# STRONG CHILDREN'S RESEARCH CENTER

***Summer 2014 Research Scholar***

**Name: Benjamin Kasper**

**School: SUNY Geneseo**

**Mentor: Dr. Gloria Pryhuber**

## ABSTRACT

Title: *Optimization of Human Lung Cell Sorting and Identification Techniques*

Background: The Molecular Atlas of Lung Development Program (Lung MAP) aims to unravel the secrets of the developing lung. The University of Rochester will serve as the Human Tissue Core (HTC). The function of the HTC is to process lung tissue received from organ procurement agencies and to provide varied lung and cell samples to four collaborating research centers. The dissociated lung tissue samples will be shipped off to research centers in a variety of ways, from heterogeneous cell mixtures to isolated cell type samples.

Objective: The goal of this project is to test and optimize lung cell sorting and identification techniques in order to develop standard operating procedures (SOPs). Experiments include mesenchymal stem cell (MSC) culture, Milltenyi MSC differentiation of cord blood and lung cell MSCs, detection of differentiated cells, magnetic column separation, fluorescence-activated cell sorting (FACS), and cytospin antibody-mediated cell identification.

Results: MSCs can be considered as such if they can be differentiated into osteoblasts, chondrocytes, and adipocytes. The Milltenyi MSC differentiation kit was successful for the cord blood differentiation. It is in progress for lung MSC differentiation; however, the osteoblasts have already been successfully stained from lung. MSCs separated by magnetic column were significantly more numerous, viable and proliferative in culture than those that passed through FACS prior to magnetic column purification. Sorted epithelial cells set on cytospin slides were probed for with lung cell specific markers CC10, AQP5, and proSP-C. First attempts at texas red conjugated secondary antibody detection of these markers were faint though visible. Additional staining optimization is required.

Conclusion:
-Milltenyi Differentiation Kit and methods for detecting differentiated mesenchymal stem cells work well for umbilical cord derived stem cells.
-Putative MSCs isolated from human pediatric lung can be differentiated toward an osteoblast and adipocyte phenotype supporting their identification as stem cells.
-FACS and magnetic column separated MSCs are less viable than those separated with only magnetic column: an important consideration for further investigation of the isolated cells.
-With future cytospin antibody detections, antigen retrieval should be considered as well as using different antibody dilutions.