Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100050893

6. **Project Number and Title of Research Project**: 6 - *Human Lung Progenitor Cells for Pulmonary Tissue Engineering and Regenerative Medicine*

7. **Start and End Date of Research Project:** 1/1/2010 - 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Peter Lelkes, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 112,152

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Wilf, Rona	Coop student	100	$ 13,882
Stabler, Collin	Graduate Student	100	$ 29,000

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Lelkes, Peter	Professor	5
Qvavadze, Teah	Technician	20
Schulman, Edward	Professor	5

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:___) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No_____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

PI moved to Temple University.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____x_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	1
Black				
Asian				
Other				
Unknown				
Total			**1**	**1**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____x_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes_____ No_____x_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes_____ No____x_____

    If yes, please describe the collaborations:

    16(B) Did the research project result in commercial development of any research products?

    Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Title and Purpose

Human Lung Progenitor Cells for Pulmonary Tissue Engineering and Regenerative Medicine - The purpose of this interdisciplinary research is to isolate and study human lung-specific somatic progenitor cells. These cells can be used to engineer high-fidelity distal pulmonary constructs (alveolar forming units, AFUs) for studying basic mechanisms of lung development and ultimately, for treating currently incurable lung diseases, such as in newborns (e.g., pulmonary hypoplasias) and/or in adults, (e.g., chronic obstructive pulmonary disease (COPD), such as emphysema). Moreover these AFUs can also lead to a commercializable tissue-engineered product for use as models in accelerated drug discovery (e.g., improved anti-asthmatics), in understanding common lung infections (e.g., pneumonia/tuberculosis), or in toxicity studies (e.g., asbestos/vapor inhalation).

Project Overview

Project goals were to be achieved through three specific aims:

Aim1: *To isolate and characterize human lung progenitor cells (hLPCs) derived from distal airways/alveoli.* Using techniques established in non-human mammalian lung, we will isolate, culture and characterize hLPCs from freeze-thawed fragments of human distal lungs, obtained from anonymous donors from the Tissue Bank.

Aim 2: *To develop an optimized regimen for the directed differentiation of hLPCs into diverse distal pulmonary alveolar cells.* The main goal of Aim 2 is to develop an optimized regimen for the directed differentiation of hLPCs into diverse distal pulmonary alveolar cells, specifically AE2 cells and endothelial cells (ECs) and other mesenchymal cells.

Aim 3*: To assess assembly of hLPC into "alveolar forming units" upon seeding into tissue-engineered permissive matrices*. In this aim, we will explore the functional assembly of hLPC derivatives into vascularized, surfactant synthesizing "alveolar forming units," similar to our prior work with fetal murine lung cells as a model system.

In this project we will synergistically leverage the unique expertise of two laboratories in an effort to propel Drexel to the apex of personalized lung tissue engineering and regenerative medicine. Moreover the availability of tissue samples (biopsies) from individual patients opens the realm of "personalized" regenerative medicine, the possibility to study patient-centered lung development in health and disease and/or producing autologous lung cells for transplantation back into the patient.

Summary of Research Completed:

Currently there is no consensus in the literature about the existence of an adult stem cell located in the lung that can differentiate into both conducting airway epithelium and alveolar epithelium. There have been reports of whole lungs from mice, rats, and sheep being digested and finding a cell deemed to be an "adult stem cell". The problem with these reports is that most of the papers only show that the cells they have isolated differentiate into conducting airway epithelium, or

express markers of such epithelium. Hence, they have isolated a cell that should be called a "Conducting Airway Epithelium Progenitor".

In order to identify adult stem cells, located in lung tissue, which could differentiate into alveolar epithelium, we began our studies by attempting to replicate the work of others. After hiring a graduate PhD student, Mr. Collin Stabler, for this project, our initial goal was to teach him how to digest rodent and human lung tissue in order to obtain the highest yield of general lung cell populations and then to focus on the special cell population that might harbor the "adult" alveolar progenitor/stem cells. Following a careful literature survey and trying out, with mixed success, some of the diverse, sometimes opposing protocols, we finally decided to develop our own methodology, by combining several individual methods in order to make a "workable" protocol. For the sake of availability all initial studies were carried out with adult rat lung. Since there is no consensus in the literature as to what is the best method to digest lung tissue, we went through a fair number of optimization steps (e.g., varying the concentration of enzymes, types of enzymes, as well as the digestion times). At this time, we now have in our hands a reproducible protocol that yields large number of mixed populations of primary adult rat lung cell isolates.

Details of our protocol were as follows:

1.) Intact rat lungs, preferable with intact vascular pedicles are harvested, washed in PBS, and transported in PBS + Pen/Stre to maintain sterility.
2.) The trachea is discarded, and the lungs are finely minced in PBS + Pen/Strep on top of ice for ~10-15min.
3.) The resultant tissue fragments are spun at 200rpm for 5min, and then re-suspended in fresh enzyme solution (1 U/ml Collagenase + 10 mg/ml Elastase), incubated in a shaking water bath and periodically triturated at 37oC for up to 45min.
4.) At various time points of enzymatic digestion (5, 10, 15, 30 and 45 min) the cell suspension is strained through a 70um cell strainer to collect "liberated" cells at various phases of enzymatic digestion. The remaining tissue fragments are then re-suspended in fresh enzyme solution. The supernatant from each of the fractions is strained through a 40um cell strainer, centrifuged at 1000 rpm for 5min, and re-suspended in culture media.
5.) Each cell suspension is then differentially plated for 30 min on gelatin-coated flasks to remove rapidly adhering mesenchymal cells (fibroblasts),
6.) The non-adhered cells are collected, plated and then cultured in a humidified 5% CO2 incubator at 37°C.

Based on the morphology of the resultant cell cultures, this protocol yields different populations of constituent cells; by choosing the right cell culture conditions post isolation, we hypothesize that we can enrich these cultures for alveolar type II epithelial cells and/or the appropriate epithelial progenitor cells. The above protocol yields whole lung populations, after one week in culture, as seen in Figures 1 and 2. Specifically, we have identified several culture conditions in which, through addition or omission of serum and specific growth factors, we can selectively modulated the cellular phenotypes.

Based on microscopic/morphologic analysis, we surmise that under optimized conditions the resultant attached cells attain an epithelial phenotype, the unattached rounded cells are

reminiscent of stem/progenitor cells growing on a tissue specific feeder layer. We are currently in the process of testing our hypothesis by analyzing these cells by flow cytometry and immunocytochemistry, using lung specific markers (see below).

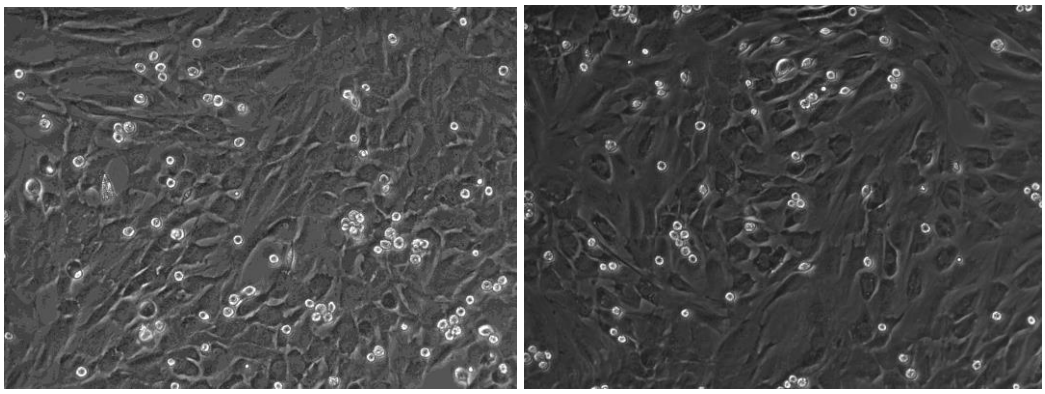

Figure 1: Primary adult rat lung cell isolates, enriched for epithelial cells and putative progenitor cells

Our PhD student, Mr. Collin Stabler developed a three-pronged approach to isolate and characterize the progenitor cells from rats, mice and humans, with the understanding that whatever can be gleaned form rodents (easier and more regularly available than human tissue) will be amenable to application to the humans system. In order to ascertain that the putative distal lung progenitor cells are indeed the elusive "Bronchio-Alveolar Stem Cells", the following modifications have been introduced in the human tissue isolation protocols:

1. Perfusion of tissue with collagenase / elastase before digestion
2. Hypotonic lysis of red blood cell after enzymatic digestion and filtering.

In the next set of experiments we used magnetic cell sorting to select for the population of interest. Most recent studies suggest that this population is characterized by being CD45-, CD31, Sca1+, CD34+. Once isolated and characterized we attempted to ascertain the alveolar epithelial phenotype will be ascertained by flow cytometry and immunofluorescent staining using as consensus markers (CCSP+, SP-C+). Through optimization of the digestion protocol we were able to isolate a heterogeneous population (Figure 2). As we were to begin immunological characterization of this heterogeneous rodent population, a pivotal, yet controversial paper was published on the isolation of human lung stem cells, which purportedly have the ability to differentiate into multiple lineages of the lung (Kajstura et al., 2011, Stripp et al., 2011, Anversa et al., 2011).

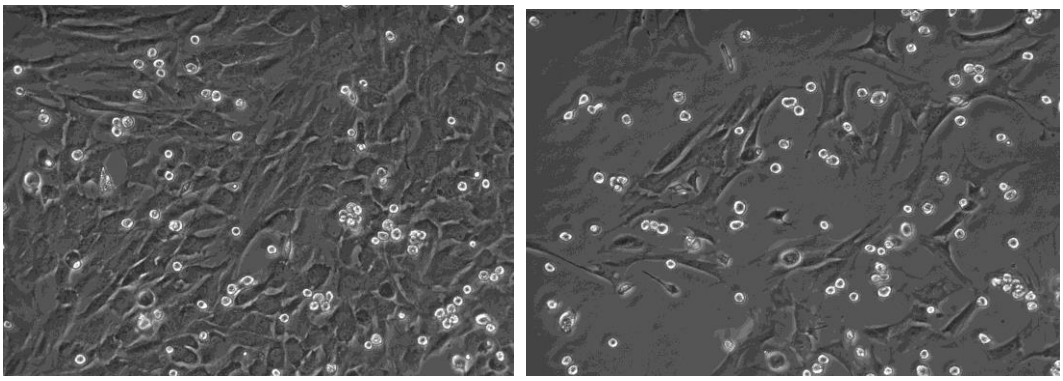

Figure 2: Rat lung cells. Both figures are at a 10X magnification (day 7 after isolation).

Based on the paper by Kajstura et al., we decided to solely focus on isolating the putative stem cells from human lungs. Through the Collaborative Human Tissue Network we have had access to a large supply of apparently healthy adult human lung tissue that can be utilized to isolate the human lung "stem cells" described in Kajstura et al. We initially used the isolation techniques developed by Dr. Ed Schulman at Drexel, which can be used to obtain many of the 40 different cell types present in the lung to isolate the CD 117^+ lung "stem cells". (The method of elutriation allowed for the separation of the isolated heterogeneous human cells into fractions based on size/mass (Figure 3). The distribution of CD117 expression that we observed would be later determined to be Mast Cells (which also have a CD117 expression profile).

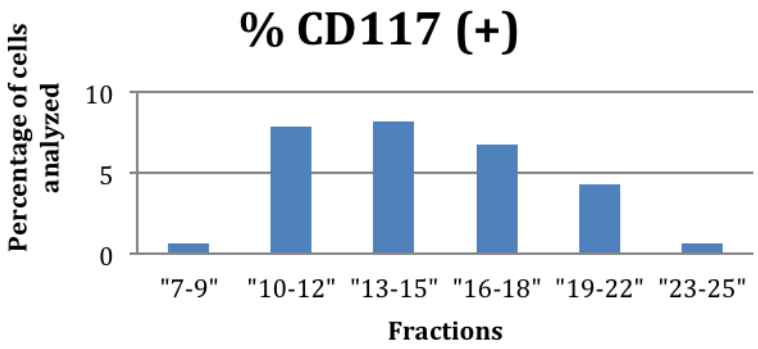

Figure 3: Expression of CD117 among elutriation fractions. Assessed by Flow Cytometry.

While this protocol yielded ample $CD117^+$ mast cells, we were unsuccessful at culturing adherent cells from these fractions for extended periods of time.

In the wake of the Kajstura paper we contacted Dr. Anversa and were given the opportunity to send Mr. Collin Stabler, to Boston in order to learn the methods for cell isolation by directly working with the researchers in the Anversa lab. The method consisted of tissue mincing, collagenase digestions, cell filtration, and culture. In faithfully and meticulously repeating the Anversa / Kajstura protocol including some non-published details communicated during Collins visit to the Aversa lab, we obtained a good cell yield upon isolation and observed extensive cell growth over extended periods of time. At 70%-80% confluent we analyzed the cells by Flow

cytometry in search of the $CD117^+$ population. Unfortunately we were unable to detect any $CD117^+$ positive cells in all the isolations that we have performed to date.

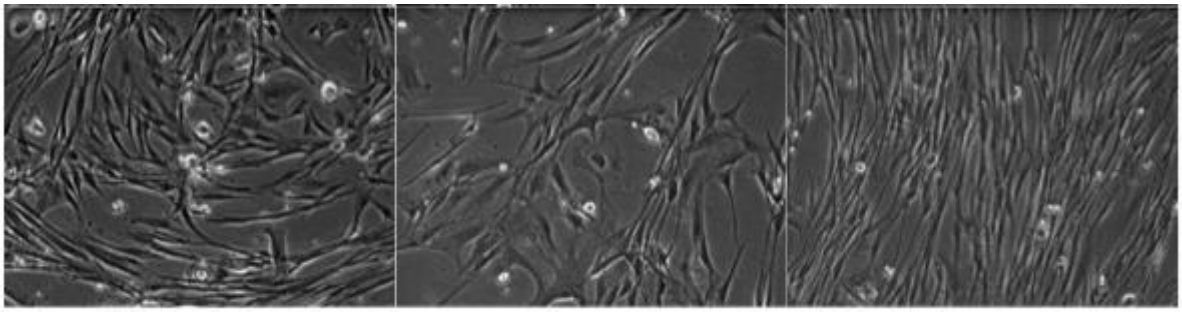

Figure 4: Phase contrast micrographs of cells isolated by Anversa technique in three different preparations (day 13 after isolation) .

At this stage, Mrs Teah Qvavadzhe, MD joined our team to try once more with more sophisticated techniques to reproduce the Anversa data. In following the Anversa protocol, we were able to isolate and culture cells from human lung, which we were not able to do previously. In our opinion the major reason for our inability to isolate $CD117^+$ stem cells from human lungs may be related to the age and health of the human lung samples at our disposal. According to Dr. Anversa's collaborators, the lungs they use are from young (infants, children) and healthy donors, whereas our samples were derived from the apparently healthy segments found in the lungs of adult lung cancer patients undergoing lung surgery.

The cells with $CD117^+$ and $CD90^-$ were sorted out from the general pool of pulmonary cells. We obtained very small cell yield after cell sorting (examples: 189 out of $6x10^6$ cells from one preparation, 405 out of $4x10^6$ cells from another preparation).

A B

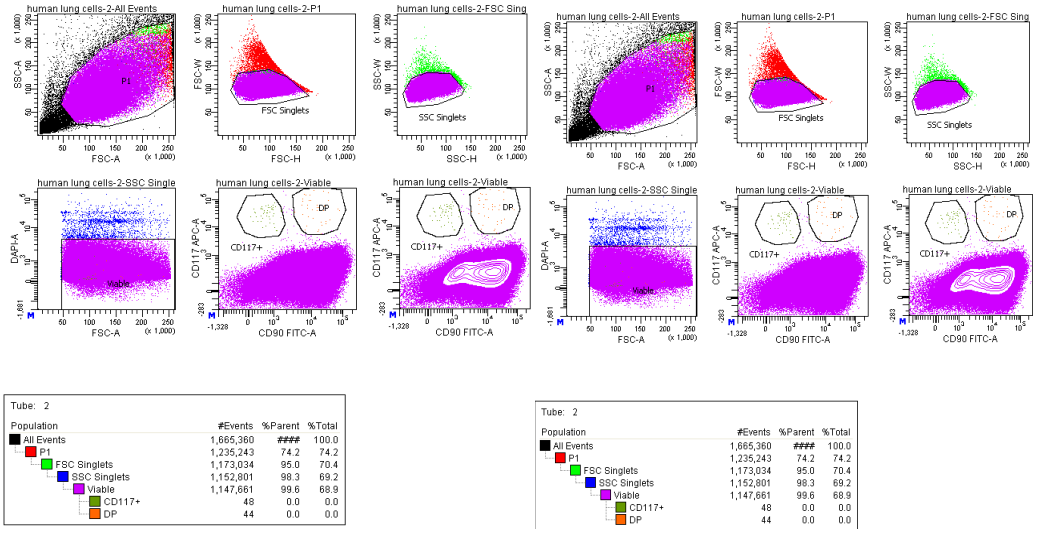

Figure 5: Human lung cell sorting analysis in two different preparations (A and B)

The sorted cells were cultured in stem cell specific culture media provided by Kajstura et al.

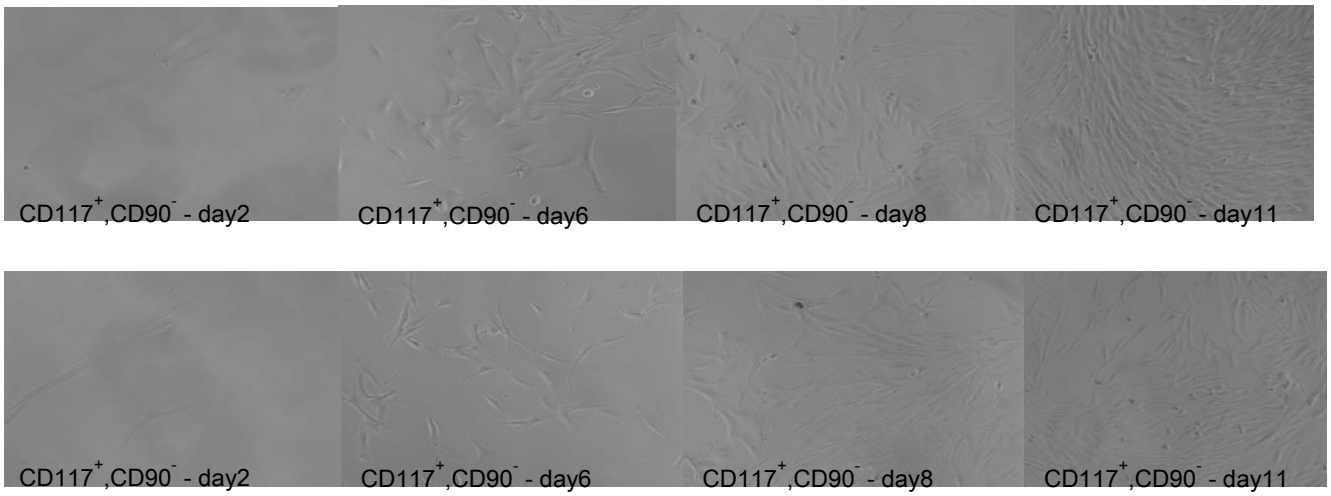

Figure 6: Phase contrast micrograph of cells sorted by Anversa technique in two different preparations (day after sorting)

Despite the very small yield, the cells survived and expanded. At 70%-80% confluence we divided them in two groups, one group was analyzed by Flow cytometry to confirm $CD117^+$ and $CD90^-$ phenotype of the cells, the other group were seeded in 96 well plate and after three days, cell culture was fixed and later stained for Immunofluorescence microscope, looking for extracellular markers such as CD117 and CD90, to confirm the presence of $CD117^+$ cells.

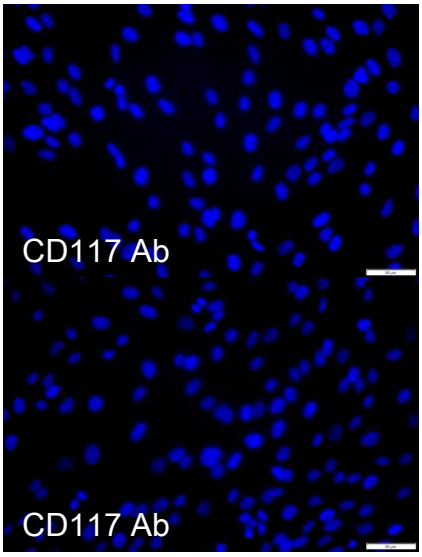

Figure 7: Immunofluorescent micrograph images of cells sorted by Anversa technique in two different preparations

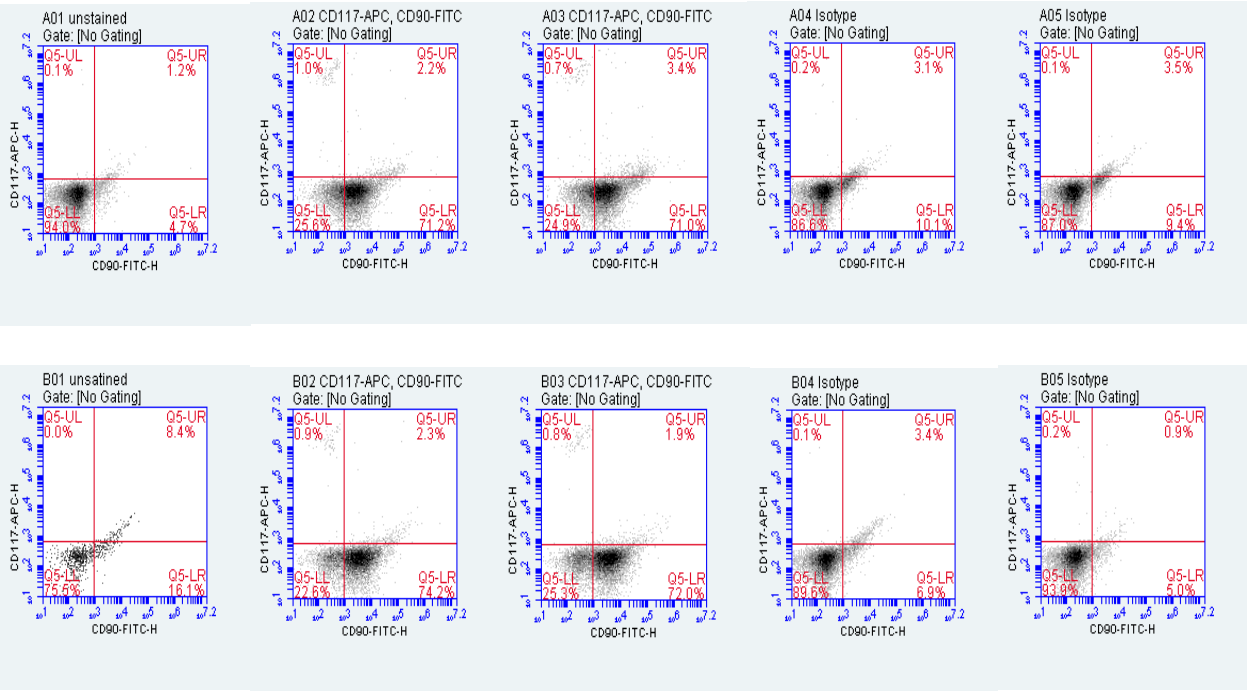

Figure 8: Flow cytometry analyses in the two different preparations

Conclusion: In following the Anversa protocol, we were able to isolate and culture cells from human lung, which we were not able to do previously. In addition we were able to sort $CD117^+$ and $CD90^-$ cells by flow cytometry and pt the cells back into culture. Despite the small yield of cells, it was possible to expand the cultures. However, upon expansion, we were no longer able to confirm presence of $CD117^+$ cells by flow cytometry and immunofluorescence microscopy. In our opinion there could be several reasons for our inability to culture $CD117^+$ stem cells from human lungs. The first one is that once put into culture, the cells could have lost $CD117^+$ expression. A second reason could be a non-specific binding during the sorting, resulting in the yield of cells not related to cells of our interest.

References:

Anversa P, Kajstura J, Leri A, Loscalzo J. Tissue-specific adult stem cells in the human lung. Nat Med. 2011;17:1038-1039.

Kajstura J, Rota M, Hall SR, Hosoda T, D'Amario D, Sanada F, Zheng H, Ogórek B, Rondon-Clavo C, Ferreira-Martins J, Matsuda A, Arranto C, Goichberg P, Giordano G, Haley KJ, Bardelli S, Rayatzadeh H, Liu X, Quaini F, Liao R, Leri A, Perrella MA, Loscalzo J, Anversa P. Evidence for human lung stem cells. N Engl J Med. 2011; 364:1795-1806.

Stripp B, Hogan B, Thannickal VJ. Lung stem cells: looking beyond the hype. Nat Med 2011; 17: 788–789.

Schulman ES, MacGlashan DW Jr, Peters SP, Schleimer RP, Newball HH, Lichtenstein LM. Human lung mast cells: purification and characterization. J Immunol 1982;129:2662-2667.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics

_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__x___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the

publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___x_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The goals of the project were not fully accomplished; however, we are not the only ones in the world who have not been able to reproduce the data from the Anversa group (Nature Medicine 17: 788, 2011)

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were

no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Peter I. LELKES	Laura H. Carnell Professor and Chair, Dept. Bioengineering and Director, Temple Institute for Regenerative Medicine and Engineering, Temple University, Philadelphia, PA
eRA COMMONS USER NAME PILELKES	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Technical University (RWTH), Aachen, Germany	MSc	1971-1974	Biophysics/Cell Biology
Technical University (RWTH), Aachen, Germany	PhD	1974-1977	Cell Biology/Biophysics
Weizmann Institute of Science, Rehovoth, Israel	postdoc	1977-1981	Cell Biology, Membrane Biophysics

A. Positions and Honors

7/01/1981	Staff Member (Scientist) - Weizmann Inst. of Sci., Dept. of Membrane Research, Rehovoth
9/01/1983	Visiting Scientist, Laboratory of Cell Biology and Genetics, NIDDK, National Institutes of Health, Bethesda, MD
7/01/1988	Associate Professor of Medicine, University of Wisconsin Medical School, Milwaukee Clinical Campus, Milwaukee, WI
7/01/1991	Associate Professor of Medicine, University of Wisconsin Medical School, Madison, WI
7/01/1993	Professor of Medicine, University of Wisconsin Medical School, Madison, WI
1997 - 1998	Forchheimer Visiting Professor, Hebrew University, Jerusalem
2000 - 2011	Calhoun Chair Professor of Cellular Tissue Engineering, School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia
2012 – to date	Laura H. Carnell Professor and Chair, Dept. Bioengineering and Director, Temple Institute for Regenerative Medicine and Engineering, Temple University, Philadelphia, PA
2004	Honorary Professor, Changchun Institute of Polymer Chemistry and Physics, Chinese Academy of Sciences
2005	Honorary Professor, University of Applied Sciences Aachen, Germany
2008	Outstanding Research Scholarship Faculty Award, Drexel University
2008	Outstanding Teach Faculty Award, School Of Biomedical Engineering, Science and Health Systems
2010	Distinguished Visiting Fellow, Imperial College London
2012	Distinguished Calhoun Scholar, Drexel School of Biomedical Engineering
2012-	Laura H. Carnell Professor and Chair, Dept. Bioengineering and Director, Temple Institute for Regenerative Medicine and Engineering, Temple Univ., Philadelphia, PA
2012	Distinguished Calhoun Scholar and Visiting Fellow, Drexel University , School of Biomedical Engineering, Philadelphia
2012	Benjamin Franklin IEEE Key Award

B. Select Peer-Reviewed Articles (2009-2013) out of a total of >175 since 1976

1. **Arien-Zakay, H., Lecht, S., Perets, A., Roszell, B., Lelkes, P.I, and Lazarovici, P**. (2009) Quantitative assessment of neuronal differentiation in three dimensional collagen gels, using enhanced Green Fluorescence Protein expressing - PC12 pheochromocytoma cells, J Mol Neurosci. 37:225-237.
2. **Jiang, J., Sun, L., Feng , H., Chen, L., Meng ,S., Feng, Z., Tao, J. and Lelkes, P.I.** (2009). Modified acellular dermal matrix for chondrocyte implantation in repairing cartilage defects of rabbits Journal of Clinical Rehabilitative Tissue Engineering Research 13: 5613-5618
3. **Roszell, B., Mondrinos, M.J., Seaton, A., Simons, D.M., Koutzaki, S.H.,. Fong, G.H., Lelkes, P.I. and Finck, C.M.** (2009) Differentiation of Embryonic Stem Cells to Definitive Endoderm and Alveolar Epithelial Type II Cells, Tissue Tissue Eng Part A. 5:3351-3365.
4. **Lecht, S., Arien-Zakay, H., Marcinkiewicz, C., Lelkes, P.I., and Lazarovici P.** (2010) Nerve growth factor-induced protection of brain capillary endothelial cells exposed to oxygen-glucose deprivation involves attenuation of Erk phosphorylation, J Mol Neurosci. 41:183–192.
5. **Lecht, S,. Foerster, C., Arien-Zakay, H., Marcinkiewicz, C., Lazarovici, P. and Lelkes, P.I.** (2010) Cardiac microvascular endothelial cells express and release nerve growth factor but not fibroblast growth factor-2, In Vitro Cellular & Developmental Biology - Animal, 46:469-76.
6. **Lecht, S., Arien-Zakay, H., Kohan, M., Lelkes, P.I., and Lazarovici, P.** (2010) Angiostatic effects of K252a, a Trk inhibitor, in murine brain capillary endothelial cells. Mol Cell Biochem. 339:201-213.
7. **Lecht, S., Arien-Zakay, H., Wagenstein, Y., Inoue, S., Marcinkiewicz, C., Lelkes, P.I,. and Lazarovici, P.** (2010) Transient signaling of Erk1/2, Akt and PLCγ induced by nerve growth factor in brain capillary endothelial cells. Vascular Pharmacology, 53:107-14.
8. **Senel-Ayaz HG, Perets A, Govindaraj M, Brookstein D, and Lelkes PI.** (2010) Textile-templated electrospun anisotropic scaffolds for tissue engineering and regenerative medicine. Conf Proc IEEE Eng Med Biol Soc. 1:255-258.
9. **Zhang Q, Mochalin VN, Neitzel I, Knoke IY, Han J, Klug CA, Zhou JG, Lelkes PI, and Gogotsi Y**. (2011) Fluorescent PLLA-nanodiamond composites for bone tissue engineering. Biomaterials. 32:87-94. Epub 2010 Sep 24.
10. **Pimton P, Sarkar S, Sheth N, Perets A, Marcinkiewicz C, Lazarovici P, and Lelkes PI.** (2011) Fibronectin-mediated upregulation of α5β1 integrin and cell adhesion during differentiation of mouse embryonic stem cells. Cell Adh Migr. 5:73-82.
11. **Han J, Lazarovici P, Pomerantz C, Chen X, Wei Y, and Lelkes PI.** (2011) Co-Electrospun Blends of PLGA, Gelatin, and Elastin as Potential Nonthrombogenic Scaffolds for Vascular Tissue Engineering. Biomacromolecules. 12:399-408.
12. **Botta GP, Reginato MJ, Reichert M, Rustgi AK, and Peter I. Lelkes, PI** (2012) Constitutive K-RasG12D Activation of ERK2 Specifically Regulates 3D Invasion of Human Pancreatic Cancer Cells via MMP-1., Mol Cancer Res. Jan 24. [Epub ahead of print].
13. **Lin, L., Perets, A., Har-el, Y., Varma., D., Li, M., Lazarovici, P., Woerdeman, D.L., and Lelkes, P.I.** (2012) Alimentary "Green" Proteins as Electrospun Scaffolds for Skin Regenerative Engineering. J Tissue Eng Regen Med. 2012 Apr 12. doi: 10.1002/term.1493. [Epub ahead of print
14. **Saglam, A., Perets, A., Canver, A.C., Li, H-L., Kollins, K., Cohen, G., Fischer, I., Lazarovici, P., and Lelkes, P.I.** (2012) Angioneural Crosstalk in Scaffolds with Oriented Microchannels for Regenerative Spinal Cord Injury Repair. J Mol Neurosci. 2012 Aug 10. [Epub ahead of print]
15. **Gincberg G, Arien-Zakay H, Lazarovici P, and Lelkes PI.** Neural stem cells: therapeutic potential for neurodegenerative diseases.Br Med Bull. 2012 Sep 17. [Epub ahead of print]
16. **Bercu MM, Arien-Zakay H, Stoler D, Lecht S, Lelkes PI, Samuel S, Or R, Nagler A, Lazarovici P, and Elchalal U.** (2012) Enhanced Survival and Neurite Network Formation of Human Umbilical Cord Blood Neuronal Progenitors in Three-Dimensional Collagen Constructs. J Mol Neurosci. 2012 Dec 12. [Epub ahead of print]
17. **Frohbergh ME, Katsman A, Botta GP, Lazarovici P, Schauer CL, Wegst UG, and Lelkes PI.** (2012) Electrospun hydroxyapatite-containing chitosan nanofibers crosslinked with genipin for bone tissue engineering. Biomaterials. Dec;33(36):9167-78.

18. **Kambach, D.M., Sodi, V.L., Lelkes, P.I., Azizkhan-Clifford, J., and Reginato, M.J. (**2013) ErbB2, FoxM1, and 14-3-3ζ Prime Breast Cancer Cells for Invasion in Response to Ionizing Radiation. Oncogene. 2013 Jan 14. doi: 10.1038/onc.2012.629. [Epub ahead of print]
19. **Botta, G.P., Reichert, M., Reginato, M.J., Heed, S., Rustgi, A.K., and Lelkes, P.I** (2013) ERK2-regulated TIMP1 Induces Hyperproliferation of K-Ras G12D-Transformed Pancreatic Ductal Cells. Neoplesia, 15:359-372.