NHLBI

REPORT OF THE

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE'S WORKING GROUP

ON

CELL-BASED THERAPIES FOR REGENERATIVE & REPARATIVE MEDICINE:

VISION, SCOPE, AND DIRECTIONS

Pooks Hill Marriott, Bethesda, MD
May 1-2, 2002

**NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE
& REPARATIVE MEDICINE: VISION, SCOPE, AND DIRECTIONS**

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NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE & REPARATIVE MEDICINE: VISION, SCOPE, AND DIRECTIONS

Executive Summary

The National Heart, Lung, and Blood Institute (NHLBI) convened a Working Group of investigators on May 1-2, 2002 to define the scientific state-of-the-art regarding cell-based therapies, discuss the implications of that knowledge for research and medicine, and identify opportunities and obstacles to successfully exploit cell-based therapies for repairing or replacing damaged, diseased, or defective tissue with new, functional tissue. The Working Group was organized by the Institute's Cell-based Therapy Group to assist in the formulation of a strategic plan for new cell-based therapies.

The Working Group agenda was organized into three primary sessions. The first session on "Tools, Technologies and other Resources" included discussions on "Sources of Cells for Cell-based Therapies" and on "Models and Functional Assessment." The second session on "Basic Science Knowledge" included discussions on "Lessons from Developmental Biology for Cell-based Therapy" and the "Immune Aspects of Cell-based Therapy." The third session on "Clinical Applications" included discussions on "Disease Candidates for Cell-Based Therapies" and on "Clinical Applications." In the final session, participants discussed recommendations for a "Master Plan: Vision, Scope and Direction" based on the summaries from the sessions on "Tools, Knowledge, Applications" as well as the group's assessment of "Opportunities, Obstacles and Implementation Strategies."

The recommendations of the Working Group have been organized into sixteen items in three broad categories depending upon whether implementation might best be initiated on a Divisional, or NHLBI-wide level, or whether multiple Institutes of the National Institutes of Health (NIH) should be involved. The complete list of these recommendations is included in section four of this report and the complete meeting agenda is included in the appendix.

The Working Group's top recommendation is for a continued, strong basic research program in stem cell biology and cellular therapy. The key recommendations for the development of new programs are for: 1) definition of the stem cell niche both structurally and functionally; 2) identification of regeneration mechanisms at both the cellular and tissue level; 3) understanding the immunogenic response to cells intended for use as cell-based therapies; 4) the development of improved non-invasive imaging techniques to track cells in vivo; 5) an original effort promoting lung stem cell research; 6) and new research on the cardiomyognic potential of stem cells. In addition, the Working Group recommended continuation of programs supporting Bioengineering Research Partnerships and Tissue Engineering grants along with new and continued support of training programs for the isolation, culture and use of stem cells. Furthermore, the Group recommended the NHLBI continue to sponsor stem cell meetings for the purpose of building an interdisciplinary community of investigators to study stem cells and cell-based therapies.

The area of embryonic stem cells and their therapeutic potential was addressed. The Working Group supported this research area and proposed an embryonic stem cell data workshop to standardize data collection and facilitate data comparisons among laboratories. A greater

availability of human embryonic stem cell lines to the scientific community was seen as essential. The Working Group saw the need for Institute-sponsored stem cell research centers, such as the Specialized Centers of Clinically Oriented Research (SCCORs) to encourage collaborative teams with multi-disciplinary basic and clinical investigators, including an assessment of stem cell delivery and safety.

The Working Group also recommended utilization of genomic and proteomic techniques to characterize the progression from a stem cell to adult cell types. A number of resource needs, including stem cell banks and animal models to establish treatment efficacy, were also identified.

**NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE
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Background

Recent discoveries provide new and unprecedented opportunities for the therapeutic use of stem, progenitor, and differentiated cells for new treatments of heart, lung, blood, and sleep disorders. In August 2001 the National Heart, Lung, and Blood Institute (NHLBI) formed the Cell-based Therapy Group in order to address stem cell and cell-based therapy issues, to track human embryonic stem cell applications and grants, and to formulate a strategic plan for the development of a research program leading to new cell-based therapies. The Cell-based Therapy Group is formulating an Institute-wide implementation plan integrating basic and translational research programs to support the development of new cellular therapies.

Potential cell-based therapies could involve a variety of cell sources. Bone marrow, peripheral blood, and cord blood stem cells have been used to treat serious blood disorders, malignant disease, and inherited diseases. Hematopoietic stem cells give rise to all blood cells but may also differentiate into other cell lineages. For example, the capacity of bone marrow derived cells to engraft as alveolar type 1 epithelial cells and as bronchial epithelial cells has been demonstrated.   Bone marrow and cord blood are also being used as a source of endothelial progenitor cells for exploratory treatments for cardiac and limb ischemia.

Mesenchymal stem cells from bone marrow have been demonstrated to differentiate into a variety of non-hematopoietic tissues including bone, cartilage, tendon, fat, skeletal and cardiac muscle, and early progenitors of neural cells. Interesting preliminary data suggests mesenchymal stem cells may promote hematopoietic stem cell engraftment and foster tolerance and facilitate transplantation of heart, lung, or blood tissues from unrelated donors.

Adult bone marrow cells have been reported as a source of cells for repairing various tissues, including blood vessels, heart muscle, skeletal muscle and nervous tissue. A novel multipotent adult progenitor cell isolated from bone marrow is reported to differentiate into cells associated with all three germ cell layers and the potential of these cells is just beginning to be explored. Adult skeletal muscle cells are being utilized and cultured as a potential cell source for cellular cardiomyoplasty for the treatment of heart disease. In addition, it has been speculated and there is preliminary evidence that cardiovascular and lung tissues may contain one or more progenitor or stem cells that could be induced to proliferate and repair cellular damage.

The National Institutes of Health is implementing a policy permitting federal funding of research using human embryonic stem cell lines from a registry of cell lines that meet the eligibility criteria. Embryonic stem cells can differentiate into cells associated with all three germ cell layers and have been shown to form cardiomyocytes and hematopoietic cells. The best source of cells and their potential for cell-based therapy is a key research question.

In order to plan for a working group for 2002, the Cell-based Therapy group convened a six-member teleconference on October 15, 2001. The six panel members were: 1) Dr. Ray C.J. Chiu in Division at Cardiothoracic Surgery at McGill University; 2) Dr. Cynthia Dunbar in the Hematology Branch at National Heart, Lung, and Blood Institute; 3) Dr. Loren Field in the

Department of Medicine at Indiana University; 4) Dr. Margaret Goodell in the Department of Pediatrics at Baylor College of Medicine; 5) Dr. Saul Sharkis in the Experimental Hematology Program at the Johns Hopkins Oncology Center; and 6) Dr. David Warburton in the Department of Developmental Biology at the Children's Hospital Los Angeles. They provided perspectives for developing a program in cellular therapies that assisted the Cell-based Therapy Group in formulating a framework for a working group to be convened in 2002. The panel also provided names of scientific experts, outlined roadblocks and priorities to be addressed, and cited resources missing from current programs.

Using this information, the Cell-based Therapy group outlined the agenda and convened a working group May 1-2, 2002 to define the state-of-the-art on cell-based therapies, discuss the implications of that knowledge for research and medicine, and identify opportunities and obstacles to successfully exploiting cell-based therapies for repairing or replacing damaged, diseased, or defective tissue with new, functional tissue. The meeting organizers included Mary Anne Berberich from the Division of Lung Diseases, Chris Kelley and John Fakunding from the Division of Heart and Vascular Diseases, and John Thomas from the Division of Blood Diseases and Resources.

The twenty-four scientific experts listed below were scheduled to participate in the working group, however, the meeting chair, Dr. Helen Blau, and the one session chair, Dr. Anthony Atalia, were not able to attend due to illness. The meeting was organized into four sessions: 1) Tools, Technologies and other Resources; 2) Basic Science Knowledge; 3) Clinical Applications; and 4) Master Plan: Vision, Scope and Direction. The entire agenda is included in the Appendix.

•	Anthony J. Atala	Harvard Medical School
•	Barbara E. Bierer	National Heart, Lung, and Blood Institute
•	Helen Blau	Stanford University School of Medicine
•	Ray C. J. Chiu	McGill University
•	Ronald Crystal	Cornell University
•	Francesco J. DeMayo	Baylor College of Medicine
•	Cynthia Dunbar	National Heart, Lung, and Blood Institute
•	Jonathan A. Epstein	University of Pennsylvania
•	Loren Field	Indiana University School of Medicine
•	Alan Fine	Boston University Medical Center
•	Donald W. Fink, Jr.	U. S. Food and Drug Administration/CBER
•	Meri Firpo	University of California, San Francisco
•	Brigid Hogan	Vanderbilt University School of Medicine
•	Mark Keating	Children's Hospital, Harvard University
•	Jane Lebkowski	Geron Corporation
•	Polly Matzinger	National Institute of Allergy and Infectious Diseases
•	Richard J. O'Reilly	Sloan-Kettering Institute of Cancer Research
•	Mark Pittenger	Osiris Therapeutics, Inc.
•	Thomas Quertermous	Stanford University School of Medicine
•	Saul Sharkis	Johns Hopkins Oncology Center
•	Catherine Verfaillie	University of Minnesota
•	David Warburton	Children's Hospital Los Angeles

- Michael West   Advanced Cell Technology
- Jeff Whitsett   Children's Hospital Cincinnati

**NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE
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State of the Science

Session I: Tools, Technologies and other Resources

Sources of Cells for Cell-based Therapies
Discussion panel: Sharkis, Verfaillie, West, Firpo, Pittenger, Lebkowski

Cells suitable for cell-based therapy must provide robust and persistent engraftment to repair injury or genetic disease. Stem cells must undergo tissue-specific differentiation either prior to transplantation or *in vivo* and be expandable to the scale required for clinical application. Safety considerations are paramount, including a lack of tumor formation and an inability for germ line transmission.

The advantages of embryonic stem cells as a cell source, include virtually indefinite growth and differentiation potential that encompasses all cells and tissues. Specific differentiation *in vitro* into cells with the phenotypic characteristics of cardiomyocytes, neural cells, insulin producing beta cells, and hematopoietic cells has been demonstrated. However, embryonic stem cells give rise to teratomas, suggesting the need for complete stem cell differentiation or the removal of any remaining undifferentiated embryonic stem cells from a graft. If human embryonic cell lines are used, they would be allogeneic to potential recipients. The generation of autologous embryonic stem cells would require nuclear transfer techniques.

An alternate cell source is somatic stem cells. These so-called "adult" stem cells could be derived from specific tissues including hematopoietic, mesenchymal, neuronal, and endothelial stem cells and possibly stem cells from the heart, lung and liver. If tissue-specific stem cells have the ability to acquire the fate of cell types different from the tissue of origin, termed "stem cell plasticity," this will further expand the utility of somatic stem cells for cellular therapy. To date most demonstrations of stem cell plasticity involve only a fraction of 1 percent of the cells in a tissue. For clinical application plasticity must be rigorously demonstrated to include a robust, persistent engraftment resulting in functional improvement.

Models and Functional Assessment
Discussion Panel: Field, Fine, Chiu, Keating, Whitsett

The development of models for functional assessment of cell-based therapies requires accurate stem cell tracking, evaluation of the stem cell differentiation, and the selection of physiologic endpoints or surrogate markers for assessing impact on function. Cell tracking or cell differentiation fate mapping has used genetic markers, such as Y or X chromosome markers or enzyme polymorphisms. The inherent error rate of these techniques is problematic when the cell frequency is low. An alternate method, transgene markers, are more difficult to use clinically and this method cannot be applied to banked samples. Metabolic tags such as 5-bromo-2-deoxyuridine (BrdU) labeling can be useful for cell transplantation studies but the

results are subjective and sensitivity may be an issue if the transplanted cells proliferate.

The assessment of differentiation or trans-differentiation events can use endogenous phenotypic markers, but multiple markers are needed to increase reliability. Lineage restricted reporter genes can provide an unambiguous assay if designed and performed correctly, but they are more difficult to use clinically and this method cannot be applied to banked samples. In more complex systems "nonspecific" complications may arise, such as transgene methylation.

Assessment of the physiologic consequences of the process requires consideration of the mechanism for the physiologic endpoint. A cell-based therapy could result in the development of functional new cells. Alternatively, the therapy could increase growth of a nearby tissue, increase tissue circulation, have a positive effect on connective tissue remodeling, or alter inflammatory or immune responses. That is, the cellular therapy may result in a direct cell-specific effect or may result in an indirect effect. Therapy may improve function over time or attenuate a decreasing physiological function. The pursuit of new cell-based therapies requires demonstration of long-term efficacy in a longitudinal study, not merely single time point measurements.

Session II: Basic Science Knowledge

Lessons from Developmental Biology for Cell-based Therapy
Discussion panel: Warburton, Hogan, DeMayo, Epstein, O'Reilly, Whittset

The disciplines of developmental biology and stem cell biology are closely linked. Issues at the core of developmental biology research are reemerging as important subjects relevant to therapeutic interventions utilizing stem cells. Recent discoveries in organ morphogenesis and stem cell-based organ regeneration support a cautious optimism about their successful application to heart, lung and blood disease. However, useful information about the possibilities for exploitation of human genome programs governing organogenesis, and by analogy, regeneration by stem cells, is as yet rudimentary. Therefore, the successful application of cell-based therapies to disease conditions is realistically limited by insufficient basic science knowledge of fundamental processes. Studies in flies and mice have identified only a few of the key genes that determine organogenesis. More basic research is required to comprehend the full repertoire of genomic and proteomic interactions that determine organ-specific form and function. Because much of the specificity is derived from temporo-spatial interaction between signals of mesodermal, endodermal or ectodermal origin, knowledge of these interactions is a necessary prerequisite to devising rational cell therapies for repair or regeneration.

Central to any discussion of cell-based therapies is the need for some agreement on the definition of a "stem cell" and how it differs from a "progenitor cell." A stem cell is a relatively undifferentiated cell that has the capacity for sustained self-renewal, often throughout the lifetime of an animal, as well as the potential to give rise to differentiated progeny. A multipotent "stem cell" can self renew and give rise to several different mature cell types. In the lung, a multipotent stem cell might give rise to ciliated cells, clara cells, alveolar type II cells, and other cell types. Due to complexity of structure, the lung, like the heart, may lack tissue-specific stem cells. A progenitor cell usually belongs to a transitory amplifying population of cells derived from a stem cell. It does not have the capacity for sustained, undifferentiated self renewal.

Unfortunately, studies on stem cell biology in the lung are nowhere near as advanced as in other endoderm-derived organs such as the intestine and pancreas. However, an investment in basic stem cell biology, along with continued progress in lung embryology, should alter this disparity. A major need has been identified as a "molecular lung anatomy project". The goal of this project would be to describe the lineages of all the different cell types in the lung and to identify the nature and function of genes and proteins expressed in them. This could be achieved by introducing "tags" or "reporters" into specific kinds of epithelial or mesenchymal cells and following their fate in embryonic lung, in normal adult lung, and in lung undergoing reparative growth. This approach is very versatile; marked cell populations could be isolated and their gene expression profiles determined, or the effect of changes in specific genes on cell behavior could be followed in vivo. This approach would generate searchable, annotated, internet accessible databases of expressed genes that can be compared to similar databases generated from stem and progenitor cells from other tissues, for instance, the hematopoietic system, maintained by Dr. Ihor Lemishka at Princeton.

Preliminary proof of the principle that several possibilities for tissue regeneration exist has been obtained for cardiac regeneration, either from resident stem cells and from homing of bone marrow or body fat-derived stem cells. However, the biology of both resident stem cells and tissue specific stem cell homing remains obscure. Specificity of homing mechanisms for bone marrow, fat or mesenchymal stem cells to specific tissues is a high priority pursuit and should be explored. The development of suitable gene markers to be able to identify stem cells and to follow their fate in vivo would represent a major advance and would accelerate discovery in therapeutic applications. An important lesson from developmental biology relevant to this potential is that there are alternative embryological origins of mature cardiovascular structure. For example, recent studies indicate that there are at least two early heart fields with cells capable of ultimately contributing to the myocardium. Likewise, both lateral plate mesoderm and neural crest cells are able to differentiate into smooth muscle, yet the respective progenitor cells are quite different. As in the lung, with its diverse assortment of cell types, it remains unclear if the intra- and extra-cellular signaling pathways that trigger terminal differentiation of these unrelated progenitors are identical or related. For stem cells derived from adult or embryonic tissue, the growth factor and signaling pathways governing the process of appropriate differentiation may not be conserved. Each cell type will need to be investigated in detail and premature extrapolation from one to the other should be avoided.

Studies on the basic biology of stem cells are needed. Areas to emphasize include:
1.   Develop RNA and protein expression profiles, cell-surface markers and/or monoclonal antibodies to define the stages of progression from a stem cell to a functional differentiated cell. (This may lack the 'hypothesis driven' label because of its inherently descriptive nature but it is a fundamental requirement for success.)
2.   Provide evidence for the existence of a stem cell in the lung. Determine how many different kinds of stem cells are present, i.e., one for each type of cell present.
3.   Characterize the stem cell markers. Determine how robust they are and whether they can be used to sort out the stem cells.
4.   Determine whether they respond to signals and whether these signals can be manipulated to promote proliferation of stem cells after injury.
5.   Determine and characterize the cytoplasmic, nuclear and genetic factors regulating stem cell phenotypes.
6.   Continue studies of basic mechanisms of tissue and organ patterning and regeneration.
7.   Develop small and large animal models of cell-based therapies with appropriate delivery devices and in vivo imaging capabilities.
8.   The "molecular lung anatomy project," using cre-lox technology for tagging presumptive stem cells and follow their course in lung development in transgenic mice, is equally applicable to heart and would provide for sharing of these mouse stem cell models and the data base of gene function and lineage specification generated from them.

Immune Aspects of Cell-based Therapy
Discussion panel: DeMayo, Matzinger, O'Reilly

One of the goals of investigating the role of the immune system in cell-based therapies is to understand how the immune system regulates cell function, circulating stem cell and progenitor cell repair, and transplantation. This includes how the immune system regulates pulmonary epithelial cell function, how the pulmonary tissue regulates the activity of the immune system in

the lung and how circulating stem cells may contribute to pulmonary cell repair in this milieu. Critical elements that control proliferation versus differentiation choices of resident heart, lung, blood stem cells are only beginning to be understood. This information is of paramount importance in keeping stem cell therapy under control. The use of animal models where gene expression and ablation can be controlled in a regulated fashion, in combination with genomic approaches will allow the development of such therapies. Further development and characterization of animal models mirroring human disease, for example, storage diseases, could be used to define the potential therapeutic effects of stem cell therapies at different stages of development. The basic science of physiologic and immunologic barriers to stem cell transplantation must also be defined. It will be important to determine whether cell-based therapies will enjoy immune tolerance and, if not, to develop an immune-tolerant universal donor pool of stem cells. An evaluation of the immunogenicity of embryonic stem cells and adult multi-potential stem cells compared to hematopoietic stem cells, differentiated cells in organ allografts, and dendritic cells could reveal whether embryonic stem cells have alternative mechanisms for inhibiting or evading alloresponsive T cells, such as those operating in the developing fetus. All this would be facilitated by establishing a gene activation registry of appropriate transgenic mouse model constructs. Development of better imaging techniques would assist in the characterization of phenotypes. Stem cell transfer likely involves definition of critical pairings of cell types essential to lineage specific stem cell differentiation. The influence of established, specialized cell niches permissive to in vivo development, organ specific differentiation and functional integration must be explored. It is critical to bear in mind that immediate clinical translation will likely be impossible or dangerous without more basic knowledge of the fundamental processes involved in the immune aspects of cell-based therapy and regenerative medicine.

Session III: Clinical Applications

In considering clinical applications for cell-based therapies, we need to reflect on lessons learned from the experience with gene therapy. First and foremost will be adequate characterization of cell preparations that are to be utilized for cell-based therapies. It is necessary that adequate characterization of cells has taken place to be able to understand and follow the fate of cells. In terms of control issues, there is concern that utilizing cells, particularly stem cells, may have unintended consequences, such as the development of malignancies or teratomas. In addition, with therapies utilizing cells, there is the possibility that too many cells will be introduced, that the wrong cell type will be utilized or incorporated, or that cells will either end up in the wrong place within the target tissue or organ or be distributed outside of the intended target with a possible adverse consequence. There may be instances, such as these, where it may not be beneficial to have the cell-based therapy continue or there may be a need for a means of cellular control. In this instance, there may be a consideration of utilizing suicide genes or other similar strategies to halt or limit the cell-based therapy.

Stem cell products have long been used and manipulated in various ways largely focused on depleting subsets of accompanying immune cells. However, the burgeoning field of stem cell biology has posited the stem cell as a resource for regenerating multiple cell types if manipulated properly *ex vivo*. Such manipulations involve complex culturing systems and a sophisticated understanding of the basic biology of the cells coupled to bioengineering expertise. In addition, recent advances with *in vivo* selection systems for genetically modified stem cells have raised the possibility of using *in vivo* selection to artificially amplify genetically modified cells in diseases (e.g., sickle cell disease, Cooley's anemia) where a therapeutic gene alone is expected to confer little growth or survival advantage. There is a clear need for facilities where basic investigators can transform validated hypotheses into clinical applications. Some of the preclinical tasks of a cell processing facility include the qualification and testing of reagents, scale-up of methods, high-end cell selection and culturing, development of standard operating procedures, ongoing process validation, and the provision of controlled good manufacturing practice (GMP) infrastructure.

An additional consideration is the need for controlled studies to monitor the delivery of cells and the use of appropriate controls. Is the effect of a cell-based therapy the result of the use of a specific stem cell or are similar results obtained using control cells that would not be expected to produce the same results. For example, in cell transplantation studies in the heart, the injection of skeletal muscle cells have been reported to improve function. However, the question remains, would cardiac function also be improved using non-muscle cells such as fibroblasts or hepatocytes?

Once the science has developed to the point of initiating clinical studies in cell-based therapies, there will be special concerns for patient safety and data integrity. Current regulation, policies and procedures in place for data and safety monitoring should suffice for clinical studies and trials of cell-based therapies. However, there may be a need for implementing the types of stringent controls on human subject research that apply to gene therapy protocols.

Disease Candidates for Cell-Based Therapies

Discussion Panel: Drs. Dunbar, Fine, Crystal, Keating, and Quertermous

The capacity of bone marrow derived cells to serve as precursors to differentiated cells of various organs has challenged long held views regarding the potential origin of somatic stem cells and the fixed nature of stem cell potential. Indeed, recent studies indicate that postnatal stem cells can give rise to tissue structures previously held to originate from only one of the different embryonic cell layers. In the mouse lung, collagen producing interstitial cells, and epithelial cells of the airway and alveolus have been shown to arise from marrow cell precursors after bone marrow transplantation or direct systemic injection. The relevance of these observations to normal organ function and the pathogenesis of disease remains uncertain. Regardless, these observations have suggested a therapeutic strategy in which marrow cells are employed as an alternative source of progenitor cells for the replacement of specific cell types damaged or lost during the course of disease. The relative ease of obtaining and purifying marrow cells makes this an attractive option.

Clinically, cell-based therapies may consist of cell transplantation strategies, cell mobilization and regeneration, or tissue engineering for replacement tissues and organs. In terms of therapeutic candidates for cell-based therapies, there are a number of possibilities. These include new bone marrow reconstitution strategies and possible nuclear transfer for autologous stem cell gene therapies for blood diseases such as sickle cell disease and hemoglobinopathies. In addition, cell-based therapies could be employed to establish hematopoietic chimerism to induce transplant tolerance. In terms of cardiovascular disease, cell-based therapies offer the potential to correct myocardial sinus and pacemaker dysfunction, and potentially to treat rhythm disturbances. Cell-based therapies could be utilized to treat congestive heart failure, either through increases in the functional capacity of the heart muscles or through replacement or repair of damaged or defective tissue. Cell-based therapy could be utilized to repair the heart soon after a heart attack, as a preventive measure to limit cardiac problems, such as the development of heart failure. Cell therapy for vascular bed could prevent heart attacks by targeting and repairing atherosclerotic vessels or be used as a means of stimulating new blood vessel formation and blood flow to the ischemic heart through the stimulation of angiogenesis.

The unique properties of the lung provide inherent obstacles, but also opportunities for a cell-based therapy strategy for diseases of this organ. One major obstacle is the slow turnover of lung cells. As a result, lung engraftment may require prior treatments to create "space" for exogenously administered cells. It is notable that the growing lung has heightened cell turnover, and thus, may be a more amenable therapeutic target than its adult counterpart. Another limitation is conceptual: a more complete understanding of the factors and cues that control lung cell differentiation would provide additional tools for a cell-based therapeutic strategy. The multitude of cell types that comprise the intact lung contribute to the complexity of this issue. Defining marrow cell subtypes that have the capacity to differentiate into particular lung cell lineages is another key issue.

The appropriate homing and insertion of exogenously delivered precursor cells to specific parenchyma sites require migration through vessel walls, basement membranes, and the extracellular space. Organ injury may improve engraftment efficiency, in part, by breaching the integrity of anatomical barriers, and by causing release of local inflammatory chemoattractant

substances. Hence, migration of marrow cells into an acutely injured heart or lung will likely be facilitated. Indeed, distal injury to lung or heart appears to significantly enhance homing, engraftment and differentiation of marrow cells into alveolar T1 or myocardial cells in mice. The unique anatomical configuration of the lung, however, suggests the possibility of direct airway delivery in conditions that are not associated with significant inflammation or alterations in barrier function. Moreover, the lung has a very extensive capillary network and receives the entire cardiac output. Thus, the overall delivery of cells to the lung may not be limiting. Rather, targeting and migration into specific micro-anatomical compartments is likely to be a formidable limitation.

These initial observations, derived from highly rarified systems, are intriguing but will require novel approaches to ascertain their true physiological significance. As a treatment strategy, cell-based therapy holds great promise for reconstituting damaged tissue. Further, this approach may provide an alternative strategy for gene delivery. However, progress to this end will depend on: 1) the identification of cell types that require replacement in specific disease states; 2) a clearer understanding of the engraftment process; 3) and the identification of factors and conditions that augment and control engraftment. Ultimately, the efficacy of cell-based therapies for lung and cardiac diseases will need to based on improved physiological function.

Significantly more information regarding the fundamental biology of stem cells is required before rational therapies can be implemented. In this regard, early years of support may be directed at specified topics, including "stem cell niche" biology and better identification of precursor cell types that have trans-differentiation potential. Further, the development of certain key technologies would propel the field forward. A focus on new non-invasive methods of tracking cells into organs would be particularly useful. Once it becomes clear whether autologous of allogeneic sources of precursor cells are adequate and necessary for cell therapies, a better understanding of transplantation immunology as it applies to stem cells may be required.

It seems most reasonable that only after the biology of stem cells is better characterized that true translational research be supported. Moving forward with clinical protocols too early may in fact set the field back, as has occurred in the area of gene therapy. Regardless, there was a sense that certain target diseases should be identified. Severe congestive heart failure and end-stage emphysema were both thought to be reasonable starting points.

Cell-based Approaches to Drug Development, Testing, Delivery
Discussion Panel: Field, Fine, Chiu, Keating, and Whitsett

The routine availability of human cell types and tissues would have a dramatic impact on the drug discovery process. To date, most human primary cell types are available in only restricted quantities from biopsy, donation, or autopsy tissues, and have limited functional and life span in culture. In more limited supply are a variety of primary cell types from individuals with well-defined disease susceptibilities or genetic backgrounds. This paucity of human cells and tissue samples complicates the discovery of drugs to treat human disease and restricts thorough preclinical efficacy and safety profiling.

For the drug discovery process today, either biochemical or cell-based assays are used to screen for lead drug candidates. In most cases, the cells used during screening are immortalized cell lines that in some instances have been engineered to express a target molecule or reporter system. These cells are plated in multi-well formats and used to screen a few compounds to full libraries of chemical entities. Fresh tissue or primary cells are generally only used in confirmatory studies when and if it is available.

For drug metabolism and toxicity testing, candidate drugs are usually tested on immortalized cell lines representing gut epithelium, renal tubular epithelium, liver and other tissues representing target and non-target organs. In many of these cell lines, especially "hepatocyte" cell lines, many of the phenotypic and metabolic activities observed in the primary adult tissue are not maintained in tissue culture limiting the true predictive value of these tests. As a result it is often difficult to get an accurate early indication of the potential toxicity of a lead compound without elaborate animal and even human clinical studies.

A renewable source of cells representing normal and diseased tissue components would greatly facilitate target validation, drug screening, and drug metabolism and toxicity testing of drug candidates. Differentiating stem cell populations, especially pluripotent human embryonic stem cells, could fulfill this need. Embryonic cells have been derived from the inner cell mass of a blastocyst and will divide essentially indefinitely *in vitro*. Under appropriate cues, these cells will differentiate into representatives of all three germ lineages of the embryo, namely the ectoderm, mesoderm, and endoderm. To date, researchers have demonstrated the differentiation of neurons, cardiomyocytes, endothelial, hematopoietic, hepatocyte-like, and insulin-producing cells from human embryonic stem cells. Efforts are now underway in an increasing number of laboratories to define conditions which will direct differentiation to particular lineages and to use these derivatives to model and construct complex tissues.

Differentiated cells derived from human embryonic stem cells, whether as purified or mixed populations of cells, should facilitate the functionalization and validation of drug targets along with the identification of candidate drugs. For instance, development of mixtures of neural components from stem cell populations could facilitate study of synapse formation, function, and degeneration, and lead to discovery of new molecules to treat neurodegenerative diseases. Combinations of neural and muscle cells could be used to investigate processes active at neuromuscular junctions with the consequent development of new therapeutics for movement disorders and diseases. The routine availability of cardiomyocytes could permit direct screening for drugs that will maintain or improve cardiac muscle contractility and prevent apoptosis or remodeling after ischemic episodes. Development of stem cell lines containing genetic predispositions to disease could be used to derive target cells that could be used in basic research and in screening activities.

Human embryonic stem cell derivatives could also facilitate the introduction of drug metabolism and toxicity testing earlier in the drug discovery process. Use of such cells could allow the earlier detection of potentially ineffective or toxic drug candidates before even animal or human testing. The establishment of embryonic cell lines representing multiple haplotypes which could subsequently be differentiated for hepatocytes, neurons, cardiomyocytes, and other cell types for drug metabolism and toxicity testing could facilitate the determination of the potential benefits and safety issues amongst different patient populations. The continuous

availability of such human cells would facilitate creation of information databases on the performance and safety of compounds, providing a reference standard for comparison for new drug candidates.

In all of the above applications, the stem cell-derived differentiated cells could be used directly in the same assays that are used routinely today in the drug discovery process. This technology would complement other technologies that are already available or are in development to monitor gene and protein changes in cells.

The successful isolation of human stem cells from adult, fetal and embryonic sources offers the promise for developing an array of novel therapeutics. Biologic therapies comprised of stem cells are expected to be effective in treating a wide variety of medical conditions through tissue regeneration and repair as well as the targeted delivery of genetic material. Stem cell-based therapies represent a complex biological entity. Following transplantation, stem cells interact intimately with, and are influenced by the physiology of the recipient. Prior to patient administration cultured human stem cells are maintained under conditions that promote either the self-renewing expansion of undifferentiated progenitors or the acquisition of differentiated properties that are indicative of the phenotype the cells will assume. Once transplanted, incompletely differentiated populations of stem cells are impacted by additional fine-tuning that occurs as a consequence of instructions received from the microenvironment of the surrounding milieu encountered within the recipient. The intrinsic capacities for self-renewal and differentiation point simultaneously to the perceived therapeutic potential for stem cells, as well as, the challenge of assessing their safety from a regulatory perspective. Regardless of the source of derivation, assessing human stem cell safety through clinical investigation will require implementation of a comprehensive strategy. Each checkpoint in the process of developing a therapeutic product comprised of human stem cells beginning with identification and evaluation of suitable sources will require careful scrutiny. Included in a global assessment are the derivation, expansion, manipulation, and characterization human stem cell lines, as well as preclinical efficacy and toxicity testing in appropriate animal models. Being able to trace back from the cellular preparation for transplantation to the source of the founder stem cell population is critical as this allows each safety checkpoint to be connected, one to the other. Assessment and analysis of stem cell safety in the clinical setting will be vitally important to the development of efficacious stem cell therapies.

**NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE
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Recommendations

The Working Groups' recommendations have been designated into three general categories depending upon whether implementation might occur on level of an individual NHLBI Division (Division Interest), or the entire Institute (NHLBI Interest), or involve multiple Institutes of the National Institutes of Health (NIH-wide Interest). In addition, it is noted whether the recommendation is for a new program, the continued support of an ongoing program, or for both. The items are listed in priority order.

1.   **Basic research on stem cell biology (NIH-wide Interest)**. The recommendation is for a strong, <u>continued</u> emphasis on the support of basic stem cell research as the basis for future preclinical and clinical advances for new cell-based therapies.

2.   **Stem cell niche (NHLBI Interest).** The Working Group recommends <u>new</u> projects on the influence of the stem cell niche or environment on the differentiation of the cells. It is felt tools are available to structurally and functionally define the stem cell niche including stem cell-stromal cell interactions.

3.   **Cellular and tissue regeneration (NHLBI Interest**). The Working Group recommended <u>new</u> studies of natural cellular or tissue mechanisms for the regeneration of cells, tissues and organs involving resident stem, precursor, and differentiated cell types.

4.   **Immune response (NHLBI Interest**). <u>New</u> research needs to be initiated on the immunogenic potential of stem and precursor cells are recommended. This will address the question of tolerance or the development of histocompatibility for engrafted allogeneic cells.

5.   **Non-invasive new imaging techniques (NIH-wide Interest).** The Working Group unanimously indicated <u>new</u> and improved methods are needed to track cell fate in vivo for animal studies and for clinical research. The Group felt advances in these techniques would greatly accelerate the pace of research.

6.   **Advancement of new lung cell-based therapies (Division Interest).** The Working Group identified pulmonary stem and progenitor cell research as key areas in need of development. The following <u>new</u> areas were recommended by the Group to as key focus areas to accomplish this goal: (1) Studies to identifying currently unidentified lung stem cells; (2) Research on the factors that control growth and development of lung and lung vasculature; (3) Development of lung cell clonogenic assays; (4) Tools for lung functional genomics such as gene inactivation technology; (5) a lung genome project modeled after the hematopoietic stem cell database; (6) a lung resource project to generate cDNA libraries from embryonic and adult lung cells; and (7) preclinical animal models and translational studies investigating stem cell repair of lung injury.

7.   **Cardiomyogenic potential of stem cells (Division Interest).** This <u>new</u> area was of interest to the Working Group due to its clear clinical relevance. To

accelerate this area, the Group recommended preclinical research in three key areas: (1) assess of the cardiomyogenic differentiation potential of various stem cell sources; (2) determination of the extent and basis of physiologic improvement as the result of the cellular therapy; and (3) determination of the mechanism of improvement and heart repair whether due to the incorporation of cardiomyocytes or due to alternate mechanisms.

8. **Tissue engineering and bioengineering (NHLBI Interest).** The Group thought these programs were playing a key role in the development of cell-based therapies and recommended <u>continued</u> support of the Bioengineering Research Partnerships and Tissue Engineering grants.

9. **Training programs (NHLBI Interest).** The Group recommended sponsorship of both <u>new</u> training programs and the <u>continuation</u> of ongoing training programs to build interdisciplinary communities of investigators who study stem cells and cell-based therapies.

10. **Stem cell meetings and workshops (NHLBI Interest).** The Group recommended both <u>new</u> stem cell workshops and the <u>continuation</u> of regular stem cell meetings sponsored by NHLBI to foster the exchange of scientific information and to building interdisciplinary communities of stem cell investigators. As the field develops, the group recommended the NHLBI sponsor the formation of workshops to address a specific preclinical or clinical issue for the purpose of developing a consensus report on the topic.

11. **Embryonic stem cells (NHLBI Interest).** The Working Group thought the <u>new</u> area of human embryonic stem cell lines offers unique therapeutic opportunities, a novel avenue for developmental biology studies, and new opportunities for preclinical testing and pharmaceutical development.   The Group recommended six research areas be pursued to promote this new area: (1) the development of somatic cell nuclear reconditioning for the generation of non-immunogenic cells for use in transplantation; (2) the establishment of protocols for large-scale cell differentiation and isolation of cells for therapeutic use; (3) the development of gene transfer protocols for use with embryonic stem cells; (4) the development of transplantation methods to remove undifferentiated embryonic stem cells from cellular products; (5) the development of cell delivery systems for clinical use; and (6) preclinical translational studies to study the use of embryonic cells as cell-based therapies.

12. **Embryonic stem cell data workshop (NIH-wide Interest).** The Group recommended a <u>new</u> workshop be held to standardize embryonic stem cell data collection and comparisons, thus enabling the direct comparison of data among stem cell laboratories. The Group felt this should include the standardization of cell reference standards and reference techniques, such as the choice of the microarray.

13. **Centers (NHLBI Interest).** The Working Group recommended <u>new</u> or <u>continuation</u> of centers, consortiums, or Specialized Centers of Clinically Oriented Research (SCCORs) to encourage collaborative teams with multi-disciplinary basic and clinical investigators. Some members of the Group thought Centers were the best approach to achieve new regenerative therapies.

14. **Clinical studies (NHLBI Interest).** The Group recommended <u>new</u> clinical studies in four areas: (1) stem cell delivery; (2) integration of cells into the target

tissues; (3) clinical outcome of the therapy; and (4) safety of cell-based therapy. The Group thought clinical trials should not be undertaken prematurely, but at the appropriate time important initial clinical disease targets include these five areas: (1) heart failure due to myocardial infarction; (2) heart failure due to cardiomyopathies; (3) severe congestive heart failure; (4) end stage emphysema; and (5) obstructive vascular diseases including atherosclerosis.

15. **From stem cell to somatic cell (NHLBI Interest).** Using genomics and proteomics for characterization of RNA and protein expression profiles, the Working Group recommended new studies to define the progression from stem cell to adult cell type, such as mature cardiac myocyte, smooth muscle, endothelial cell, pneumocyte, and blood cells. The Group felt basic developmental studies were a key to accelerating new therapies.

16. **Resource needs (NHLBI Interest).** The Group identified the following seven new research resources needed to remove potential roadblocks in the field: (1) stem cell banks to increase the availability of cells to all investigators; (2) bioinformatics and informatics network to promote sharing stem cell data, e.g., microarray analysis data; (3) central resources for major equipment, e.g., cell sorters and confocal microscopes, not available at individual laboratories; (4) a registry of transgenic mice for use in stem cell research studies; (5) core facilities for functional genomics and proteomics to increase the availability of these tools; (6) a stem cell website providing information for both investigators and patient groups; (7) availability of good manufacturing practice (GMP) grade mediators and factors needed for culture of stem cells in vitro; and (8) the development of animal models to establish efficacy and assess physiologic utility for repair, to develop delivery devices and imaging capabilities, and to develop models to mirror human diseases.

NHLBI WORKING GROUP: CELL-BASED THERAPIES FOR REGENERATIVE & REPARATIVE MEDICINE: VISION, SCOPE, AND DIRECTIONS

Pooks Hill Marriott, Bethesda, MD
May 1-2, 2002

May 1

7:45 AM	Coffee/Continental Breakfast	
8:15	**Welcome and Charge to group**: Dr. Alving, Deputy Director, NHLBI	
8:30	Current state of the science	Dr. Blau

Session 1   Tools, Technologies and other Resources   Dr. Sharkis

9:00	Sources of Cells for Cell-based Therapies	Dr. Verfaillie
9:15	Discussion Panel: Drs. West, Firpo, Pittenger, Lebkowski	
10:30	**BREAK**	
10:40	Models and Functional Assessment	Dr. Field
10:55	Discussion Panel: Drs. Fine, Chiu, Keating, Whitsett	
12:00	**WORKING LUNCH**	

Session 2   Basic Science Knowledge   Dr. Warburton

1:00 PM	Lessons from Developmental Biology for Cell-based Therapy	Dr. Hogan
1:15	Discussion Panel: Drs. De Mayo, Warburton, Epstein	
2:20	**BREAK**	
2:30	Immune Aspects of Cell-based Therapy	Dr. Matzinger
2:45	Discussion Panel: Drs. De Mayo, Bierer, O'Reilly	
3:50	**BREAK**	

Session 3   Clinical Applications: **Dr. Crystal**

4:00	Disease Candidates for Cell-Based Therapies
4:15	Discussion Panel: Drs. Dunbar, Fine, Crystal, Keating, Quertermous
530	**ADJOURN DAY 1**

May 2

	Session 3 Clinical Applications (cont.)	Dr. Crystal

7:45 AM	**Coffee/Continental Breakfast**
8:15	Cell-based Approaches to Drug Development, Testing, Delivery__Dr. Lebkowski
8:40	Discussion Panel: Drs. Crystal, Fink, Pittenger
9:40	**BREAK**

	Session 4 Master Plan: Vision, Scope and Direction	Dr. Blau

10:00	**Session Summaries**: Tools, Knowledge, Applications Discussion Panel: Dr. Sharkis, Hogan, Crystal
11:00	Group Discussion: Opportunities, Obstacles, Implementation Strategies Speakers and Invited Discussants
12:00	**WORKING LUNCH**: Recommendations for NHLBI programs in cell-based therapies
1:00	**MEETING ADJOURNED**