of current issues led by a panel featuring representatives of the NHLBI, U.S. Food and Drug Administration (FDA), and each of the sponsoring nonprofit respiratory disease organizations. The panel consisted of Carol Blaisdell (NHLBI), Amy Farber (Founder and CEO, LAM Treatment Alliance), Donald Fink Jr. (FDA), Christine Kelley (National Institute of Bioimaging and Biomedical Engineering), Michael Matthay (on behalf of the ATS), Neal Pellis, Ph.D. (National Aeronautics and Space Administration), Daniel Rose (CEO, Pulmonary Fibrosis Foundation), and Adam Wanner (Alpha-1 Foundation). The conference concluded with vigorous discussion on future research and funding priorities, led by Darwin Prockop (Texas A&M). As in previous conferences, discussion was spirited in all areas. 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 results obtained with the trial of MSCs in COPD and the increasing body of data demonstrating efficacy in other inflammatory and immune-mediated lung injury disease models suggest 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.

It was acknowledged by all participants that *ex vivo* lung bioengineering and further investigation into the role of endogenous lung progenitor cells remain timely and exciting areas 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. 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 online supplement. In addition, a companion article provides a comprehensive review of the relevant literature spanning the period between the 2011 workshop through December 2012. The combined references for both this conference report and from the comprehensive review are found after the review. In accordance with recent guidelines from the ATS and other Respiratory Disease organizations, the terms "Clara cell" and "Clara cell secretory protein" have been replaced by the terms "Club cell" and "Club cell secretory protein (CCSP)," respectively (5, 6).

Detailed Session Summaries

Session 1: Endogenous Lung Progenitor and Lung Cancer Stem Cells

The endogenous lung progenitor cell and lung cancer stem cell session at this year's conference highlighted two complementary approaches to studying lung epithelial progenitor cells. The first approach revolved around the discipline of developmental biology and emphasized the importance of identifying new markers of potentially very heterogeneous populations of progenitor cells that occur at distinct positions along the respiratory tree. This approach relies on the precise identification of unique markers of different progenitor cells and, in turn, permits the creation of murine genetic models to lineage trace the fate of any one particular progenitor cell type identified by such a unique marker in an injury of one's choice. The creation of such lineage-tracing mice also permits the sorting, purification, and ultimately genetic modulation of these progenitor cells in murine disease models. The second approach borrows from the remarkable advances made in hematopoietic stem cell biology and relies on the use of multiparameter fluorescence-activated cell sorter (FACS) sorting and functional colony-forming cell assays to identify and prospectively isolate increasingly enriched populations of candidate progenitor cells, which can be hierarchically ordered on the basis of their proliferative and differentiation potential and growth factor requirements in culture. This second approach has the advantage that it can be applied to human systems as easily as it can be to the mouse. A take-

Table 2. Overall conference summary recommendations

Basic

- For studies evaluating putative engraftment, advanced histologic imaging techniques (e.g., confocal microscopy, deconvolution microscopy, electron microscopy, laser capture dissection, etc.) must be used to avoid being misled by inadequate photomicroscopy and immunohistochemical approaches. Imaging techniques must be used in combination with appropriate statistical and other analyses to maximize detection of rare events.
- Continue to elucidate mechanisms of recruitment, mobilization, and homing of circulating or therapeutically administered cells to lung epithelial, interstitial, and pulmonary vascular compartments for purposes of either engraftment or immunomodulation.
- Encourage new research to elucidate molecular programs for development of lung cell phenotypes.
- Continue to refine the nomenclature used in study of endogenous and exogenous lung stem cells.
- Comparatively identify and study endogenous stem/progenitor cell populations between different lung compartments and between species.
- Increase focus on study of endogenous pulmonary vascular and interstitial progenitor populations.
- Develop robust and consistent methodologies for the study of endogenous lung stem and progenitor cell populations.
- Develop more sophisticated tools to identify, mimic, and study *ex vivo* the relevant microenvironments (niches) for study of endogenous lung progenitor/stem cells.
- Continue to develop functional outcome assessments for endogenous progenitor/stem cells.
- Elucidate how endogenous lung stem and progenitor cells are regulated in normal development and in diseases.
- Identify and characterize putative lung cancer stem cells and regulatory mechanisms guiding their behavior.
- Continue to elucidate mechanisms by which embryonic and induced pluripotent stem cells develop into lung cells/tissue.
- Develop disease-specific populations of ESCs and iPSCs, for example for CF and α_1 -antitrypsin deficiency, with the recognition that no strategy has yet been devised to overcome the propensity of ESCs and iPSCs to produce tumors.
- Continue to explore lung tissue bioengineering approaches, such as artificial matrices and three-dimensional culture systems, for generating lung *ex vivo* and *in vivo* from stem cells, including systems that facilitate vascular development.
- Evaluate effect of environmental influences, including oxygen tension, and mechanical forces, including stretch and compression pressure, on development of lung from stem and progenitor cells.
- Identify additional cell surface markers that characterize lung cell populations for use in visualization and sorting techniques.
- Strong focus must be placed on understanding immunomodulatory and other mechanisms of cell therapy approaches in different specific preclinical lung disease models.
- Improved preclinical models of lung diseases are necessary.
- Disseminate information about and encourage use of existing core services, facilities, and web links.
- Actively foster interinstitutional, multidisciplinary research collaborations and consortiums as well as clinical/basic partnerships. Include a program of education on lung diseases and stem cell biology. A partial list includes NHLBI Production Assistance for Cellular Therapies (PACT), 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. Preclinical models will provide proof of concept; however, these must be relevant to the corresponding human lung disease. Disease-specific models, including large animal models where feasible, should be used and/or developed for lung diseases.
- Basic/translational/preclinical studies should include rigorous comparisons of different cell preparations with respect to both outcome and toxicological/safety endpoints. For example, it is not clear which MSC or EPC preparation (species and tissue source, laboratory source, processing, route of administration, dosing, vehicle, etc.) is optimal for clinical trials in different lung diseases
- Incorporate rigorous techniques to unambiguously identify outcome measures in cell therapy studies. Preclinical models require clinically relevant functional outcome measures (e.g., pulmonary physiology/mechanics, electrophysiology, and other techniques).

Clinical

- 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 biologic materials, such as lung tissue, BAL fluid, blood, etc., 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 vs. host disease will provide novel and invaluable information about potential MSC effects on development and the clinical course of bronchiolitis obliterans.
- Work with industry to have access to information from relevant clinical trials.

Definition of abbreviations: ACRC = Asthma Clinical Research Center; ALI = acute lung injury; ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; CF = cystic fibrosis; ESC = embryonic stem cell; GMP = good manufacturing process; iPSC = induced pluripotent stem cell; MSC = mesenchymal stromal (stem) cell; NCRR = National Center for Research Resources; NHLBI = National Heart, Lung, and Blood Institute; NIH = National Institutes of Health.

home message of the conference was that there needs to be a confluence of these two modalities of study in the next decade,

mirroring what has been accomplished in the field of hematopoiesis. For now, the major barriers to progress include the lack

of definitive markers to segregate progenitor cells at different levels of the airway tree, whether by FACS or genetic

approaches, and most importantly the availability of informative gold-standard functional *in vivo* assays for stem cells akin to bone marrow transplantation to demonstrate the true regenerative potential of any purified cell population from mouse or human.

Wellington Cardoso opened the section with a thoughtful review of the state of the field in lung progenitor cell biology, arguing that it has been a challenging task to define the properties of lung progenitor cells due to the great structural complexity and diversity of the cell types and the slow turnover of the respiratory epithelium under homeostatic conditions. He noted that most studies therefore focus on candidate endogenous progenitor or stem cells using models of adult lung injury and repair (7). Although these models provide important insights into the pathways and cellular activities involved in the lung's regenerative response, they may not necessarily reflect the behavior of the endogenous progenitors in the uninjured lung (8). Based on lineage tracing and injury-repair models, three different cell types have been accepted classically as endogenous progenitors of the adult respiratory system: basal and Club cells in airways and type II cells in the alveoli. There is evidence that these cell types actually encompass distinct subpopulations with different features and functions. Club cells, for example, are a highly heterogeneous population of facultative progenitors in intrapulmonary airways in rodents and humans, being able to self-renew and give rise to ciliated cells. This heterogeneity is manifested in multiple ways, such as in their ability to undergo mucous metaplasia (9) or survive naphthalene injury and mount a regenerative response (10). These properties are associated with the regional distribution of these cells and seem to depend on interactions with the microenvironment, through mechanisms still largely obscure. Studies to further explore heterogeneity of basal, Club, and type II cells and their precursors are needed and will be of great value.

Dr. Cardoso also noted that new candidate adult lung progenitors have been recently reported. A population of $\alpha 6\beta 4+$ cells present in the normal adult mouse lung at the bronchioalveolar duct junction and alveoli has been shown to possess the

capacity for proliferation and further differentiation *ex vivo*. When mixed with embryonic lung cells in serial dilutions, they form well-defined airway and saccular structures in kidney capsule assays. Moreover, they participate in the regenerative response of the lung epithelium post-bleomycin injury. A novel population of progenitor cells has been also described in submucosal glands of the murine trachea. These cells survive hypoxic-ischemic injury and play a role in repair of the submucosal glands (11). Additionally, a single CD49f(bright)/Sca1(+) /ALDH(+) basal cell has been reported to generate rare label-retaining cells and abundant label-diluting cells. The property of self-renewal of these cells was tested by serial passage at clonal density and analysis of clone-forming cell frequency. A single clone could be passaged up to five times (12). Dr. Cardoso also noted that recent claims of a human lung stem cell cKit+ capable of regenerating all elements of the injured mouse lung have been controversial (13).

Dr. Cardoso finally highlighted the Notch pathway as crucial actor in cell fate choice in differentiating airway progenitors during development or regeneration. Murine models show that disruption of Notch signaling alters dramatically the balance of ciliated and secretory cells (14–16). Alterations in the Notch pathway have been associated with aberrant programs of differentiation in human lung conditions, including COPD and cancer (17, 18). Notch is likely to mediate the responses of the endogenous progenitors to the microenvironment critical to control cell fate. Although differences exist between human and animal models, the characterization and in-depth knowledge of developmental signaling in different models will further the current understanding of endogenous adult progenitors in the lung.

Dr. Emma Rawlins then described her work in embryonic lung development. Her group has shown that during lung branching morphogenesis the distal-most ID2+ epithelial cells are a multipotent progenitor population. These cells both self-renew during development and give rise to all of the different lung epithelial cell types (19). However, these multipotent embryonic progenitors are not maintained after birth. By contrast, in the adult lung, multiple progenitor cell populations of

more restricted potency are necessary for maintenance and repair of the epithelium (20). In distinction to the molecular mechanisms governing embryonic progenitor cell behavior, the mechanisms governing these adult progenitor cell populations remain largely obscure. Dr. Rawlins noted that adult lung stem cell biology can build on the data that have been collected by lung developmental biologists. Indeed, many of the same molecular mechanisms governing both embryonic and adult lung progenitor cells and embryonic gene regulation networks can reappear in the adult lung pathologies, including cancer (21). Finally, it was pointed out that knowledge of the mechanisms that control embryonic lung development will provide a framework for driving the differentiation of iPSCs into lung progenitors (22).

Ivan Bertoncello then reviewed the significant progress made in recent years in characterizing and analyzing the proliferative potential and organization of candidate epithelial stem/progenitor cells in the adult lung using FACS-based cell sorting approaches and *in vitro* colony-forming assays. The Bertoncello laboratory has described a rare population of EpCAM^{pos}Sca-1^{low} $\alpha 6^{\text{pos}}\beta 4^{\text{pos}}\text{CD}24^{\text{low}}$ lung epithelial stem/progenitor cells, which give rise to airway or alveolar lineage-restricted colonies as well as colonies of mixed lung epithelial cell lineages when cocultured in a three-dimensional matrigel assay together with supporting mesenchymal stromal cells and cytokines (23). Parallel studies, and the refinement of cell separative strategies used by other laboratories, have since shown that candidate stem/progenitor cells isolated from different regions along the proximodistal lung axis share a similar if not identical biomarker repertoire. These include bronchioalveolar stem cells (BASCs) (24), bronchiolar progenitor cells (25), and multipotent $\alpha 6^{\text{pos}}\beta 4^{\text{pos}}$ cells, recently shown to recapitulate lung morphogenesis in a sub-kidney capsule lung organoid transplant assay (26). Dr. Bertoncello argued that although this reductionist approach provides powerful tools to monitor the status of stem/progenitor pools in the normal and diseased lung, and to identify regulatory factors, cytokines, and pathways that specify their fate, much still remains to be done to precisely understand what these

assays are telling us about the organization, regulation, and regenerative potential of endogenous lung stem cells *in situ*. In doing so, he identified a number of questions and gaps in our knowledge about the properties of candidate stem/progenitor cells that need to be resolved to assess the usefulness of these surrogate assays in identifying targets around which cellular therapies for lung disease can be tailored. For example, current protocols have enabled us to prospectively isolate cell fractions highly enriched for colony-forming cells but have not enabled us to resolve colony-forming cells of differing developmental potential. This is consistent with the premise that stem and progenitor cells are characterized by the expression of multiple markers that collectively specify the stem cell state but are not unique to individual stem/progenitor cell types. But, it is unclear whether the readout of index assays is indicative of cofractionation of heterogeneous progenitor cells expressing common biomarkers or the stochastic commitment of homogeneous progenitor cells to different fates in response to different microenvironmental cues.

Dr. Bertoncello also reminded the audience that the regenerative capacity of stem and progenitor cells *in vivo* is determined not solely by their intrinsic developmental potential but also by the reciprocal signaling between stem/progenitor cells and their niche microenvironment, which includes stromal and extracellular matrix (ECM) elements, accessory cells, as well as soluble and insoluble factors that also respond to insult or injury, temporally and dynamically altering the regulatory properties of the stem cell niche (27, 28). Consequently, *in vitro* clonogenic assays are powerful but imperfect tools for modeling stem/progenitor cell hierarchies, and there is a pressing need for the development of “gold standard” *in vivo* assays akin to hematopoietic stem cell transplant assays to establish the precise relationship between candidate stem/progenitor cells identified *in vitro* and regenerative cells *in vivo*. He concluded by raising the issue that targeting the dysregulated lung epithelial/progenitor cell niche may prove as important as targeting epithelial stem/progenitor cells in developing cell-based therapies in lung regenerative medicine (29).

Dr. Barry Stripp kept up with this theme and argued that there are regionally

distinct regenerative zones that function in concert to maintain and repair the epithelium of postnatal conducting airways (25). The Stripp laboratory has used lineage tracing coupled with cell fractionation approaches to understand how progenitors from different airway regions behave in a defined culture environment. His laboratory's data suggest that the positional identity of regionally distinct airway progenitor cells is controlled through intrinsic mechanisms. Gene expression profiling was used to define candidate molecular markers that distinguish regional progenitor cells and provide new tools for lineage tracing and functional analysis *in vivo*. Parallel studies performed using human lung tissue suggest that principles developed through use of mouse models are broadly applicable to other mammalian species, including humans.

Dr. Susan Reynolds then focused on a specific subset of basal epithelial progenitor cells, the facultative basal progenitor (FBP), and roles for β -catenin in regulation of FBP proliferation and differentiation (30). Using tracheal air-liquid interface cultures, Reynolds showed that these cultures could be divided into two phases: basal cell proliferation, which results in expansion of the basal cell population, and basal cell differentiation into ciliated or Club-like cells. Using genetic stabilization of β -catenin in basal cells, her laboratory demonstrated that β -catenin-dependent signaling had a minor impact on the level of proliferation that resulted in population expansion. However, stabilized β -catenin shortened the post-confluence period of basal cell proliferation. She speculated that this effect was due to initiation of basal cell differentiation. Stabilization of β -catenin enhanced basal-to-ciliated cell differentiation twofold but completely blocked basal-to-Club-like cell differentiation. These results argued that β -catenin regulated basal cell fate by favoring the generation of ciliated cells over Club cells. A loss-of-function allele was also used to demonstrate that β -catenin was necessary for early but not late basal-to-ciliated cell differentiation and basal-to-Club-like cell differentiation as well as basal cell proliferation and long-term survival of basal cells.

Finally, Dr. Carla Kim described her initial isolation of BASCs via isolation of Sca1-positive, CD31/CD45-negative cells by FACS and her demonstration that these cells

can self-renew on mouse embryonic fibroblast feeder layers or differentiate on a two-dimensional Matrigel setting (31). The Kim Lab has more recently developed a means to better enrich for BASC by FACS sorting CD31/45-negative, Sca1-low, CD24-low, Epcam-positive cells, offering an improved method of isolating putative lung stem cells, which is in good agreement with others' marker profile for lung stem cells (work of Stripp and Bertoncello laboratories). Dr. Kim then referenced that the Hogan Laboratory has recently shown with CCSP-CreERT2;LSL-YFP mice lineage-tracing strategies that CCSP lineage-marked cells do not contribute to alveolar epithelia during development, suggesting that BASCs do not play an important role in maintaining alveolar cell types during steady-state homeostatic conditions. Additionally, she referenced these studies demonstrating that CCSP-positive cells cannot give rise to alveolar lineages after treatment with hyperoxia. She verified that her group does not observe a BASC response to hyperoxia treatment. However, she hypothesized that BASCs play a key role in the response of adult lungs to particular contexts of bronchiolar and alveolar injury, such as the naphthalene and bleomycin response. To further test this hypothesis, her group used CCSP-CreER;LSL-YFP mice and performed lineage tracing studies with bleomycin treatment. Importantly and in contrast to the Hogan Laboratory hyperoxia results, Kim has determined that there is a significance increase in CCSP lineage-tagged cells in the alveolar space after bleomycin treatment (Kim Lab, unpublished). This is consistent with her hypothesis that BASCs are capable of differentiation to alveolar epithelia *in vivo* in particular contexts. Alternatively, the data may also indicate Club cells have this capacity. To distinguish between these possibilities, it will be necessary to achieve BASC-specific genetic marking *in vivo*. Dr. Kim also described her identification of an important new role in adult cells for Bmi1 in the repression of genes that are typically regulated by imprinting mechanisms (24). Expression of p57, Igf2, and other imprinted genes was higher in BASCs compared with AT2 cells, was required for wild-type lung stem cell self-renewal in culture, and correlated with the ability of stem cells to

effectively repair damaged lung epithelium *in vivo*.

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

This session was focused on the development of induced pluripotent stem cell lines from normal donors and patients with disease to serve as a renewable source of cells. In addition to the widely anticipated use of these cells for transplantation or regenerative medicine applications, it was clear that a more immediate impact of iPSC technology is the generation of respiratory and other lineage cells from normal donors and patients with disease to study *in vitro*. Examples were provided for the use of iPSC-derived respiratory cells to study early human development, including germ layer and cell lineage commitment, and differentiation. Other studies generated iPSC lines from patients with disease to study cellular mechanisms of disease, evaluate gene therapies, and screen drugs, as outlined below for each presentation.

In the first presentation in this session, Dr. Jeffrey Whitsett (Cincinnati Children's Research Foundation) provided an overview of the large number of respiratory disorders that could potentially benefit from the development of respiratory cellular repair and regenerative medicine applications in the lung, including bronchopulmonary dysplasia (BPD), COPD, ARDS, cystic fibrosis (CF), and others. He highlighted the complex structure and physiology of the lung at the organ and cellular levels, which need to be considered and understood before undertaking cell-based regenerative therapies in the lung. Specifically, cellular therapies for lung disease would need to ensure repair or normalization of surfactant homeostasis, alveolar–capillary integrity, mucociliary clearance, host defense, normal cell proliferation, differentiation, and repair processes. He then used alveolar homeostasis as an example of the high level of cellular organization and regulation required to produce and maintain normal tissue or repair damaged tissue. He provided many examples of disease models in which an imbalance in the spatial and temporal, proliferation or differentiation, or cellular interactions of cells in the alveolus can lead to injured or dysfunctional tissue. This presentation highlighted the need for potential cellular

therapies to produce a tightly controlled regulation of multiple cell proliferation and differentiation and the establishment of cell contact with other lineages in the alveolus to repair lung tissue, without introducing serious consequences.

The next presentation by Dr. Gustavo Mostoslavsky (Boston University) provided an overview of the methodologies for reprogramming and generating induced pluripotent stem cells, including a review of the rapid pace of innovations in reprogramming. He presented an example of reprogramming and iPSC line generation and testing using a single excisable lentiviral vector (STEMCCA), developed in his laboratory, that reprogrammed fibroblasts and other cells with a high efficiency (32). The expression cassette can be removed after a transient expression of cre recombinase to produce iPSCs without the transgene cassette. He also showed transcriptional analysis demonstrating that cells that had the transgene cassette removed were very similar to embryonic stem cells. He provided an overview of the developmental differentiation approach in which *in vitro* differentiation protocols are developed to match the conditions and factors that regulate embryonic development for specific embryonic germ layers and tissues. For example, definitive endoderm can be induced by addition of activin A, and further differentiation into specific lineages can be induced by altering the environment, such as the addition of fibroblast growth factor 10 (FGF10) to induce lung differentiation. He then discussed studies from his laboratory in which iPSC lines from patients with many genetic diseases have been generated, including CF and α_1 -antitrypsin deficiency, and highlighted the power of using these iPSC lines to model disease. He concluded his presentation with a comment that despite the rapid pace of iPSC research, clinical application of these cells is many years away, and many studies will be required to determine the safety of transplanting iPSC-derived cells. He cautioned that the “stem cell tourism,” whereby patients are receiving experimental therapies in uncontrolled and unregulated environments, has the potential to hurt patients receiving the stem cells and creates risks for the field of stem cell research to lose credibility with the public after potential medical disasters

Dr. Ali Samadikuchaksaraei (Imperial College of London) spoke about his strategy of optimizing the production of alveolar epithelial cells from mouse and human ESCs. He reviewed his work optimizing many factors in the culture system, including growth factors, conditioned medium and cell lysates from different respiratory cell types and populations, and embryonic lungs (33, 34). He then focused on his development of a bioreactor system to scale-up and simplify the production of respiratory cells by encapsulation of embryoid bodies, culture in rotating bioreactors, and addition of conditioned media, which produced alveolar type I and II cells that could be maintained for 100 days. He outlined his current focus of optimizing differentiation by building an *in vitro* respiratory unit system through the addition of other respiratory cell populations, such as epithelial, mesenchymal, and endothelial cells, as well as optimizing other culture parameters, including nutrition, pH, and oxygen tension.

Dr. Darrell Kotton (Boston University) presented his studies using a developmental approach to generate respiratory cells from mouse ESCs. This approach uses a stepwise differentiation of ESCs and iPSCs through definitive endoderm to foregut endodermal progenitors and subsequently to respiratory progenitors. The first stage of this process is efficient, with greater than 80% of the cells effectively differentiated toward definitive endoderm using activin A in embryoid bodies. His studies focused on optimizing the second phase of differentiation from definitive endoderm to respiratory progenitors through the use of a mouse ESC reporter line in which green fluorescent protein was knocked into the *Nkx2.1* locus. In this study, differentiation conditions that promote respiratory progenitor differentiation could be readily screened by fluorescence microscopy or flow cytometry, and the conditions supporting optimal differentiation could readily be identified through the expression of green fluorescent protein. They used this system to determine that inhibition of bone morphogenic protein (BMP) and transforming growth factor (TGF)- β signaling reduced “posterior” patterning and led to increased ventralization of foregut endoderm, resulting in an increase of *NKX2.1* endodermal progenitors to 20%. These *Nkx2.1* progenitors were similar to

primordial lung progenitors by gene expression profile. These progenitors could then be induced to respiratory differentiation, as evident from the expression of a wide variety of respiratory epithelial markers after further differentiation in FGF2 and FGF10. Future studies will work on the later differentiation events in fetal lung development to identify the signals regulating the development and contribution of the primordial lung progenitors to continued respiratory development (35).

Dr. Amy Wong (The Hospital for Sick Children) presented studies on the development of iPSCs from patients with CF to serve as cellular models of disease, drug discovery, and regenerative medicine. She described the development of a step-wise differentiation system that mimics lung development to generate human airway epithelium to test drugs for CF. The differentiation system used carefully timed addition of morphogens to direct differentiation through definitive endoderm and anterior ventral foregut endoderm, embryonic lung progenitor, and immature lung growth. The cells were then cultured at air-liquid interface to generate a heterogeneous, polarized, and functional airway epithelium. These cultures developed dome-like structures with an adsorptive epithelium capable of ion and fluid transport. The epithelium that formed was similar to large airways with expression of genes consistent with basal cells (p63), goblet cells (MUC5), and ciliated cells (FOXJ1). The Cystic Fibrosis Transmembrane Regulator (CFTR) was localized to the apical membrane, as in the native airways. They next developed iPSC lines from patients with the d508 CFTR mutation from patients with CF (36). Dr. Wong demonstrated that these lines were pluripotent and could generate a functional epithelial layer. These differentiated airway epithelial cultures were then used to screen chemical compounds capable of stabilizing the mutant CFTR at the apical membrane and identified one candidate drug. Ongoing studies will evaluate whether this stabilization and localization of CFTR also resulted in functional ion channels. Future studies will use these iPSC-derived epithelial layers to screen and identify novel CFTR correctors and potentiators.

Dr. Hans-Willem Snoeck (Mount Sinai School of Medicine) focused on improving the generation of anterior foregut endoderm from human pluripotent stem

cells. He highlighted that there are many studies demonstrating the efficient generation of hindgut (intestine), mid-gut (pancreatic endoderm), and posterior foregut endoderm (hepatocytes). However, the generation of anterior foregut lineages has been more challenging. He showed that this is due in part to a mid/posterior bias of activin A-induced endoderm. He screened for signals to enrich anterior endoderm formation in human ESC differentiation and reduce ectoderm and mesoderm differentiation. His studies demonstrated that dual inhibition of TGF- β and BMP signaling after the formation of activin A-induced endoderm resulted in a highly enriched population of anterior foregut endoderm. Subsequent addition of Wnt3a, keratinocyte growth factor (KGF), FGF10, epidermal growth factor, and BMP4 to the culture induced cellular lineage differentiation toward an anterior ventral foregut phenotype (22). Subsequent screening of 400 culture conditions on the anterior foregut endoderm identified an optimal combination for the production of alveolar epithelial type II cells. The optimal conditions included continued Wnt, FGF10, and KGF addition to the cultures from Days 10 to 19. This study demonstrated that optimal culture requires highly spatial-temporal addition of factors to mimic embryonic development and achieve optimal differentiation into respiratory epithelium.

Dr. Brian Davis (University of Texas Health Science Center) presented his studies on the generation of iPSCs from patients with the inherited respiratory diseases surfactant B deficiency and CF as well as gene correction of the mutant genes. He presented studies in which he generated an iPSC line from a dI507/dF508 CFTR compound heterozygote. The iPSCs were then subject to zinc finger nuclease (ZFN)-mediated gene correction that used a donor construct that corrected the CFTR gene and introduced a puromycin selection cassette into the adjacent intron 10. After transfection, the iPSCs were selected with puromycin and molecularly screened to identify clones that had undergone ZFN editing to correct the CFTR genes. They also demonstrated that the corrected cells expressed the wild-type CFTR after differentiation into an airway epithelial layer. He next presented their studies developing iPSCs from patients with surfactant protein B deficiency with ongoing

studies performing ZFN gene correction of the surfactant protein B mutation. Future studies will include generation of alveolar type II epithelial cells and evaluation of surfactant production in the gene-corrected cells.

In summary, this session highlighted that embryonic stem cells and induced pluripotent stem cell models have advanced rapidly and are now being used to study normal and diseased human respiratory development and lineage commitment. The differentiated progeny of iPSCs are being used as a source of normal and diseased cells to identify signaling pathways and cellular physiology of the disease as well as evaluate gene- and drug-based therapeutics for respiratory disease. Although it is likely that iPSCs may also serve as a source of cells for replacement or regenerative therapies for lung injury or disease in the future, a significant amount of progress on the generation of specific lineages as well as extensive studies on the efficacy and safety of this approach is required before this goal is realized.

Session 3: Bioengineering Approaches to Lung Regeneration: Structure and Matrix

The purpose of this session was to review recent developments in the pursuit of tissue-engineered lung using decellularized lung scaffolds as the platform on which pluripotent cells can be seeded. Dame Julia Polak, M.D., D.Sc., from Imperial College London began the session by teleconferencing in to the meeting in real time from London; she reviewed the limitations of artificial membrane oxygenators and artificial lungs. She then gave an overview of the current state of the field, pointing out that acellular lung matrices used in bioreactors may be the way forward for generating bioartificial lung units. It should be noted that, after her talk, Dame Julia was able to respond to audience questions and comments over the teleconference link almost as if she was in the same room as everyone else, despite actually being separated from the rest of the audience by thousands of miles.

Dr. Paolo Macchiarini from the Hospital Clinic de Barcelona then gave a presentation entitled "Cell Therapy and Bioengineered Replacement of the Airways," in which he discussed the evidence showing that stem cells can be successfully applied to airway transplants for adults and children (37, 38). He then

extrapolated this to the vision that stem cell therapy might be extended to treat untreatable end-stage lung diseases. He pointed out that although there is still much debate about exactly which kind of cell should be used (embryonic, adult stem cells, etc.), in fact, the adult body has continual stores of stem cells in specialized niches that are regularly recruited to repair damaged tissues. Phase I-II clinical trials are now using dormant stem cells taken from a patient's body to repair tissues and organs. This has the advantage of significantly reducing ethical and regulatory barriers to treatment.

Dr. Joan E. Nichols of the University of Texas Medical Branch at Galveston presented a talk entitled "Acellular Lung Scaffolds: Influence in Cell Differentiation and Evaluations of Mechanical Integrity," in which they focused on cell matrix interactions as critical factors in the design of functional engineered tissues. In particular, fabrication of biomaterials for use in an engineered lung must consider the role of the ECM in determining the differentiation of endothelial and epithelial cells. A major stumbling block in this field to date has been the production of suitable biocompatible scaffolding material that supports but does not restrict lung function. For example, such biomaterials must possess degradation profiles and elastic properties similar to that of normal lung ECM while avoiding the development of inflammation fibrosis. For these reasons, the way forward may be to use natural lung ECM obtained via decellularization of whole lungs (39–41).

Dr. Christine Finck of the Connecticut Children's Medical Center presented on "Embryonic Stem Cells in Tissue Engineered Scaffolds." She began by pointing out that preterm delivery affects more than 10% of all births and accounts for 70% of perinatal mortality and that the ability to use stem cells to regenerate functional alveolar tissue could have a major impact on premature lung disease. Key to achieving this goal is to have stem cells differentiate successfully into desired cell phenotypes, something that depends on the growth factors used and the nature of the cell culture surfaces. Again, decellularizing intact lungs provides a means of obtaining culture surface having the desired properties, although the optimal approach to this has not been completely delineated. Decellularized lungs retain their

native collagen and elastin structures while losing nuclei and glycosaminoglycans. Inoculating decellularized rat lungs, for example, with different mixtures of fetal rat lung homogenates, endothelial cells, and A549 carcinoma cells has resulted in successful recellularization, and there is evidence that inoculation with mESC leads to differentiation into a variety of mature lung cell types. Importantly, biologic scaffolds require continuous nutrient infusion. They also reported that when sheep lungs were decellularized and orthotopically transplanted into a pneumonectomized sheep and the vasculature reanastomosed, the lungs could be ventilated without major air leaks without rupture of the scaffold.

Dr. Andrew M. Hoffman of Tufts University reported on "Design of Biological Scaffolds that Promote Engraftment and Repopulation of Lung Scaffolds with Mesenchymal Stromal Cells" and began by pointing out that cultured mesenchymal stromal cells (MSCs) do not readily engraft the lung because they lack specific adherence factors for other stromal or parenchymal cells. He then focused on cell surface receptors as critical factors for the production of viable stem cell scaffolds and reported that MSCs and fibroblasts exhibit a high degree of similarity in surface phenotype, implying that universal scaffolds may be possible for MSCs. After presenting a number of research findings related to specific ECM surface receptors and associated signaling pathways, Dr. Hoffman concluded by stating that use of RGD-imbued scaffolds will likely contribute to improved engraftment *in vivo* and bioengineering (i.e., recellularization) of lungs *ex vivo*.

Dr. Peter I. Lelkes of Drexel University presented on "Decellularization and Beyond: Scaffolds and Stem Cells," focusing particularly on biomimetic scaffolds to facilitate alveolar morphogenesis and cell sourcing to populate such scaffolds. He began by discussing the use of natural and synthetic biomaterials for generating nanoscale-diameter fibers and composites for emulating the bioactive, "intelligent" structure and function of extracellular matrices. Such scaffolds can be used to manipulate various biological processes, such as the induction of branching morphogenesis. He then pointed out that cell sourcing for lung tissue engineering and regenerative pulmonary medicine may

be improved by directed differentiation and organotypic functional assembly of stem and progenitor cells. Applications include generating vascularized three-dimensional pulmonary constructs from mixed populations of fetal murine pulmonary cells, permissive natural matrices and biomaterials, and growth factor-containing culture media. Dr. Lelkes also discussed the maintenance and integration of these structures after ectopic implantation *in vivo*. He concluded by describing recent studies of murine embryonic stem cell differentiation *in vitro*.

Dr. Angela Panoskaltis-Mortari, Ph.D., from the University of Minnesota, gave a presentation entitled "Using Decellularized Matrices for Bioengineering the Lung," in which she presented her work on acellular ECM scaffolds and their use in bioreactors to evaluate the potential of multipotent stem cells to regenerate lung tissue (42). When seeded into these scaffolds under the appropriate growth conditions, iPS can express several lung markers, which illustrates the potential for regenerating lung tissue using autologous iPS cells on decellularized matrices to avoid immune-mediated rejection on subsequent transplantation. Nevertheless, the optimization of this process will require much future research.

Finally in this session, Zachary Borg, B.A., from the University of Vermont talked about "Optimizing Lung Decellularization and Recellularization with Stem Cells" and began by pointing out the current chronic shortage of donor lungs for transplant. He then summarized the current state of research into the use of decellularized lungs from autopsy seeded with stem cells to generate new lungs as well as recent results from primate and nonprimate lungs. This included a report of successful implantation of recellularized lung scaffolds into rats. Nevertheless, this field of research is still very much in its infancy, and much work remains to be done to determine the optimum conditions for regenerating lungs by recellularizing acellular scaffolds.

Session 4: Bioengineering Approaches to Lung Regeneration: Function

The goal of this session was to review the progress that was made in bioengineering of the lung, with a specific focus on functional assessment of lung tissue. The

session dealt with four primary topics: whole-organ engineering with decellularized scaffolds, bioinspired microdevices, mechanobiology, and advanced imaging modalities. The session began with Doris Taylor, Ph.D., who provided a general overview of the field of organ decellularization. Over the past decade, there has been a shift from the use of simple sheets of decellularized tissue to decellularization of complex tissues and organs that preserve the three-dimensional geometry of the starting tissue (39). This has been made possible by using the vasculature of the tissue or organ to deliver various decellularization agents to the cells, effectively reducing the diffusion distance and increasing the transport of cellular material from the tissue. Dr. Taylor notably pioneered this whole-organ engineering approach with the development of a decellularized rat heart (43), and a variety of other tissues have followed, including liver and lung (41, 44–46). Perfusion of the vasculature of the heart with detergents (particularly sodium dodecyl sulfate) resulted in a tissue with no visible histologic evidence of nuclei and significant DNA removal, without a significant loss of glycosaminoglycans (39). The decellularized heart served as a suitable substrate for attachment of various site-appropriate cells, including cardiomyocytes and endothelial cells. Cardiomyocytes showed isolated regions of synchronous contractility, and the addition of endothelial cells significantly reduced the thrombogenicity of the ECM scaffold. These results show that whole-organ decellularization is promising for development of a functional cardiac transplant in the future. Dr. Taylor has extended this work in the rodent model to the human case, with successful decellularization of a human heart with cardiac structure and large and small vessels left intact.

There are a variety of aspects of the ECM that must be considered when selecting a donor tissue for decellularization. Ultimately, this organ decellularization and recellularization relies on the bioactivity of the matrix to allow cell attachment, proliferation, and differentiation, and these cues are dependent on the composition and passive mechanical behavior of the resulting scaffold. Studies are needed to fully describe the changes in the ECM of tissue as a function of sex, age, and disease, especially because donor tissues are likely to

come from older individuals with more or less history of pathology. Likewise, processing has a variety of effects on the ECM that are not fully understood. Any changes to the ECM associated with these conditions could have an effect on the cells that are seeded into the construct or that migrate into the construct from the recipient. Standardization of processing techniques is going to be necessary in the near future to make reasonable comparisons.

Due to the preservation of ECM structure and composition through decellularization, ECM scaffolds serve as interesting test beds for studying the potential for cells to regenerate tissue. Work by Dr. Taylor and others showed that ECM from different organs has potent effects on the gene expression of seeded cells. In particular, the gene expression of cardiomyocytes differed greatly when seeded on heart-, liver-, and kidney-derived ECM. Some combination of the specific organization of the ECM and the ECM composition appears to allow cells to retain their original phenotype. Although there is some early evidence that ECM may facilitate targeted differentiation of stem and progenitor cells, there is much work to be done to substantiate this claim (47).

An important point of caution was presented regarding ECM scaffold remodeling. ECM scaffolds tend to be subject to cell infiltration followed by ECM degradation, remodeling, and deposition. This is in contrast to the desired goal of generating transplantable organs that will resume homeostasis without loss of structure. The current state of the art in this area is still too early stage to fully address this question regarding the potential for ECM scaffolds to serve as framework for future organs.

Later in the session, Drs. Harald Ott, M.D., and Laura Niklason, M.D., Ph.D., both reviewed their breakthroughs in lung tissue engineering using decellularized lungs as a substrate for tissue assembly. In 2010, both groups independently engineered lung tissue and performed short-term orthotopic implantation in rat models (48, 49). Dr. Ott focused on the many questions that will need to be addressed before widespread application of lung tissue engineering can be realized (50). His group has successfully decellularized large cadaveric lungs, but as described by Dr. Taylor, there is work yet to be done to

determine if there is an effect on the species of lung, the age of the donor, and the disease state of the tissue. Furthermore, the optimal conditions for harvesting the tissues have not been explored. It is unknown whether there are differences in the ECM from living and deceased donors or how long after death the organs persist with warm or cold ischemia before use. Furthermore, methods for organ preservation have been unexplored to date. Dr. Ott agreed that the field would need to move to a consensus about the methods for decellularization of lung tissue as well as sterilization methods. Ultimately, storage conditions and shelf life will need to be investigated.

Questions regarding source and number of cells needed for repopulation of decellularized lungs loom large. Primary cells are attractive but will be difficult to obtain in sufficient numbers to generate an organ and prompt concerns about immunogenicity. Stem cells may be an alternative, but the ability to obtain enough cells remains a challenge, and there are ethical concerns about the use of embryonic and fetal stem cell populations. Induced pluripotent stem cells are another possible cells source. In all cases, methodologies will need to be developed to drive stem cells into lung cell lineages for this approach to be feasible. Furthermore, it is unclear whether stem cells should be used in their undifferentiated state and allowed to differentiate in response to the cues from the ECM and cell culture or whether they should be differentiated before seeding within the ECM.

Bioreactor development is going to be another key step in the progress of organ engineering. The optimal configuration, culture protocol (e.g., media, mechanical conditioning), and monitoring regimes (e.g., metabolism, morphologic endpoints) need to be determined. The bioreactors must also be designed to be scalable not only from smaller organs to larger organs but also from single to mass production. Once all of the conditions for culture are determined, an important question will be how long the construct should be allowed to mature before implantation. The time for maturation raises the question of engineering versus regeneration. If the engineered organ is intended to experience further *in situ* development to gain full function, then a shorter culture period may be appropriate. This would be the

regenerative paradigm. In contrast, tissue engineering would reach for the goal of a transplantable organ that would resume homeostasis on implantation. For this approach, a much longer and more involved culture system would be required.

Finally, determining the most appropriate model for transplantation will be crucial to have the best chance of success. Animal models will be necessary to enable determination of the effects of the immune response to the graft and whether homeostasis can be achieved. It will also be necessary to determine whether the grafts can tolerate ventilatory support. Depending on how quickly mucociliary clearance function is restored, provisions may need to be made for palliative care.

Dr. Ott's most recent work drives home the need for further investigation of these questions (50). Building on his initial work, the model was expanded to include evaluation of orthotopic implanted single lobe grafts over a period 14 days. The engineered lung constructs showed similar oxygen transport capacity as comparable to a cadaveric lung transplant for a period of 7 days, but function declined over the second week due to graft consolidation and inflammation. By the end of the study, the engineered lung construct showed no ventilation or clearance and showed evidence of infection.

Dr. Niklason shared her progress in the development of functional lung constructs. Dr. Niklason described the use of decellularization methods based primarily on the use of vascular and airway perfusion with CHAPS detergent that has been shown to preserve the structure of the lung ECM (48, 51). The cell source used by Dr. Niklason was a mixed population of neonatal rat lung cells. Based on histologic data defining the phenotypes of the cells and the composition of the adjacent matrix, it was found that either the mixed cell populations tended to sort themselves to "zip codes" in the matrix, or the cells attached randomly to the ECM and remodeled it to generate an appropriate niche. The cells tended to show increased expression of regenerative markers in the early time frame after seeding into ECM scaffolds, and over a period of 12 days the phenotype changed to express more terminally differentiated, site-appropriate cells. This was all performed in Dulbecco's modified Eagle medium with 10% fetal bovine serum and no other airway-specific

markers, suggesting that the ECM is able to drive tissue assembly.

The next major thrust of this section dealt with bioinspired microdevices. The first talk was by Dr. David Hoganson, who described his work in the development of microfluidic systems to generate a lung assist device (52). Lung assist devices have been in development for decades, and the first device (Novalung) is now being used in the United States (53). The typical design for these devices use high-density parallel hollow fibers, which poses significant deviations from the physiologic flow in bifurcated vascular networks of the lungs. Using microfluidics, very small channels were formed by polydimethylsiloxane onto silicon molds by lithography to establish a vascular network and alveoli chamber separated by a gas-permeable membrane. The design of the vascular network allowed for dramatic increase in surface area, which is critical for adequate gas exchange, while maintaining physiologic flow and shear conditions to prevent thrombosis. The three-dimensional vascular networks can be micromachined with approximately 2- μ m precision. Empirical experiments have shown that the results match the predictions for computation fluid dynamics analysis. Further work is being performed to optimize the gas transport kinetics by modifying the channel shape and size, and selection of different substrates (54–56). For example, the use of PTFE films was found to increase the carbon dioxide permeability by 300 times. The empirical studies also showed the importance of manufacturing, as a small manufacturing defect led to an increased platelet adhesion and thrombosis. Future developments will also include biomimetic scaffolds to enhance endothelialization of the channels.

Dr. Daniel Huh presented his work on the development of a "breathing lung on a chip" (57, 58). This approach also used photolithography to form microfluidic channels from silicone but takes advantage of the natural elasticity of silicone to allow for application of stretch to cells within the system. This allows for testing lung cells in a system that mimics the stretch of respiration. The configuration of the system included alveolar epithelial cells on the air side of the membrane and microvascular endothelial cells on the fluid side of the membrane. The membrane had micropores to allow for transport

across the membrane. Unlike the approach described by Hoganson and colleagues, which was primarily intended to support oxygenation function for a diseased lung, this "lung on a chip" approach was intended to recapitulate the organ level function of the lung in a very small scale. Studies showed that the configuration was able to restore alveolar–capillary barrier function and that the morphology of the cells in response to mechanical loading mimicked that observed *in vivo*. Furthermore, activation of endothelial cells with tumor necrosis factor- α led to adhesion of circulating neutrophils within the microchannels. Neutrophils were then shown to transmigrate to the apical surface of the system. The system was further extended to include bacteria within the apical compartment that induced activation of the endothelium followed by attachment and transmigration of neutrophils. The system was also tested with various nanoparticles to test toxicity. Silica nanoparticles and carboxylated Cd/Se quantum dots were both shown to induce inflammation and increases in reactive oxygen species production but only in the presence of accompanying mechanical strain. The mechanical strain-induced response appeared to be specific to these nanoparticles, as other nanomaterials did not show the same effect. This suggested that care must be taken when deciding whether to use mechanical ventilation in patients exposed to these materials and demonstrated the potential for these microdevices to impact clinical care.

Dr. Dan Tschumperlin, Ph.D., followed with a broader look at recent developments in mechanobiology of the lung, in particular the importance of matrix stiffness and the response of cells to stiffness (59). Recent advances in imaging techniques have made it possible to interrogate the micromechanics of the lung as never before. A recent report using optical sectioning microscopy showed the heterogeneous distention of alveolar segments in response to inflation, with significantly greater distention of type 1 epithelial cells as compared with type 2 cells (60). This finding emphasized the importance of the composition, structure, and stiffness of the ECM on the phenotype and behavior of cells. Dr. Tschumperlin's group recently measured the stiffness of normal and fibrotic mouse lung matrix on the microlevel using atomic force

microscopy and investigated the effects of stiffness on lung fibroblasts (61). ECM in fibrotic lesions showed a sixfold higher stiffness than in the surrounding lung parenchyma, which is a larger difference than observed in macroscale measurements. In *in vitro* experiments, the stiffness levels corresponding to normal lung parenchyma (~0.5 kPa) led to more rounded cells that tended to show decreased proliferation, increased apoptosis, and decreased collagen synthesis. In contrast, stiffness levels ranging from 3 to 15 kPa, which corresponded to the stiffness of fibrotic lesions, showed the opposite trends. Increased stiffness also led to decreased matrix metalloproteinase expression. It is important to note that the shift toward more fibrotic fibroblast phenotype occurred at stiffness levels just slightly above those observed in normal tissue, suggesting that the native ECM suppresses fibrosis, and only a slight injury could perpetuate the formation of the lesion. PGE2 was found to mediate the fibroblast response to stiffness. ROCK down-regulated COX-2 to suppress PGE2 synthesis, thereby leading to a more fibrotic fibroblast phenotype. However, not all cells respond to stiffness in the same way. Another study investigated the response of various cancer cells and fibroblasts to varying stiffness, and “rigidity-dependent” and “rigidity-independent” growth profiles were observed, with low growth rates on soft gels due in part to selective alterations in the cell cycle progression (62). The rigidity-dependent cells also tended to show less spreading and migration on soft gels. On implantation, the rigidity-independent lines were able to grow more robustly on normal lung matrix. Clearly, matrix stiffness and mechanobiology are important areas for continued investigation in lung biology.

Finally, Dr. Jason Woods, Ph.D., presented developments in *in vivo* imaging of the lung. Assessment of conditions like inflammation can only be tested indirectly through pulmonary function testing or through invasive bronchoalveolar lavage. The current state of the art in evaluating lung morphology is histologic analysis, which has the limitations of being constrained to two-dimensional planar sections of tissues and is destructive in nature. In both cases, new minimally invasive imaging modalities that allow for three-dimensional visualization of the lung would be advantageous. A recent study

evaluated the potential for positron emission tomographic imaging with [18F] fluorodeoxyglucose ([18F]FDG) to monitor inflammation in the lung (63). A small volume of endotoxin was instilled into a limited region of the lungs of healthy patients. At 24 hours after endotoxin instillation, [18F]FDG-positron emission tomographic was performed and was able to show with acuity the presence of inflammation at the instillation site. The rapid uptake of [18F]FDG for acute and chronic inflammation suggests that it will be a useful biomarker to assess the efficacy of antiinflammatory therapies in conditions like CF, ARDS, and pneumonia.

Another technique described by Dr. Woods for visualizing the lung microstructure was hyperpolarized gas magnetic resonance imaging. Hyperpolarized magnetic resonance imaging with ³He or ¹²⁹Xe works by measuring the random atomic motion (diffusion) of the gas molecules (64, 65). Because airway structures will limit the diffusion of gas, measurement of the atomic motion will provide information about the lung microstructure. Both gases have been used in humans, large animals, and, more recently, small animals in both healthy and diseased conditions and have provided important insights into the three-dimensional structure of the lungs (64–73). The usefulness of this approach to treatment of human disease is obvious, with greater ability to determine the changes in lung microstructure as a function of disease and over time. The expansion of this technique to mouse models opens up huge opportunities to study the architectural changes in the lung as a function of disease and treatment with all of the advantages of small animal models.

This session showed how advances in the field of regenerative medicine, microscopy, and imaging are changing paradigms regarding the study the mechanisms and progression of disease and development of strategies to repair or replace injured lung tissue. These new approaches are providing opportunities for the field to move toward a more rigorous study of the structure–function relationships in the lung.

Session 5: EPCs, MSCs, and Cell Therapy Approaches for Lung Diseases

The general theme for this session was the evolving use of MSCs and EPCs for the prevention and/or treatment of important

diseases of the lung, ranging from ALI/ARDS to pulmonary hypertension. This session primarily focused on the potential of these “stem” cell populations to function in immunomodulatory and/or tissue repair roles rather than on their regenerative medicine capacities *per se*.

The first speaker in the session was Dr. Darwin Prockop, M.D., Ph.D. (Texas A&M Health Science Center College of Medicine, Temple, TX) who addressed the question of whether studies using therapeutic administration of MSCs could ultimately translate to the development of novel protein or small molecule therapies for ALI. Dr. Prockop began his presentation with the observation that although MSCs have been reported to produce beneficial effects in a wide variety of disease models, the precise mechanism(s) by which MSCs exert these effects remain(s) unclear. He suggested three possible paradigms that could be responsible for the observed beneficial effects: (1) MSCs could provide a microenvironment or “niche” providing cell-to-cell contact to nourish resident cells, (2) MSCs could engraft and differentiate to replace injured cells in the target tissue (i.e., regenerative medicine), or (3) MSCs could express and secrete/release specific gene products with the potential to decrease or reverse disease-related pathophysiology in the host via a paracrine mechanism.

Dr. Prockop considered paradigm one to be highly unlikely to account for MSC beneficial effects in lung diseases. Although intravenously administered MSCs are initially “trapped” in the lung, imaging studies have failed to document significant long-term pulmonary engraftment of MSCs (74, 75). In the lung, he believes that paradigm three is most likely, and the identification of factors secreted by MSCs may enable the development of novel specific protein- or small molecule-based therapies for ALI. His group has recently identified two new MSC-derived gene products as candidate molecules. TSG-6 is an antiinflammatory protein secreted by MSCs that has been shown to play an important role in MSC-mediated improvement of experimental myocardial infarction in the mouse (76). A subsequent study demonstrated that MSC-derived TSG-6 attenuates experimental zymosan-induced peritonitis via a paracrine effect to decrease proinflammatory responses in

resident macrophages activated by Toll-like receptor 2/nuclear factor- κ B signaling (77). The second recently identified MSC-derived molecule discussed by Dr. Prockop was stanniocalcin 1 (STC-1), which has been shown to have antioxidant and antiapoptotic properties (78). Dr. Prockop concluded with his opinion that a careful and comprehensive analysis of the activated MSC transcriptome and “secretome” (i.e., secreted gene products) is likely to yield potential candidates for translational development of novel protein-based strategies for the treatment of ALI. It is highly possible that the beneficial effects of MSCs in diseases associated with inflammation and tissue injury are not due to a single soluble factor secreted by MSCs but rather to a combination of MSC-derived factors that target various components in the pathophysiology of the disease process. Furthermore, MSCs may have to be activated by the host microenvironment to express specific factors responsible for their beneficial immunomodulatory and/or reparative properties.

Dr. Prockop also mentioned that he now works exclusively with human MSCs in mouse models, due to the inherent instability of mouse-derived MSCs and associated inconsistent experimental results. Dr. Michael Matthay, another speaker in this section (*see below*), commented that his group also uses human MSCs in mouse models of ALI (and other mammalian models of ALI) with consistent therapeutic effects.

The next speaker in this session was Dr. Jacques Galipeau (Emory University Winship Cancer Institute, Atlanta, GA), who provided a cautionary discussion of the immune plasticity of MSCs and the associated impact on the design of MSC-based preclinical and clinical studies. He emphasized that human and rodent MSCs differ in their respective immune properties and urged caution when attempting to translate findings in murine models to clinical settings, such as graft-versus-host disease and Crohn disease (79, 80). Furthermore, although MSCs are generally viewed as nonimmunogenic, “immunoprivileged” cells with immunosuppressive and antiinflammatory properties suitable for use in allogeneic cell therapy, there is evidence that MSCs can function under certain circumstances as antigen-presenting cells and effectively

promote alloimmunization-type responses and even lead to loss of tolerance to self-antigen (79, 81–87). Because these observations have serious implications for the use of MSCs in clinical cell-based therapeutic regimens, Dr. Galipeau suggested that further consideration be given to strategies using autologous human MSCs in clinical trials. However, loss of antiinflammatory and/or immunosuppressive functional activity in stored (e.g., cryopreserved) human MSCs represents a potential hurdle of major significance in the development of autologous MSC therapy. Data were presented demonstrating that it may take 24 hours or longer after thawing for cells to regain full function.

Dr. Donald Phinney (Scripps Research Institute, Jupiter, FL) expanded on the implications of functional heterogeneity in MSCs for clinical therapies in his presentation. He and his colleagues have developed high throughput assays to evaluate and quantify the trilineage differentiation potential of human MSCs at the clonal level (88). These assays demonstrated that human MSCs represent a heterogeneous population of clonal progenitors with varying potency and biological/physiological properties. Functional studies revealed clonal variation in growth and survival rates, which correlated with differences in the expression of TWIST, TWIST2, and BMI1 (88, 89).

In a severe combined immunodeficiency mouse model of ALI, Phinney and colleagues have observed that secretion of IL-1 receptor antagonist (IL-1RA) plays an important role in the ability of MSCs to improve outcome (89). This antiinflammatory activity was suppressed by pretreatment of MSCs with FGF2, which suppressed expression of TWIST. These findings suggest a critical role for TWIST in the regulation of multipotent clonal expansion and antiinflammatory activity in MSCs. Dr. Phinney emphasized that variable biologic capacities in MSCs can have serious consequences for studies of their potential for the treatment of disease states. The biological properties and behavior of MSCs are influenced by media supplements used in the cultivation of MSCs, level of expansion of MSCs, and the initial plating density of MSCs. As of July 2011, more than 180 trials using MSCs have been completed or are underway. Variable, and sometimes inconsistent, degrees of

therapeutic efficacy have been reported. The selection of specific clones during isolation, expansion, and cultivation *in vitro* can critically affect subsequent functions of MSCs in both preclinical studies and clinical trials.

Dr. Michael Matthay (University of California—San Francisco, San Francisco, CA) discussed studies performed by his group investigating the potential therapeutic use of allogeneic human MSCs (hMSCs) for the treatment of ALI. In a murine model of experimental gram-negative pneumonia, hMSCs administered either intravenously or intratracheally reduced the severity of ALI (including modulation of proinflammatory responses and restoration of normal lung fluid balance), and improved survival (90). MSCs also enhanced the phagocytic activity of resident monocytes/macrophages, resulting in enhanced bacterial clearance *in vivo* consistent with findings recently reported in experimental sepsis (91, 92). Another mechanism contributing to hMSC-mediated enhancement of bacterial clearance is the release of soluble antimicrobial peptides, including LL-37 (cathelicin B and lipocalin-2) by MSCs (93, 94). In a study of *ex vivo* perfused lung, intravenous hMSCs reduced lung endothelial permeability changes induced by either LPS or live *Escherichia coli*, primarily via antiinflammatory and tissue reparative paracrine mechanisms involving the release of hMSC-derived factors, including keratinocyte growth factor, angiopoietin-1, and the aforementioned antimicrobial peptides (93, 95). Thus, multiple pathways are likely responsible for the observed hMSC-mediated treatment effects in ALI and sepsis, including pathways that reduce inflammation and tissue injury as well as pathways that enhance repair of damaged endothelium and lung epithelium.

In collaboration with Dr. Dan Traber (University of Texas—Galveston, Galveston, TX), Dr. Matthay and colleagues are currently completing evaluation of hMSCs in a large animal model of ALI in sheep. These studies involve measurement of pulmonary and systemic hemodynamics and respiratory function in preparation for a clinical trial testing clinical grade cryopreserved allogeneic hMSCs (supplied by the NHLBI-sponsored Production Assistance for Cell Therapies [PACT] Program at the University of Minnesota)

for the treatment of severe ALI/ARDS. This planned clinical trial would focus on the safety and respiratory efficacy endpoints, such as oxygenation and pulmonary dead space fraction.

Next, Dr. Sam Janes (University College London, London, UK) presented work from his laboratory investigating the use of MSCs to deliver gene therapies for the treatment of cancer involving the lung. Dr. Janes presented data showing that lentiviral vectors can be used to transduce MSCs effectively and safely. Using this system, he discussed studies using MSCs engineered to express the proapoptotic molecule TRAIL in an inducible manner using the TET-ON regulation system to kill cancer cells both *in vitro* and *in vivo* in a mouse model of metastatic lung disease (96–98). Further discussion focused on the regulatory hurdles and advancements needed to translate this novel type of antitumor therapy in the clinical setting (96).

Dr. Meagan Goodwin and Dr. Daniel Weiss (University of Vermont, Burlington, VT) addressed the use of MSCs for the treatment of obstructive airway diseases in the next portion of this session. Specifically, the reported beneficial immunomodulatory effects of MSC therapy were discussed in the context of rodent models of asthma, bronchiolitis obliterans (BO), and COPD (99–122). A small clinical trial (10 patients) investigating the potential of MSCs, collected from related or unrelated HLA-identical or HLA-mismatched donors, to treat BO after lung transplantation is ongoing at Prince Charles Hospital, Perth, Australia. A clinical trial of autologous MSCs in COPD has been initiated at the University of Leiden (The Netherlands) examining the effect of administration of autologous MSCs before lung volume reduction surgery for severe pulmonary emphysema. Finally, Dr. Weiss served as an investigator on a phase II, multicenter, double-blind, placebo-controlled, industry-sponsored (Osiris Therapeutics, Inc.) clinical trial of allogeneic MSCs to treat moderate to severe COPD. This recently completed study randomized 62 patients from six sites to MSC therapy versus placebo. The patients received a total of four intravenous infusions over the course of 4 months and were followed for 2 years after the first infusion (4). The data are currently being analyzed, but Dr. Weiss reported that no

safety concerns arose during the course of the trial. He concluded that obstructive airway diseases are viable targets for MSC-based therapeutics. In addition, he emphasized that mechanistic studies with proper controls are essential in both preclinical and clinical studies to further advance the field.

The next speakers in this session were Dr. Mervin Yoder (Indiana University School of Medicine, Indianapolis, IN) and Dr. Bernard Thebaud (University of Alberta, Edmonton, AB, Canada), who spoke on the topic of human EPCs. Dr. Yoder focused on the controversies surrounding the definition, biological properties, and functions of EPCs (123–125). The term EPC was first used more than 15 years ago to refer to circulating cells with endothelial cell-like characteristics and surface markers in the human bloodstream that could augment blood flow to sites of experimental ischemia in rodents. Subsequent studies have defined a number of subtypes of circulating EPCs in normal human subjects and in individuals with a variety of clinical disorders. Most investigators attribute the hematopoietic component of the bone marrow as the origin of EPCs. It appears as though the vast majority of EPCs represent cells of the hematopoietic lineage, but most current evidence indicates that EPCs do not differentiate into functional endothelial cells. Rather than serving as endothelial cell progenitors *per se*, EPCs exhibit proangiogenic activity and function to promote vascular regeneration. Dr. Yoder suggested that a number of alternative designations better define the vast majority of cells classified in the literature as EPCs. More accurate, alternative terms for EPCs include circulating progenitor cells, circulating angiogenic cells, circulating proangiogenic hematopoietic stem and progenitor cells, circulating myeloid cells, and Tie2-expressing macrophages. Dr. Yoder presented new evidence that a very rare circulating endothelial colony forming cell (ECFC) does exist with clonal proliferative potential, cell surface antigens characteristic of mature endothelial cells but not blood cells, and capacity to form capillary lumens *in vitro* and blood vessel formation *in vivo*. He concluded his talk with recent data demonstrating that ECFCs form human vessels *in vivo* when injected into immunodeficient mice. The physiological properties of the ECFC-

derived capillary plexus were shown to be modulated by ECM, mural cells, and Notch signaling.

Dr. Thebaud discussed the potential use of ECFCs to treat bronchopulmonary dysplasia (BPD) in newborns (126–134). He presented evidence that ECFCs exist in the developing lung and that ECFC function is impaired in experimental O₂-induced BPD. Furthermore, human umbilical cord-derived ECFCs were sown to lung structure and function and decrease pulmonary hypertension in experimental O₂-induced BPD. He concluded that ECFCs represent a promising novel treatment strategy for clinical BPD and related disorders characterized by arrested alveolar development and pulmonary hypertension.

This session concluded with a talk presented by Dr. Duncan Stewart (Ottawa Hospital Research Institute—University of Ottawa, Ottawa, ON, Canada), who presented an update on EPC-based therapies for pulmonary arterial hypertension (PAH) (135). In preclinical studies, administration of syngeneic EPCs can prevent the development of experimental PAH in the rat. More importantly, EPCs transfected with endothelial NO-synthase (eNOS) were able to reverse established PAH and prolong survival (136). Dr. Stewart is currently directing a “first-in-human” dose escalation study, the Pulmonary Hypertension and Cell Therapy (PHACeT) trial, investigating the safety of eNOS-transfected autologous EPCs in patients with severe, refractory PAH. Although enrollment in this single-center trial has proven challenging, preliminary data suggest potential improvements in functional and hemodynamic parameters in treated patients. These improvements appear to be related to NO, the eNOS transgene product. These promising results have spurred planning for an upcoming, rigorously designed randomized, blinded phase II clinical trial in patients with PAH to be performed in multiple centers across North America (PHACeT-2).

Session 6: Perspectives from the NHLBI, FDA, and Respiratory Disease Foundations: Summation and Directions

Dr. Carol Blaisdell, National Heart, Lung and Blood Institute. “Building the Foundation for Lung Regeneration—

NHLBI Perspective” described the NHLBI-supported science in lung progenitor/stem cell biology, which consisted of less than a handful of projects in 2001 and has grown to nearly 70 grants in fiscal year 2010. The program is examining several aspects of lung stem cells, such as the pluripotency of lung resident cells during development and after lung injury, the impact of mesenchymal stromal cells in preclinical studies of lung repair, and the potential of decellularized scaffolds for lung regeneration. Clinical research in lung stem cell therapies along with the knowledge gained by understanding the basic biology of the lung stem cell populations will be needed to translate to diagnostic and therapeutic strategies in the future.

Dr. Amy I. Farber, Ph.D., LAM Treatment Alliance. “Return on Investment: A Case Study in Driving Science Focused on Patient Impact” described that lymphangioleiomyomatosis (LAM) is a multisystem disease predominantly affecting women of childbearing age. LAM occurs as a sporadic disorder or in association with tuberous sclerosis complex (TSC), a genetic disease affecting multiple organs including the lungs, kidneys, skin, eyes, and brain. TSC and LAM have been associated in some cases with mutations in the TSC1 or TSC2 tumor suppressor genes. Pulmonary LAM is likely more sex-specific than breast cancer. LAM is frequently fatal due to respiratory failure caused by worsening airflow obstruction.

The LAM Treatment Alliance is the largest LAM research organization in the world, driving an aggressively funded, high-impact research strategy and fostering innovative patient partnerships to effectively treat and eradicate LAM in time for patients living with the disease today. The Alliance is hopeful that collaborations with those working in the stem cell area will provide insights into key discovery and engineering efforts focused on overcoming hurdles and answering questions regarding the cell of origin, challenges to isolating, growing, and analyzing LAM cells, and tissue- and cell-based therapeutics.

Dr. Daniel M. Rose, M.D., CEO, Pulmonary Fibrosis Foundation (PFF), described that the PFF was founded in September 2000, with missions of finding a cure for IPF, advocating for the pulmonary

fibrosis community, promoting disease awareness, and providing a compassionate environment for patients and their families. IPF is a chronic diffuse interstitial lung disease of unknown etiology characterized by inflammation and fibrosis of the lung parenchyma. The disease is probably underdiagnosed and underreported. In the United States, the prevalence has been estimated at 56 cases per 100,000 individuals, with a slightly higher incidence among men. The mortality rate at 5 years is estimated to be 50 to 70%. The diagnosis can be made with high-resolution computed tomography scanning or surgical lung biopsy. Biopsy specimens from individuals with IPF typically will show the histological appearance of usual interstitial pneumonia. It is hypothesized that the disease is associated with active alveolar injury and abnormal wound healing. Presently there are no effective pharmacological therapies.

Dr. Donald W. Fink, Jr., Ph.D., Center of Biologics Evaluation and Research, FDA. “FDA Perspective: Stem Cell-Based Products and Lung Disease” described the expectation that cellular biologic therapies consisting of or derived from ESCs, fetal stem cells, adult stem cells, or iPSCs (stem cell-based products) will provide effective treatments for a variety of current unmet medical conditions including debilitating lung diseases continues to drive expanding research efforts. Evaluating the safety profile of an investigational stem cell-based product intended for early-phase clinical testing is the responsibility of the FDA Office of Cellular, Tissue, and Gene Therapies, which possesses more than 2 decades’ experience regulating cellular therapies, including stem cell-based products. Since conclusion of the 2009 conference, the FDA has received and reviewed numerous investigational new drug applications involving stem cell-based therapies for a number of indications, including critical limb ischemia, ischemic stroke, spinal muscular atrophy, articular cartilage defects, macular degeneration, spinal cord injury, healing of chronic wounds, and cardiac disease. Notably absent are early-phase clinical safety trials for lung disease. Recent publication of evidence for the presence of resident human lung stem cells could spur progress in this area of unmet medical need.

Dr. Adam Wanner, M.D., Alpha-1 Foundation. “Role of Venture Philanthropy in Finding New Therapeutic Solutions for Alpha-1 Antitrypsin Deficiency” described that α_1 -antitrypsin deficiency (Alpha-1) is a rare genetic disorder that can lead to potentially fatal chronic lung disease in adults and chronic liver disease in children and adults. Although currently available therapies may halt the progression of lung disease, there are no effective treatments for the liver disease, and a cure for Alpha-1 remains elusive. Academic research, to a great extent supported by the Alpha-1 Foundation, has identified novel therapeutic targets but is not in the business of developing new therapies. Big pharmaceutical companies, on the other hand, have the means of introducing new drugs and other treatments to the market but until now have been reluctant to invest in new therapeutic solutions for rare diseases.

The Alpha-1 Project, the Alpha-1 Foundation’s venture philanthropy program, is designed to accelerate the process of new drug development and ultimately develop a cure for Alpha-1. The rationale for the program is based on exciting recent research that demonstrates how the lung and liver disease of Alpha-1 come about and on the biotechnology community’s growing interest in Alpha-1 and its treatment. The Alpha-1 Project website (www.thealpha-1project.com) provides additional information on the program and how to apply for funding.

Summary and Conclusions

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

lung injury and further provide a sound scientific basis for therapeutic use of stem and cell therapies in lung diseases. ■

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Joaquin Cortiella, M.D., M.P.H.
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Thomas Gilbert, Ph.D.
David Hoganson, M.D.
Peter Lelkes, Ph.D.
Joan Nichols, Ph.D.
Laura Niklason, M.D.
Angela Panoskaltis-Mortari Ph.D.
Julia Polak, D.B.E., M.D., D.Sc.

MSCs/ESCs/Clinical Trials

Armand Keating, M.D.
W. Conrad Liles, M.D., Ph.D.
Michael Matthay, M.D.
Duncan Stewart, M.D.
Mervin Yoder, M.D.

Endogenous Progenitors/ESCs/iPS Cells

Ivan Bertoncello, Ph.D.
Carolyn Lutzko, Ph.D.
Darrell Kotton, M.D.
Carla Kim, Ph.D.
Jay Rajagopal, M.D.
Emma Rawlins, Ph.D.
Sue Reynolds, Ph.D.
Barry Stripp, Ph.D.
Rick Wetsel, Ph.D.

A list of participants, travel award winners, executive summaries of speaker

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