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14. ABSTRACT

Acute lung injury (ALI) is a complex condition associated with diffuse injury to the distal alveolar epithelial gas exchange surface, resulting in marked impairment in the ability to oxygenate blood. This condition commonly afflicts patients with cancer. In this regard, cancer patients are especially susceptible to developing ALI due to the immunosuppressive and toxic effects of chemotherapy and the debilitating effects of cancer. The over-arching goal of our application is to develop ventilatory and cell based strategies to treat the ALI syndromes. In Project 1, we proposed to develop a novel mode of ventilation (variable ventilation) that will minimize the injurious effects of conventional mechanical ventilation in patients with ALI. In the past year, we have successfully developed software to allow for programming the titration of inspiratory flow and inspiratory time on the ventilator to patient comfort for variable ventilation. Most notably, we have obtained an approved IND from the FDA to proceed with the human study as originally proposed. To proceed, we plan to submit and obtain approval of the FDA's required changes in our protocol with our local institutional IRB. Once this has been achieved, we will request DoD human subjects review and approval. In the preclinical Project 2 we proposed to develop a cell based therapy for ALI. In the past year, we have successfully developed protocols for the differentiation of embryonic stem cells into distal lung alveolar epithelial cells. . These cells were found to engraft in a de-cellularized lung preparation with the morphological appearance of alveolar epithelium. In the future, we hope to utilize these derivative lung epithelial cells to treat ALI induced in mice..

15. SUBJECT TERMS

Lung injury, cancer, ventilator, stem cells

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Introduction

The over-riding goal of this application has been to develop new treatments for patients with acute lung injury syndromes (ALI). As we have previously note, ALI is the end-result of a variety of a clinical scenarios. These include: sepsis, exposure to toxins, trauma, multiple blood transfusions, and long bone fractures. Patients afflicted with cancer are particularly susceptible to developing ALI as a result of the immunosuppressive effects of chemotherapy, which leads to severe infections along with the debilitating consequences of cancer on overall well-being. While poorly understood, it is also the case that certain chemotherapeutic agents, themselves, cause a syndrome with features of ALI. The pathology of ALI is very complex and includes features of acute inflammation and distortion of lung cellular integrity. A salient feature is diffuse injury to the alveolar epithelial gas exchange surface. From a physiological point-of-view, this results in a marked impairment in the ability to oxygenate the blood. Type I cells, which comprise the vast majority of the gas exchange surface are particularly susceptible to injury.

To meet our goal, this grant has 2 Projects. In Project 1, we proposed to build upon findings from animal and *in silico* studies to determine whether we can develop a better method for mechanical ventilation of patients with ALI. This need reflects that fact that conventional methods of ventilation, themselves, appear to worsen underlying ALI by both causing further damage to lung epithelial cells and also by further amplifying inflammation. Specifically, we proposed to evaluate the efficacy of so-called *variable ventilation* in patients with ALI relative to conventional ventilation. Based on pre-clinical experiments, this ventilator mode is less injurious to the lung. In Project 2, our goal is to develop a cell based therapy with the intent of reconstituting the damaged alveolar gas exchange surface. A prominent feature of this Project is to clarify what are the most appropriate and scalable cell populations that can be used to treat and replace injured alveolar epithelium Part of defining this population involves evaluating the efficacy of this reparative cell population in treating mice with ALI. In this report, I will discuss the progress and achievements for both of these 2 Projects.

Body of Progress Report

Below is a summary of the progress and achievements for the 2 Projects that comprised the original parent proposal.

Project 1

As we discussed in earlier reports and letters, there were some unforeseen delays in the initiation of the Clinical Study comparing variable ventilation to conventional ventilation in patients with acute lung injury. These delays included completing a legal agreement with the ventilator manufacturer (Covidien^R), and in overcoming several distinct software programming issues. One vexing problem has been building in a capacity to titrate has been securing approval of an IDE from the FDA for this work.

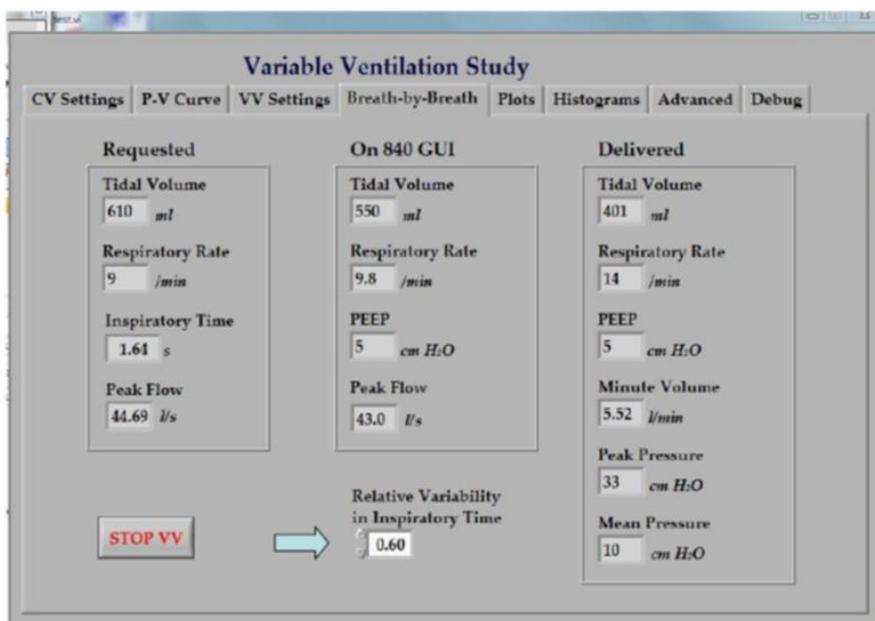


Figure 1: Window VV program (left to right) showing the parameters for the next breath to be sent to the ventilator, the parameters for the current breath, and the parameters for the last delivered breath. The blue arrow shows the control to adjust partitioning parameter.

In the past year, we have made significant headway in resolving these issues. Most notably, we have successfully developed software to allow for programming the titration of inspiratory flow and inspiratory time on the ventilator to patient comfort (see Figure 1). In view of this and other achievements, we have obtained an approved IDE (attached in *Appendix*) from the FDA to proceed with the study. As a result, we hope to proceed this year with the first-in-human trial of variable ventilation for treatment of acute lung injury in humans. We fully recognize that for this to occur, we will need to submit and obtain approval of the FDA's required changes in our protocol with our local institutional IRB. Once this has been achieved, we will request DoD human subjects review and approval.

After obtaining these approvals, we will commence with the first-in-human trial of variable ventilation in acute lung injury. We fully expect to be able to recruit sufficient numbers of

patients over the next year for this exciting study to ensure a meaningful analysis and comparison of variable to conventional ventilation in acute lung injury in humans. It is important to note that we have secured a supplemental grant for approximately \$100,000 from the Coulter Foundation. This reflects our success in developing this alternative means of ventilation and our resolution of technical issues that have prevented us from proceeding with the clinical trial. These newly secured supplemental grant funds will be used specifically to support and ensure completion of this proposed human study, which will be performed on patients in the Boston Medical Center.

Project 2: We will establish a pre-clinical program conducted in laboratory mice with the objective of developing cell-based treatments for ALI.

The long-term goal of this project is to develop a cell-based therapy that can replace and reconstitute the injured lung epithelium. It would be preferable that such a therapy employ autologous cells so as to obviate immune issues that might arise during administration of exogenous therapeutic cells. As discussed previously, we developed a mouse that has a knockin GFP into the Nkx2-1 locus. This was a very labor intensive effort whose success opens up a variety of possibilities for the objectives set forth in this Project.

Expression of the NKx2-1 gene is required for both lung and thyroid epithelial development. Expression of GFP can thus serve as a surrogate marker for NKx2-1 expression. In this context, cellular expression of GFP along with demonstrating expression of lung specific genes permits us to follow and develop in vitro protocols for the differentiation of cells into alveolar epithelium.

We therefore derived embryonic stem cells (ESCs) from Nkx2-1 GFP mouse as a starting point and have been able to identify conditions that support derivation of alveolar epithelium (see Figure 2, next page).

These findings represent a major advance for the field. This work shows that mouse ESCs under specified conditions can differentiate into cells that express lung epithelial genes. This has been further examined by globally examining the transcriptome of these ESC derived lung epithelial cells in comparison to regular lung epithelium. These data indicate a clear relationship between the 2 cell populations and provide further evidence that we have successfully derived lung epithelial cells from mouse ESCs. Notably, these cells engraft in a de-cellularized rodent lung preparation in a pattern suggestive of type II cells and type I cells. This protocol can also be utilized for iPS cells.

By establishing conditions that promote lung epithelial differentiation, we may be able to design therapies that are directed at facilitating the differentiation of endogenous lung epithelial progenitors. We have furthered this work by showing that these lung epithelial derived cells can engraft in a de-cellularized rodent lung.

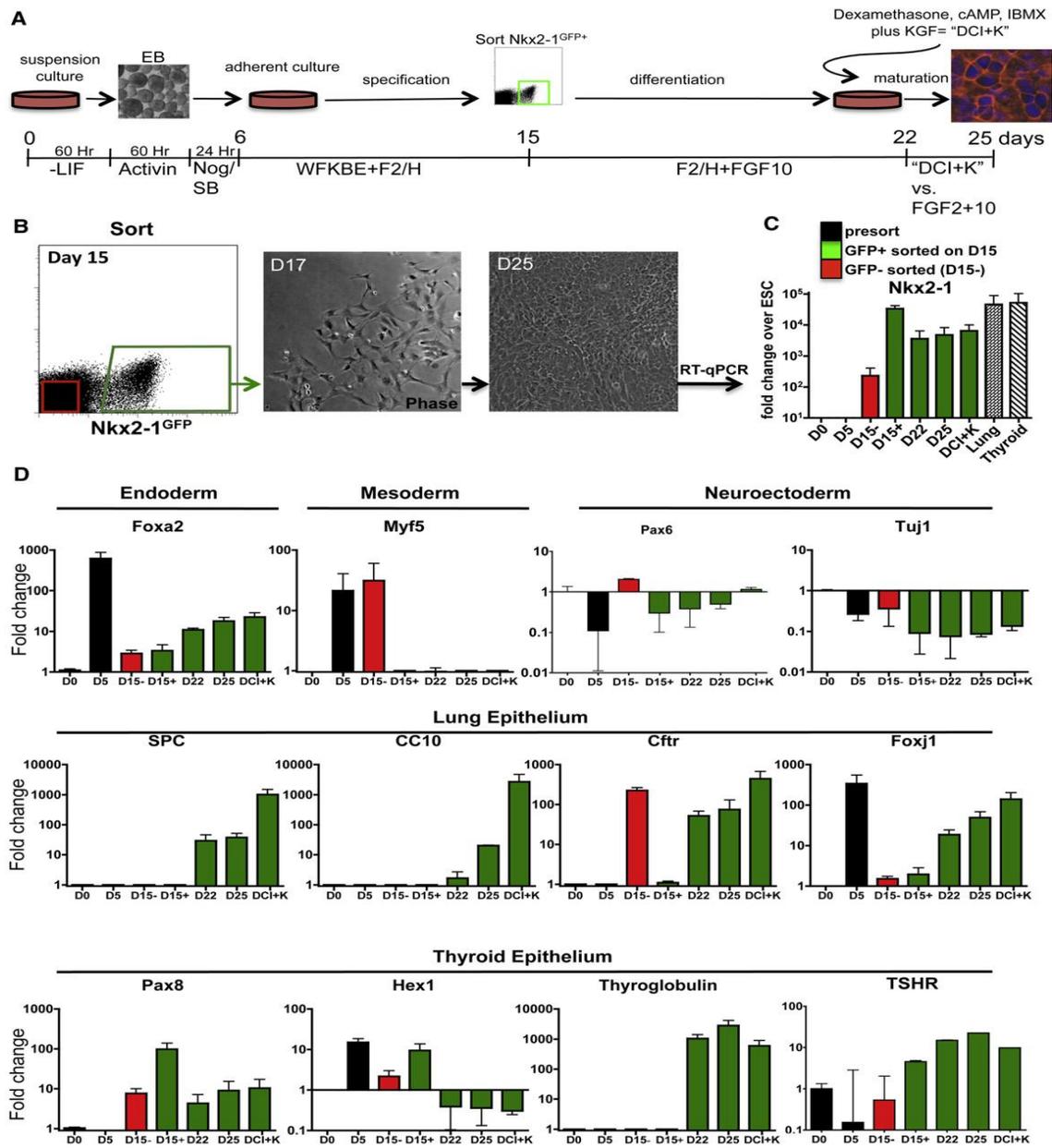


Figure 2: (A) Schematic of culture protocol for directed differentiation of ESCs into Nkx2-1GFP⁺ cells. (B) Sort gate used to purify GFP⁺ cells for replating and outgrowth. (C) Expression of Nkx2-1 mRNA and indicated marker genes (D) for each cell population, quantified by real time RT-PCR. E18.5 lung and thyroid RNA extracts served as positive controls. Bars indicate average fold change in gene expression over ESCs \pm SEM ($n = 3$ independent experiments). DCI+K = cells exposed to lung maturation media from day 22–25.

Key Research Accomplishments

- Developed software has a built in capacity to regulate inspiratory time and flow rations.
- Secured approval of the FDA IDE application
- Maintained Institutional Review Board approval of Phase I variable ventilation study.
- Developed protocols with clinical and research staff to improve compliance with the control and research arms of the study
- Obtained a supplemental grant from the Coulter foundation to support the clinical study
- Successfully developed protocols to obtain alveolar epithelial cells from mouse ESC cells
- Developed in vitro engraftment model for alveolar epithelial progenitors using de-cellularized rodent lung

Reportable Outcomes

- 1) Manuscript published on iPS-derived lung epithelial cells
- 2) Approval of IND to initiate clinical project
- 3) Development of training protocols for clinical project.
- 4) Strategic plans for patient recruitment being developed

Conclusion

We have made considerable progress in both Projects of the original parent grant. As in prior years, we have continued to optimize the software necessary for performing the clinical study proposed in Project 1. It is our belief that this has been fully completed. As a consequence we have successfully obtained an IND to initiate our clinical study. In anticipation of this study, we are training personnel to ensure that this study is performed safely and effectively. This study will commence once we have obtained the necessary IRB approvals.

In Project 2, we have been able identify conditions that facilitate the differentiation of ESC and IPS cells to lung epithelium. This is truly a major advance for the field. To help do this we have developed and employed an in vitro engraftment assay. This involves the application of iPS and ESC derived lung cells to de-cellularized rodent lungs. This assay permits the rapid and reproducible assessment of the ability derived cells to assume alveolar epithelial fates in a real lung. The next step is to inject cells into a live mouse that has ALI. These studies have also highlighted for us the identity of factors and conditions that control the differentiation of lung epithelium form progenitor cells. This information may also prove useful in the development of strategies to treat ALI.

APPENDIX

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

MAR 15 2012

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Room -WO66-G609
Silver Spring, MD 20993-0002

Boston University School of Medicine
George T. O'Connor, M.D., M.S.
Professor of Medicine
715 Albany St., Room R304
Boston, MA 02118

Re: G110202
Variable Ventilation Using the Covidien Puritan-Bennett 840 Ventilator
Dated: February 13, 2012
Received: February 15, 2012
CMS Reimbursement Category: A1 (for procedures to request re-evaluation of the categorization decision, please see the appropriate enclosure)
Annual Report Due: One Year from the Date of This Letter.

Dear Dr. O'Connor:

The Food and Drug Administration (FDA) has reviewed your investigational device exemptions (IDE) application to conduct a feasibility study for a significant risk device. A feasibility study is a preliminary study which is not expected to provide the primary support for the safety and effectiveness evaluation of a medical device for the purposes of a marketing application. Your application to conduct a feasibility clinical investigation is approved, and you may begin your investigation at Boston Medical Center, Boston, Massachusetts you have obtained institutional review board (IRB) approval and submitted certification of IRB approval to FDA. Your investigation is limited to 2 institutions and 16 subjects.

We would like to point out that FDA approval of your IDE application to conduct a clinical investigation does not imply that this investigation will provide reasonable assurance of the safety and effectiveness of your device or assure a determination of clearance/ approval for your premarket submission.

You should also give serious consideration to the following item which is considered essential for the analysis of your data for the purposes of clearance/approval of a premarket submission:

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Use of corticosteroids will diminish the inflammatory response, therefore decreasing biomarker levels. Steroid use will therefore mask biomarker levels associated with tissue injury / infection, to an extent that is unknown. If enrollment includes patients on corticosteroids, then please remove biomarkers as secondary outcome. Otherwise, if biomarkers are included, subject selection should be refined to patients not administered steroid therapy. Furthermore, please stratify patient data according to corticosteroids use.

FDA encourages sponsors to collect clinical trial data in accordance with the Guidance for Industry: Collection of Race and Ethnicity Data in Clinical Trials (<http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126396.pdf>) and to enroll patients that would reflect the demographics of the affected population with regard to age, sex, race and ethnicity. Reference is made to 21 CFR 812.25(c) regarding description of patient population and to 21 CFR 814.15(d)(1) with regard to the need for data, including foreign data, to be applicable to the U.S. population and U.S. medical practice. We recommend that you include a background discussion of prevalence, diagnosis and treatment patterns for the type of disease for which your device is intended. This should include sex- and race-specific prevalence, identification of proportions of women and minorities included in past trials for the target indication, and a discussion of your plan to address any factors identified or suggested, which may explain potential for under-representation of women and minorities, if applicable. We recommend that you include a summary of this information in your protocol and investigator training materials. Consideration should be given to enrollment of investigational sites where recruitment of needed populations for study can be more easily facilitated.

Future correspondence concerning this application should be identified as an IDE supplement referencing the IDE number above, and must be submitted in triplicate to:

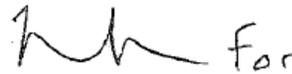
U.S. Food and Drug Administration
Center for Devices and Radiological Health
IDE Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Information to help you understand the function and duties of a sponsor, titled, "Sponsor's Responsibilities for a Significant Risk Device Investigation," is available at: <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm049859.htm>. Additionally, information which you should provide to participating investigators, titled, "Investigators' Responsibilities for a Significant Risk Device Investigation," is available at: <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm049864.htm>.

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If you have any questions, please contact Mr. Chan Lee at (301) 796-6267.

Sincerely yours,

Handwritten signature of Anthony D. Watson, appearing as "ADW for".

Anthony D. Watson, B.S., M.S., M.B.A.

Director

Division of Anesthesiology, General Hospital,

Infection Control and Dental Devices

Office of Device Evaluation

Center for Devices and

Radiological Health