

Novel approaches to scleroderma lung disease using animal models and biomarker studies**Rationale for the Proposal**

This research proposal focuses on scleroderma lung disease. **Our two complementary research objectives are to develop a novel mouse model of scleroderma lung disease, and to identify biomarkers in a scleroderma patient cohort.** Lung disease develops in most patients with scleroderma and represents a major unmet medical need. Lung disease is poorly understood and has no effective treatment. Moreover, in 20% of the patients, lung disease is progressive and associated with poor survival. In this proposal, we aim to identify risk factors that predict progressive lung disease in scleroderma, and develop a disease model in order to gain a better understanding of the underlying pathogenetic mechanisms. Our integrated clinical and laboratory scleroderma program pursues research at the cellular, molecular and genetic levels, focusing on patient-oriented approaches to discover and develop effective therapies. The proposed studies build on our research expertise and resources, including existing infrastructure and patient cohort.

Objectives

In order to accelerate progress toward improved outcomes for scleroderma lung disease, we propose the following two complementary objectives: **First**, we will use mice to establish a novel disease model that recapitulates progressive scleroderma lung disease more accurately than current animal models permit. This will provide an invaluable research tool a) for identifying the molecular basis of lung fibrosis, and b) for preclinical therapeutic studies. **Second**, we will use patient samples and clinical information to identify biomarkers, defined as soluble proteins whose levels in the circulation correlate with and can predict the presence of lung disease and its course. Currently we lack biomarkers that could be used to identify scleroderma patients are highest risk for lung disease, or provide information regarding the extent of lung disease, and its progression or regression during therapy. We propose to study multiple putative biomarkers in our well-characterized scleroderma patient cohort, and correlate biomarker levels and activity with validated metrics of lung disease activity and severity, along with other clinical parameters. In addition to their clinical utility, novel biomarkers can also provide important insights into disease pathogenesis that we will explore in subsequent research.

Methodology

Aim 1 will establish a novel mouse model for scleroderma lung disease that will be informative for pathogenesis studies and preclinical treatment development. Using normal mice, we will induce chronic lung fibrosis by injecting bleomycin directly into the skin. This approach differs from the current animal models of lung fibrosis induced by intratracheal bleomycin installation directly into the lungs, which results in an acute ARDS-like pathology unlike that of scleroderma lung disease. Subcutaneous bleomycin causes lung fibrosis via endothelial cell injury, which is congruent with scleroderma lung disease, whereas intratracheal bleomycin causes epithelial cell-driven lung fibrosis distinct from scleroderma. Our preliminary findings in a small number of mice show that the subcutaneous bleomycin model is characterized by insidious (rather than acute) lung disease that more closely mimics scleroderma lung disease than existing mouse models. Since the subcutaneous bleomycin model faithfully recapitulates the

pathogenesis, evolution and progression of scleroderma lung disease, it is a more useful and specific experimental model than currently existing models.

Aim 2 focuses on biomarkers for scleroderma lung disease. Biomarkers are serum components that can be readily measured and used in both clinical research and clinical practice to identify patients at risk for lung disease, assess disease extent, severity and activity, and monitor for progression or regression with treatment. Moreover, biomarkers are also indispensable for therapeutic trials to be successful. There are currently no validated biomarkers for scleroderma lung disease. We will measure a series of proteins and bioactive lipids in the serum, and correlate levels with clinical parameters. In initial studies we will measure proteins and lipids selected on the basis of their potential relevance to disease pathogenesis. The list includes SAA, SAP, adiponectin, SPD, KL6, chitinase, IL-1 and MCP-1, as well as scleroderma-specific autoantibodies. We will optimize appropriate ELISA and/or Multiplex assay methodologies for accurate determination of each biomarker. In initial studies, serum from healthy individuals and 50 scleroderma patients representing early and late disease, and limited and diffuse subsets, will be studied. We already have obtained institutional approval for collecting serum and clinical information from consenting patients. The levels of these biomarkers determined as above will then be correlated with indices of lung disease activity and severity, including PFT and HRCT, in a cross-sectional study design. Data will be analyzed using appropriate statistical tests, including Kruskal-Wallis, Spearman's rank correlation, and Bonferroni correction for multiple comparisons. These hypothesis-generating initial pilot studies will then be further pursued in a longitudinal design using a larger cohort of patients. More definitive studies will be pursued under a new NIH grant.

Significance and Future Directions

Results from these pilot studies will contribute to improved understanding of scleroderma lung disease, and identify and validate potential biomarkers to be studied in depth in subsequent research. The present studies will facilitate the development of targeted therapies for scleroderma lung disease. Moreover, the results will ultimately enable us to design biomarkers as tools for individualizing scleroderma therapy, and for identifying patients at risk for lung disease or for progression. Based on our results, we will plan an NIH grant application to pursue in-depth mechanistic studies and longitudinal biomarker discovery and validation studies for scleroderma lung disease in a larger cohort of up to 500 patients.

Proposed Budget

PI (oversees all aspects of project)	1% effort	\$2,100
Study Coordinator (biorepository for biomarker studies)	15% effort	\$7,000
Lab technician (performing Biomarker assays)	75% effort	\$30,000
Lab technician (mouse model validation)	50% effort	\$20,000
Fringe Benefits - 24.5%		\$14,479
Reagents, mice		\$10,000
Total Direct Costs (indirects not included in this total)		\$83,579