Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature that ultimately leads to right heart failure and death. PAH may be idiopathic (IPAH) or associated with a variety of other diseases, including scleroderma. PAH related to scleroderma (PAH-SSc) occurs in approximately 10-30% of patients with limited cutaneous scleroderma and is a leading cause of death in this population. Survival of patients with PAH-SSc is markedly worse compared to patients with IPAH or other forms of PAH, despite the advent of therapies targeting pathways involved in the putative pathogenesis of the disease. Multiple clinical trials of these novel agents have shown improvement in clinical outcome measures, including survival, six minute walk distance (6MWD), and quality of life in IPAH, but minimal improvement in PAH-SSc.

The reasons for worse survival and attenuated response to targeted therapy are unclear. A prior study from our group demonstrated a higher prevalence of non-systolic dysfunction of the left ventricle in patients with PAH-SSc; however, this difference did not explain the higher mortality seen in the PAH-SSc group. Coronary vascular disease, malabsorption of oral therapy due to gastrointestinal disease, and sub-clinical interstitial lung disease have been suggested as potential contributing factors to the attenuated response and worse survival. Structural differences of the pulmonary vasculature and the right ventricle in PAH-SSc compared to IPAH and their contribution to the worse response to therapy and poorer survival are the focus of our institution’s Specialized Center for Clinically-Oriented Research (SCCOR) grant, Molecular Determinants of Pulmonary Arterial Hypertension (P50HL084946), for which I serve as Co-Investigator. Using the infrastructure that this large, programmatic grant provides, I will study a different component of cardiac function; neurohormonal activation, as the focus of my recently-awarded Mentored Patient-Oriented Research Career Development Award from the NIH entitled, “Neurohormonal Activation in Scleroderma-Related Pulmonary Arterial Hypertension” (K23 HL092287).

RESEARCH ACTIVITIES

While the planned research activities for the proposed LRP funding period are similar to the prior funding period, the current research plan reflects on-going projects, utilizing the data obtained from studies conducted during the prior LRP funding period. The focus of my research activities continues to be on determinants of survival in PAH-SSc. Importantly, my projects examining the role of the neurohormonal axis in PAH-SSc have been funded both by the NIH (as a K23 award) and the Pulmonary Hypertension Association, thereby ensuring extra-mural resources and support during the proposed LRP funding period.

I. Molecular Determinants of Pulmonary Arterial Hypertension (P50HL084946)
The Molecular Determinants of Pulmonary Arterial Hypertension Program is an institutional SCCOR grant, headed by my primary mentor, Paul M. Hassoun, MD. The objectives of this 5-year award include development of reliable measures of right ventricular (RV) and pulmonary vasculature (PV) function, characterization of patterns of gene expression and identification of gene polymorphisms associated with susceptibility to PAH in SSc, and utilization of therapy targeting the interaction between RV and PV in SSc. I serve as co-investigator for these aims.

Project 1: Novel Imaging to Assess RV-PV function in PAH-SSc
PAH leads almost uniformly to death through right ventricular failure or sudden death. The hypothesis of the SCCOR study is that divergent response to therapy and overall worse outcome in PAH-SSc, as compared to IPAH, is related to more severe structural changes involving the PV and the RV, resulting in marked RV-PV dysfunction. Currently available outcome measures do not allow dissection or consideration of the components of the cardiovascular response, in particular RV function, PV remodeling, and the interaction of the RV and PV. We have identified significant differences in Pressure-Flow (P/F) relationship between IPAH and PAH-SSc patients during right heart catheterization and in non-invasive assessment of RV function with tricuspid annular plane systolic excursion (TAPSE) measurement obtained with transthoracic echocardiogram. PAH-SSc patients have a predominance of
Pulmonary vascular concentric and obliterator lesions in addition to the plexiform lesions observed in IPAH which may explain striking differences in P/F relationships. Similarly, inflammatory and scar lesions (detectable by MRI) involving the RV are more common in PAH-SSc than in PAH patients, and may explain differences in RV function detected by TAPSE measurement in these two groups. We hypothesize that these structural changes involving the RV and the pulmonary vasculature (PV) lead to overall severe right ventriculo-pulmonary vascular (RV-PV) dysfunction in PAH-SSc. We will test this hypothesis using a cross-sectional study design in which RV-PV function will be assessed in PAH-SSc and IPAH by P/F slope calculations via invasive hemodynamic measurements, echocardiographic (including TAPSE measurements) and Magnetic Resonance Imaging (MRI) techniques. We have begun enrollment in this trial. To date, 43 subjects have completed the protocol. Goal recruitment is 100 PAH subjects; 50 IPAH and 50 PAH-SSc. My responsibilities for this project include: oversight of subject recruitment along with collection and analysis of data from RHC, and manuscript preparation.

Project 2: Gene Expression in PAH-SSc
It is currently thought that the development of PAH requires a genetic susceptibility coupled with one or more additional factors such as a drug exposure (e.g., anorexigens) or a viral infection (e.g., HIV disease). In addition, inflammation may play a major role in the development of PAH in diseases such as systemic sclerosis. Patients with PAH-SSc are heterogeneous with respect to the development of PAH, i.e., a subset of patients develops very severe PAH; alternatively, a fair number of SSc patients with limited (about 60-70%) and systemic (over 80%) disease never develop PAH or only have mild disease. These observations are highly consistent with the likelihood that variants in specific candidate genes drive both susceptibility and severity of the phenotype in PAH-SSc. Therefore, we postulate that (a) polymorphisms in select genes alter the susceptibility of certain individuals with scleroderma toward developing PAH, and (b) these and other polymorphisms also modify severity of disease. Our studies will employ high-throughput genomic technologies to examine the patterns of gene expression in peripheral blood mononuclear cells (PBMCs) obtained from SSc patients with and without PAH, and patients afflicted with IPAH. Patterns of expression will be analyzed within each clinical condition to determine both concordantly and discordantly regulated gene clusters, linking these gene clusters with clinical outcome measures (e.g., 6MWD), and determine the gene profiles associated with the development of PAH in SSc. Our preliminary data suggest differential gene expression between IPAH and PAH-SSc and within PAH-SSc when stratified by disease severity in genes that are thought to be important in the pathogenesis of PAH. Ultimately, we will add these relevant, highly filtered genes to our candidate gene list and examine polymorphic variants of these genes in order to validate their role in susceptibility to PAH in SSc. We have collected over 750 samples of patients with SSc, PAH-SSc, and IPAH who have been well-characterized clinically by both our collaborators in Rheumatology and our pulmonary hypertension team. Gene expression analyses are ongoing; genetic variant analyses, specifically single-nucleotide polymorphisms, have been performed and are currently undergoing analysis. My responsibilities with this project include: oversight of subject recruitment, collection and analysis of demographic and clinical data, and manuscript preparation.

Project 3: A Randomized, Double-Masked, Parallel Group Study of Ambrisentan, Tadalafil, or Both in Patients with PAH-SSc
Over the past two decades, therapy for pulmonary arterial hypertension (PAH) has been targeted at the endothelin and prostacyclin pathways, which have been implicated in the remodeling of the pulmonary vasculature (PV). However, there is no evidence that these standard therapies for PAH have altered PV and/or right ventricle (RV) remodeling or offered significant beneficial effects in patients with pulmonary arterial hypertension associated with systemic sclerosis (PAH-SSc) in whom mortality remains exceedingly high (3-year survival of less than 50%). We hypothesize that improvement in this group of patients will only be achieved with therapy directly targeted at RV-PV dysfunction. Tadalafil inhibits phosphodiesterase type 5 (PDE5) which is abundant in the lung and is the main enzyme responsible for cGMP hydrolysis. The resulting increase in cGMP probably mediates the relaxant and antihypertrophic actions of nitric oxide and natriuretic peptides in vascular tissues, and exerts a direct antihypertrophic action on cardiac muscle. In addition, open-label studies suggest that PDE5 inhibitor
(PDE5I) treatment reduces pulmonary artery pressure and improves cardiac index. A recent small randomized study comparing PDE5I with the endothelin receptor antagonist bosentan indicates that PDE5I added to conventional treatment for PAH reduces RV mass and improves cardiac function and exercise capacity in patients with PAH. Animal data has shown that PDE5I prevents maladaptive cardiac hypertrophy in experimental PAH. The goal of this study is to test the hypothesis that PDE5I therapy will improve RV function in PAH-SSc patients. This will be a 32-week, randomized, double-masked, parallel group study comparing the effects of tadalafil monotherapy, bosentan monotherapy and combination therapy with tadalafil and bosentan in patients with PAH-SSc. Standard outcome measures such as six-minute walk distance (6MWD), NYHA classification, and hemodynamic measurements will be assessed, as well as novel functional measures of RV-PV function including the TAPSE and contrast-enhanced cardiac MRI. This design (excluding a placebo-placebo arm) was selected for ethical concerns and to provide optimal efficiency and active therapy to all study subjects. It also allows for comparisons between the two monotherapy groups and with combination therapy. We have received IRB approval for this study and are actively recruiting with a goal enrollment of 75 patients (25 in each group). This will be the largest study of oral therapy in patients with PAH-SSc. **My responsibilities for this project, shared with other co-investigators, include: oversight of subject recruitment, clinical assessments, collection of data, and data analysis. I will be primarily responsible for data synthesis and manuscript preparation.**

**II. Neurohormonal Activation in PAH-SSc (K23 HL092287)**

Despite a preponderance of evidence supporting a central role of neurohormonal dysfunction in heart failure due to left heart disease, little attention has been paid to its potential role in the pathophysiology of PAH and right heart failure, particularly with respect to PAH-SSc. Patients with scleroderma have underlying autonomic dysfunction that is present prior to the development of clinically-apparent pulmonary, renal, or cardiac complications. Microvascular dysfunction, evidenced by skin sclerosis and Raynaud’s phenomenon, develops prior to other clinical manifestations and may be related to aberrations in autonomic function. Esophageal dysmotility and gastroparesis are likely to result from autonomic disturbances. Conventional autonomic testing and heart rate variability testing has demonstrated impaired cardiovascular reflexes, reduced sympathetic skin responses, and increased plasma catecholamine levels in SSc patients. Further, the higher prevalence of left heart disease in PAH-SSc compared to IPAH may influence neurohormonal function as has been shown in heart failure associated with preserved left ventricular function. Dysregulation of the neurohormonal axis in PAH-SSc could potentially explain differences in response to therapy and survival between PAH-SSc and IPAH. Further, therapeutic pathways, previously found to be clinically relevant in left heart failure, may be potential targets for treatment of PAH-SSc. **We hypothesize that neurohormonal activation is up-regulated in PAH-SSc compared to IPAH and that this up-regulation contributes to the increased mortality in these patients.** Therefore, we propose a prospective cohort study of patients with PAH-SSc and IPAH to address three specific aims (Figure 1). **I will serve as Principal Investigator for this study.**

**Specific Aim 1:** To define whether activation of the neurohormonal system differs between idiopathic pulmonary arterial hypertension and scleroderma-associated pulmonary arterial hypertension as assessed by 1) serum markers including epinephrine, endothelin-1,
N-terminal pro-brain natriuretic peptide, amongst others; 2) heart rate variability by 24-Holter monitoring; and 3) NHA gene expression profiling of right ventricular tissue.

**Specific Aim 2:** To establish whether the differences in neurohormonal activation between idiopathic and scleroderma-associated pulmonary arterial hypertension are associated with the risk of hospitalization and death in these populations.

**Specific Aim 3:** To determine if polymorphisms in genes of the neurohormonal axis, including β₁-adrenergic receptor, β₂-adrenergic receptor, α₂c-adrenergic receptor, angiotensinogen, angiotensin receptor type 1, angiotensin-receptor converting enzyme, adrenomedullin, resistin, and tumor necrosis factor, differ between IPAH and PAH-SSc.

Characterization of potential differences in NHA between IPAH and PAH-SSc is not part of the SCCOR program. However, the K23 proposal focusing on neurohormonal activation in PAH, while ambitious, is quite feasible because 1) the patient population studied is unusually motivated and willing to participate in clinical trials and 2) the SCCOR grant provides a vast infrastructure for patient recruitment, clinical investigations, and genetic analyses. **As Principal Investigator, I will be responsible for all facets of the proposed study, including subject recruitment, data collection and management, data analysis, and reporting of results.** The resources of the SCCOR, including research coordinators, database management, and statistical support, will be available to assist me with this project.

**RESEARCH ENVIRONMENT**

I am fortunate to conduct my research at the extremely rich environment in the Division of Pulmonary and Critical Care Medicine at Johns Hopkins University School of Medicine. My clinical research activities are supported by an extensive institutional infrastructure, including ample office space equipped with a Dell Pentium computer (Optiplex 745) with intranet and internet access, a Hewlett Packard Laserjet printer, a Dell laptop computer (Latitude X1), phone, fax machine and other office tools. I have an exceptional Mentoring Team, and am working directly with staff dedicated to clinical research, including an administrative assistant, research coordinators, and biostatisticians. The tight physical layout of the campus, daily shuttle and courier services will continue to facilitate multi-disciplinary collaboration between campus scientists.

**Institutional Infrastructure:**

The Johns Hopkins University School of Medicine and Department of Medicine (JHU-SOM) has a long-standing tradition of excellence in clinical training and biomedical research. Today, the Johns Hopkins Hospital is ranked consistently as one of the best in the country with a clear focus of integrating research with clinical medicine. The departmental leadership fosters scientific activity, encourages scientific interaction and fully supports this initiative.

The Division of Pulmonary and Critical Care Medicine has a long tradition of NIH-sponsored research. These efforts are supported by a strong divisional infrastructure. The Program has 55 full-time Faculty, with a wide variety of research interests. The newly established Center for Translational Respiratory Medicine has created strong programs in functional genomics that in turn have established a cDNA array facility, a proteomics core, and bioinformatic expertise within the Division, and a new genotyping data analysis core. This facility will promote clinical research to study mechanisms of disease in specific patient populations. Further, ongoing studies in the areas of patient and physician compliance, bioethics, bronchoscopy, clinical therapeutics, quality of care and outcomes expand the realm of clinical research to address individual patient-centered issues in pulmonary disease.

The Johns Hopkins Pulmonary Hypertension Program, headed by my senior mentor, Dr. Paul Hassoun, is one of the largest clinics in North America devoted to patients with pulmonary hypertension. Approximately 200 new patients are evaluated each year with 500 follow-up visits annually. In
particular, the Program cares for a large number of patients with PAH-SSc who are referred by the Johns Hopkins University Scleroderma Center. Co-directed by Dr. Frederick Wigley, Division of Rheumatology, and Dr. Robert A. Wise, Division of Pulmonary and Critical Care Medicine, this is a collaborative, multidisciplinary center where patients with scleroderma are evaluated, treated, and enrolled in research projects. Approximately 85 new patients with definite scleroderma are seen per year with over 900 patients enrolled in the registry. Given the intensity of the medical therapy and the lack of alternative specialized centers for pulmonary hypertension in the mid-Atlantic region, patients are rarely lost to follow-up.

The Pulmonary Hypertension Program clinical team consists of Dr. Hassoun, Dr. Reda Girgis, Dr. Ari Zaiman, Dr. Stephen Mathai, three physician research coordinators, one research nurse coordinator, and several office managers. This team meets weekly to discuss patient care issues, research in progress, and potential new research projects. I meet with Dr. Hassoun on a biweekly basis to discuss the progress of my clinical projects and frequently discuss issues with Dr. Girgis. I attend in the Pulmonary Hypertension Clinic one day a week. As a member of the team, I have unlimited access to the resources of the Pulmonary Hypertension Program.

The Johns Hopkins Bloomberg School of Public Health is rich in resources that will be accessed through my work on the proposed research. The Department of Biostatistics is committed to applying statistical science to the solution of public health problems. I will be working with leaders from this Department through the proposed research.

The William H. Welch Medical Library is located on the medical campus and contains over 300,000 bound volumes, 2,300 audiovisual programs, and receives over 2,800 biomedical periodicals. WelchWeb offers online access to over 2400 journals, 300 full text electronic books and 230 databases, including MEDLINE, PREMEDLINE, EMBASE, CINAHL, and the Cochrane Library. Online services are available 24 hours a day through either the university network or through the use of JHSecure (virtual private network).

Intellectual Resources
I have been fortunate to receive exceptional clinical and research training during my post-doctoral fellowship in an environment of dedicated and experienced mentors. We have assembled a mentoring team of established faculty with many years of productive research experience and substantial prior mentoring experience. Together, we have published several manuscripts reporting the findings from our collaborative work. Each individual has distinct, complementary strengths in relevant areas of research. In addition, each serves as an excellent role model for my career development into an independent investigator.

Mentor
Paul M. Hassoun, MD (Mentor): Dr. Hassoun is a Professor of Medicine in the Pulmonary Division of the Johns Hopkins School of Medicine. He is the Director of the Johns Hopkins Pulmonary Hypertension Program and Principal Investigator for our institution’s SCCOR program in pulmonary hypertension. He has been my primary mentor since 2003, when I began working with the Pulmonary Hypertension Program. He is well known for his clinical research examining phenotypic and genotypic differences between IPAH and PAH-SSc and is an expert in vascular biology. He will assume responsibility for guiding my research progress as related to the clinical research projects in the SCCOR program (Molecular Determinants of Pulmonary Arterial Hypertension) and the investigation of the role of neurohormonal activation in PAH. He will also guide me through my training plan and extramural funding process for the duration of the K23 award.

Advisory Committee
Kathleen Barnes, PhD (Advisor): Dr. Barnes is an Associate Professor and the Mary Beryl Patch Turnbull Scholar in the Department of Medicine. She is the director of the Johns Hopkins Bayview Medical
Center Genetic Research Facility and the director of the Genomics Core. She is internationally recognized for her expertise in genetic and genomic studies, particularly in pulmonary disease. She currently collaborates with our Pulmonary Division on the SCCOR on Molecular Approaches to Ventilator-Associated Lung Injury and the SCCOR on Molecular Determinants of Pulmonary Arterial Hypertension. Dr. Barnes will perform the genetic analyses proposed and provide expertise in the interpretation of these results.

Robert Wise, MD  Professor of Medicine in the Johns Hopkins University Division of Pulmonary and Critical Care Medicine will provide advice on clinical study design, as well as provide overall career guidance. Dr. Wise has provided valuable feedback by reviewing grants and manuscripts. He has also helped me to network and meet leaders in clinical research at national meetings, such as the American Thoracic Society and American College of Chest Physicians. He will continue to serve in this capacity, offering the necessary guidance to help my transition to an independent investigator.

Thomas Louis, PhD  Professor in the Departments of Biostatistics at the Johns Hopkins Bloomberg School of Public Health will provide guidance on the application and interpretation of statistical testing and analyses. He will have frequent scientific contact with the laboratory, clinical and epidemiologic projects and cores and so will have a seamless connection to the specific aims proposed in my K23 application.

Career Development Activities
During upcoming year, there will be several means of fostering development into an independent clinical investigator: 1) Direct one-on-one mentoring by members of the mentoring group 2) Coursework 3) Development and conduct of the research project, including direct contact with the participants 4) Dissemination of results through manuscript preparation and presentations at national meetings 5) Participation in conferences and didactic training. In addition to learning from my mentors, I will take advanced coursework in genetics, survival analysis, and longitudinal data analysis at the Johns Hopkins Bloomberg School of Public Health, which will build on the foundation provided by the completed coursework during my Masters Program. I will participate in weekly conferences within the Pulmonary Division and will present my own research at these conferences several times during the year. I will also present my findings at national/international meetings.

Summary
My overall career goal is to become an independent clinical investigator with specific expertise in the pathogenesis of pulmonary arterial hypertension. The vast infrastructure provided by the SCCOR program enables the interaction of multi-disciplinary experts skilled in genetics, epidemiology, biostatistics, and clinical investigation, thereby creating a rich environment for opportunity and growth as a clinical researcher. The research that I will pursue in the upcoming year is a logical extension of the collaborative work that is ongoing at our institution and will provide an invaluable base upon which I can develop into an independent investigator.