

Transplant & Preparation Center

Raymond L. Benza M.D. FACC

**Allegheny General Hospital
Transplant and Preparation Center**

At AGH, we have established a team to facilitate the rapid collection of lungs removed at transplant from patients with primary (idiopathic) pulmonary arterial hypertension (PPAH) and other pulmonary diseases which can be used as controls for the study of PPAH through a unique collaboration with our local organ Procurement organization, CORE. To permit comparisons, equivalent tissue will be collected from the same areas of each lung and uniquely labeled as to site/source in the lung. Specifically, endothelial cells and smooth muscle cells will be collected for culture from arteries and separately from veins and transferred directly to the Cell Culture Facility.

Arteries, veins, and supporting tissues are dissected and transferred for processing and storage to the Tissue Processing Core at the University of Alabama at Birmingham (UAB) for later molecular analysis at the gene expression level and proteomic analysis. Proteomic analysis also will include obtaining tissues to be fixed in various fixatives for immunohistochemical studies of protein expression and research involving in situ hybridization and real time quantitative PCR.

Supporting lung tissues for studies in molecular biology will be collected and snap frozen at the collection site. These tissues will be transferred for processing and storage to the Tissue Processing Core at UAB.

James West, PhD

**Vanderbilt University Medical Center
Transplant and Preparation Center**

Vanderbilt University Medical Center has one of the largest cohorts of heritable pulmonary arterial hypertension (PAH) patients in the world, and a comparably large cohort of idiopathic and secondary PAH patients in clinic. We have historically had about two transplants per year across PAH classes, as well as processing lungs from matched non-PAH controls from unused donors. We have a team of seven ready to process lungs whenever they become available: this allows each lung section to have two team members working simultaneously to ensure the most rapid possible processing for the highest possible quality of explanted tissues.

Our work with human tissues primarily focuses on metabolic defects. While we first identified these as a direct effect of mutations in the heritable PAH gene *BMPR2*, we have found that these defects are present across all classes of PAH. A shift to aerobic glycolysis, an increased reliance on glutaminolysis, a failure of anapleurosis, and reduced ability to use lipid oxidation for energy are all part of a change in metabolic program that appear to be fundamental to the development

of disease. None of these alterations are changed by any existing therapy. Our goals are first to develop biomarkers based on these defects to allow us to determine when therapies are impacting the fundamental molecular defects in disease, and second to determine entry points for therapies designed to restore normal metabolic function.

Serpil Erzurum, M.D. and Suzy Comhair, M.D.

Cleveland Clinic Foundation Pulmonary Hypertension Studies Requiring Human Lung, Blood and Bone Marrow Transplant and Preparation Center

1.) IPAH Endothelial Cell Abnormalities

IPAH is characterized by impaired regulation of both pulmonary hemodynamics and vascular growth. Our preliminary data show that primary pulmonary artery endothelial cells (PAEC) from IPAH lung have enhanced proliferation, migration and abnormal tube formation in vitro. The signal transducer and activator of transcription (STAT) 3, recently identified as a critical regulator for angiogenesis, is persistently activated in IPAH PAEC, but not in control cells.

We hypothesize that the pathogenesis of IPAH stems from abnormal endothelial cells, which may derive from aberrant endothelial progenitor cells (EPCs), and result in autonomous growth signals resulting in increased proliferation, and deregulated angiogenesis. We plan to quantitate proliferation, migration and tube formation of IPAH PAEC, and levels of circulating and bone-marrow derived EPCs, in comparison to healthy and disease controls.

To identify mechanisms that account for the altered biology of IPAH cells, we focus our investigations on the evaluation of the factors leading to the low nitric oxide (NO) in IPAH, in particular post-translational regulatory pathways of NO synthesis, including factors affecting substrate arginine levels. We propose the network collection of pulmonary artery endothelial cells from explanted lungs of IPAH and donor lungs not used in transplantation, blood for EPC measures, serum and plasma analyses of factors that regulate NO synthesis, and bone marrow for evaluation of endothelial progenitor cells. Our signal transduction studies also encompass evaluation for type II bone morphogenetic protein receptor (BMP2) mutations [described in (2) below].

Finally, our previous studies showed an inverse correlation of NO to pulmonary artery pressure. So, we are conducting a longitudinal study of IPAH patients to test our hypothesis that determinants of NO synthesis, i.e. arginase, methylarginines and arginine, predict outcomes in IPAH. Measurement of IPAH patients' exhaled NO prior to transplantation is suggested for the network cohort. Our studies aim to determine if there are inherent alterations in IPAH endothelial cells, progenitor cell contribution, and identify the causal mechanisms, which may potentially lead to novel therapies for treatment of IPAH.

2.) Genetic Association Studies in IPAH

Mutations of the bone morphogenetic protein receptor II (BMP2) gene are now recognized to underlie at least 75% of familial PAH cases. Similar mutations are also identifiable in 10-40% of sporadic idiopathic (IPAH) cases. However, the etiology in the majority of IPAH remains unexplained and is likely a combination of genetic and environmental factors. Genetic association studies offer the ability to delineate some of these risk factors and have greatest power when a large number of samples can be uniformly analyzed. The establishment of the IPAH research network therefore provides a unique opportunity to exploit these techniques.

We propose to collect DNA samples from IPAH patients, together with controls matched for age, sex and ethnic background, in order to conduct a collaborative genome-wide association study. While the main focus of the IPAH network is on patients undergoing lung transplantation, it would be highly advantageous to extend the scope for DNA collection, recruiting as many patients as possible, regardless of treatment regimen and transplant status.

Such an approach would both increase the power of studies conducted in the near term, but also lay the foundations for a longitudinal study that might ultimately identify correlates between genetic status and disease progression.

George Noon, M.D.

**Baylor College of Medicine
Transplant and Preparation Center**

Patients are referred to The Methodist Hospital and Texas Children's Hospital for lung transplant evaluation. They undergo a thorough physical evaluation and testing, meet with pulmonologists, surgeons, transplant coordinators, and the transplant research coordinator. After their testing is complete, they are presented to the thoracic transplant patient selection committee. If the patient is deemed an acceptable candidate for pulmonary transplant, and has financial and social clearance, the transplant coordinators will list the patient with UNOS (United Network Organ Sharing).

At each institution, the physicians and transplant coordinators have access to the lung transplant list. The patients name and diagnosis are included on the list. All patients on the transplant list with idiopathic pulmonary artery hypertension or primary pulmonary hypertension will be eligible to participate in the study.

When a suitable organ donor is identified by an organ procurement organization for a patient listed at one of these institutions, a call is placed to the transplant coordinator. The coordinator receives a report on the donor. The coordinator contacts the pulmonologist and the surgeon and a decision is made to accept or reject the donor offer. If the organ is accepted, the transplant coordinator admits the recipient to the implanting hospital and the patient is prepped for surgery.

The transplant coordinator communicates with the harvesting surgeon, the implant surgeon and OR personnel to coordinate the time the recipient goes into the operating room for transplant.

The transplant coordinator will collect patient data that includes results of pre-operative tests, medical history, and demographics. This data will be sent to the DCC. Patient information will be identified by a code and not by name outside of the hospital. The transplant research coordinator will ensure that all procedures run smoothly and the collection of data is correct, prospective and secured in a password-protected database in a locked office.

Patient codes will be issued and used for the collection of all tissues and will be used for transmission of patient data (excluding patient name) using this same patient code to the Data Base Collection Center. Only the transplant research coordinator will have access to codes used to identify individual patients.

The person responsible for collecting the tissues will immediately obtain tissues from the operating room during the explantation procedure. This includes collection of blood and tissue followed by immediate processing of tissue necessary to maintain integrity of histology, mRNA, DNA, proteins and cells. The processed samples will then be shipped to the appropriate and designated processing center via Federal Express.

Our original grant submission detailed the exact manner that we would use for processing the tissues and fluids. However, this group will comply with the primary protocols of the Data Bank and Coordinating Center.

Marlene Rabinovitch, M.D.

Stanford University–UCSF–University of Toronto Transplant and Preparation Center

Patient Population: Lungs removed at transplantation will be procured in collaboration with UCSF and University of Toronto. Those procured from UCSF will be prepared at Stanford and those from the University of Toronto will be prepared at that center. We anticipate 3-5 lungs a year for IPAH, 5-6 for secondary PAH (Eisenmenger's), 5-6 for secondary PAH (chronic lung disease) and 5-6 matched non-PAH controls.

Both Stanford and the University of Toronto have extensive databases from which clinical information (75 data points/patient at Stanford) can be retrieved. This will serve both for entry into the DCC as well as for future use by Network Investigators.

Procurement of Tissue for the Various Centers: Lung tissue from the right upper lobe (RUL) will be procured for light and electron microscopy (LM, EM) as well as in situ hybridization and from the right middle lobe (RML) for genomic and proteomic studies. The anterior right lower lobe (RLL) will be prepared for immunohistochemistry and laser capture microscopy and studies of the microvasculature with Dr. Donald McDonald as consultant. A portion of the left upper lobe (LUL) will be used for organ culture and the left lower lobe (LLL) for harvest of cells. In addition, bronchoalveolar lavage fluid and cells as well as plasma and DNA will be frozen for future analyzes.

To prepare tissue for LM, EM and in situ hybridization, the RUL will be fully inflated (20-32 cm H₂O) and the PA perfused free of blood. The arterial system is fixed by perfusion at the in vivo pressure with universal fixative (1% glutaraldehyde in 4% formaldehyde) and the airway then fixed by perfusion at the inflation pressure. Fixation is maintained for 24-48h depending on the amount of tissue. Three sections are retained to archive the tissues according to the structural changes observed by Movat pentachrome staining. The rest of the fixed tissue will be sent to the Processing Center and embedded in paraffin for light microscopy and for in situ hybridization, in epon for EM or in lowicryl for immunoEM.

To prepare tissues for immunohistochemistry and laser capture microscopy, RLL tissue will be inflated with air and the PA perfused for 2 min with 1% paraformaldehyde and then immersed in fixative for 1h prior to preparation of cryostat sections in OCT. for the Processing Center. Part of the tissue from the posterior segment of the RML will be frozen in liquid N₂ and then at -80°C for DNA, RNA and protein analyses at the Genomic and Proteomic Centers.

For detailed studies of the microcirculation, the tissue is cannulated and then perfused for 5min at the in vivo mean pressure using 1% paraformaldehyde, 0.5% glutaraldehyde in PBS then with PBS for 1min, PBS + 1% BSA for one min, biotinylated lectin (5-10 µg/ml) for 1 min, then PBS+ BSA and then PBS for 1 minute each. The tissue is permeabilized by overnight incubation in PBS containing 0.3% Triton X-100, then incubated with avidin-peroxidase complex for 24h and then reacted with DAB.

We will continue organ culture studies from a small piece of the LUL from which proximal PA and distal tissue with intra-acinar PAs are placed in collagen gels and exposed to agents to study the extent of regression of the lesions observed. The LLL tissue is distributed to the Cell Center.

Keith Wille, M.D.

University of Alabama, Birmingham Transplant and Preparation Center

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