Pulmonary Vascular Disease Phenomics Program - PVDOMICS

PVDOMICS

Study Protocol
Version 1.1

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1. Introduction (1-12)

This NHLBI PVDOMICS protocol represents the core working protocol jointly created by the six centers awarded the NHLBI U collaborative grant under RFA-HL-027 (Columbia/Cornell, Johns Hopkins, Brigham and Women's Hospital, Mayo Clinic, University of Arizona Tucson, and Vanderbilt University) together with the project Data Coordinating Center (DCC) (Cleveland Clinic) and the NHLBI. The need for such a project grew out of recognition of the prognostic impact of pulmonary hypertension (PH) and right ventricular (RV) failure across a wide spectrum of disease states that are currently classified under the Nice modification of the World Health Organization (WHO) classification system.

It is recognized that patients with various forms of heart and lung disease exhibit varying degrees of pulmonary vascular disease, leading to pulmonary vascular remodeling, pulmonary hypertension, and right ventricular dysfunction. The genetic, molecular, and cellular processes driving these phenomena are not well understood. Rapid advances in high throughput omic methodology, combined with powerful bioinformatics and network biology capability, have created the opportunity to conduct studies that broadly search for homologies and differences across the spectrum of disease states associated with pulmonary hypertension, and determinants of the spectrum of pulmonary vascular disease and RV compensation that accompanies these conditions.

The 2010 NHLBI Pulmonary Vascular Strategic Plan identified the development of a comprehensive cohort to define phenotypes integrating Omics technologies and systems approaches as a top priority(2). The current WHO PH classification, based solely on clinical/hemodynamic subsets, limits our ability to customize treatment for an individual patient, or to assign meaningful clinical phenotype designations, *e.g.*, Long-term survivor or Maladaptive RV Hypertrophy (1). **The overall goal of the PVDOMICS network is to perform comprehensive phenotyping (demographic, physiologic, clinical chemistries, and imaging) and endophenotyping (genomic, proteomic, metabolomic, coagulomic, cell and/or tissue based) across the World Health Organization (WHO) classified PH clinical groups 1 through 5 as well as intermediate phenotypes (including those without overt PH) in order to deconstruct the traditional classification and define new meaningful subclassifications of patients with PVD.** The long-term goal is utilization of endophenotypes/biomarkers for early diagnosis, at-risk screening, and personalized approaches for interventions and/or preventions of PVD.

The PVDOMIC protocol is designed to lead to this comprehensive understanding of patients with pulmonary vascular disease based on phenotypes and endophenotypes.

2. Objectives (1-12)

2.1 Specific Aims:

The first aim will be a natural product of the protocol, to identify the molecular basis of pulmonary vascular disease regardless of WHO clinical classification, by comparison of current subsets of PH patients with healthy subjects and with non-PH diseased comparators. The second aim is to discover biological measures of disease and therapeutic responses that may be useful not only in diagnosis but also as outcome measures in treatment and possibly prevention trials.

1. Create an advanced description of structural and functional abnormalities of the heart and pulmonary circulation in patients with PVD to define novel phenotypic clusters of PVD.

    a. Compare and contrast imaging assessment with Echo, CT and MRI, clinical and hemodynamic and gas exchange data

    b. Compare and contrast invasive exercise assessment with imaging, volume loading and baseline catheterization data and etiology.

2. Create a detailed molecular endotype of all PVD patients, including genomic, transcriptomic, proteomic, metabolomic, cell biomic and coagulomic metrics.

    a. Test for known PH mutations, new genetic variants and genomic correlations with all PVD and PH Group designations, including acute vasodilator responders, and appropriate controls.

    b. Compare and contrast transcriptomic, proteomic, metabolomic, cell biomic and coagulomic data in all categories of demographic features, known etiology (such as genetic) exercise physiology, pharmaceutical management and outcomes (where feasible).

    c. Compare all omics data without regard to PH Group designation to generate a new, more accurate classification of pulmonary vascular disease leading to PH.

3. Cross-validate variants of PH between PVDOMIC genetic data with that of the Nichols R24 and other available databases.

2.2 Hypotheses

2.2.1 General Hypotheses

1. Epidemiological, biological, metabolomic and hemodynamic features will allow differentiation of phenotypic similarities and differences among current World Symposium PH Group categories of PH. These insights will lead to newer classification of PH based on shared biological features.

2. The molecular basis of pulmonary vascular disease of all etiologies will be discovered by integration of biological markers with careful phenotyping of all patients with PH and comparing this data with healthy subjects and with non PH patients as diseased comparators, such as emphysema and interstitial lung disease.

3. Racial and gender-related (or ancestry) genetic variation in phenotype, natural history and responses to therapy will be discovered and lead to more precise diagnostic and therapeutic approaches.

4. The response, adaptation and dysfunction of the right ventricle (RV) will be elucidated by careful phenotyping, including specialized imaging and –omic correlations.

5. Exercise pathophysiology will lead to improved early diagnosis of PVD, elucidation of RV- pulmonary vascular interactions, RV functional reserve, failure, and response to therapies.

6. The biological and genetic features of patients with combined pulmonary venous hypertension and PAH will lead to better differentiation of these two etiologies of PH, and of shared biological mechanisms.

7. Epigenetic and RNA variants will influence the development, severity and type of PVD and reveal therapeutic responses and form a basis for new therapies.

2.2.2 Specific Hypotheses

Examples of specific hypotheses that can be tested in the study are below.

1. Racial and ethnic (or ancestry) differences in transcriptomics, epigenetics and mitochondrial haplotypes will inform PH pathogenesis by similarities and differences among similar phenotypes with PH.

2. Patients with left heart dysfunction and PH will have patterns of omic measures that predict the presence of combined PH. Patients with combined PH in WHO Group 2 will have patterns of genomic vulnerability similar to patients with Group 1 PH, different from Group 2 patients without severe PH.

3. Patients with parenchymal lung disease and moderate to severe PH will have omic signatures that reflect not only underlying pathogenesis (emphysema or fibrosis) but will have patterns of genomic vulnerability similar to patients with Group 1 PH.

4. Patients with exercise-induced PH will have omic features that are similar to those with Group 1 PH, different from those with normal exercise hemodynamics.

5. Connective tissue disease patients with PH (largely systemic sclerosis) will have endothelial dysfunction measured as abnormalities in nitric oxide production, arginine/ornithine/citrulline metabolism and vasoactive mediators that are similar to patients with Group 1 PH and different from scleroderma patients without PH.

6. Genomic, transcriptomic and metabolomic patterns will distinguish the degree of right ventricular compensation for a similar degree of right ventricular afterload in patients with pulmonary hypertension, regardless of the underlying WHO category.

2.3 Study Design (13, 14)

2.3.1 Organizational Structure of the Study

The study will be governed by a Steering Committee comprised of the Principal Investigators (PIs) of the PVDOMICS clinical centers and the DCC, the NHLBI Project Scientists participating in the PVDOMICS, and the Steering Committee Chair of the PVDOMICS. The Steering Committee has the primary responsibility for the study protocol, monitoring study conduct, and reviewing data prior to reporting study results. It is also responsible for determining policies in such areas as access to participant data, ancillary studies, publications and presentations. Day-to-day decision-making is vested by the Steering Committee in an Executive Committee consisting of the Steering Committee Chair, NIH Project Scientists, DCC PI, and two rotating clinical center PIs. Study oversight is also provided by an Observational Safety and Monitoring Board (OSMB) appointed by the NHLBI (See Section 6.6). Proposals for ancillary studies of high scientific merit are encouraged to further enhance the scientific value of the main study and to optimize the yield from collected data, images and biospecimens (see Section 7). Upon conclusion of the study, data will be archived and shared according to NIH policies.

There will be many central core facilities for PVDOMICS. These include ones for imaging, lung physiology, clinical chemistry, right heart catheterization and cardiac pulmonary exercise tests. These cores will provide central direction, personnel training, and supervision to the study, while supporting ascertainment of high-quality standardized data. Study coordination, centralized data management, biospecimen management and repository, and statistical collaboration will be provided by the PVDOMICS DCC at the Cleveland Clinic. Also, the

DCC's Biorepository Core will have several omics cores to aid in carrying out the omics analyses and interpretation. These cores include those for genomics and transcriptomics, proteomics, metabolomics, cell biomics and coagulomics

2.3.2 Interactions with Other NHLBI Awards and the Pulmonary Hypertension Association

The overall goal of the PVDOMICS network is to perform comprehensive phenotyping (demographic, physiologic, clinical chemistries, and imaging) and endophenotyping (8, 15, 16) (genomic, proteomic, metabolomic, cell and/or tissue based) across the World Health Organization (WHO) classified PH clinical groups 1 through 5 in order to define new subclassifications of patients based on characteristics that are associated with mechanisms of pathogenesis (17). The network plans to leverage the synergistic scientific and operational strengths of two NHLBI-awarded investigator-Initiated Resource-Related Research Project Application (R24) grants on PH to support, facilitate and accelerate PVDOMICS goals: (1) Pulmonary Hypertension Breakthrough Initiative (PHBI) (18, 19) and (2) the National Biological Sample and Data Repository for PAH (20). The latter is led by Dr. Nichols [PI] at Cincinnati Children's Hospital Medical Center, and represents collaboration between academic PH centers across the United States to collect a cohort of PAH patients to identify novel pathways or genetic factors contributing to the disorder. They will collect and maintain biological material and generate genetic data from 3000 WHO Group 1 PAH patients. Dr. Nichols will be invited to participate in PVDOMICS Steering Committee meetings in order to optimize interactions and leveraging of ideas and data. The PHBI aims to accrue PH lung and heart with detailed clinical annotation of specimen lung and heart tissues and human primary endothelial and smooth muscle cells derived from explanted PH and control lungs, which will be available for translational approaches to endophenotype/biomarker discovery in PVDOMICS. Researchers in the PHBI overlap with membership in PVDOMICS, including Dr. Geraci [PI] and Drs. Comhair, Aldred and Erzurum. The PHBI R24 protocols, data, samples and cells will be leveraged for optimal productivity of PVDOMICS. Specifically, Drs. Aldred and Geraci run the PHBI Genomics and Mutation Analysis Cores, the goals of which are to develop a genomic catalog of PAH specimens by mutational and genomic analysis of DNA and RNA from pulmonary and cardiac tissues. Explant lung tissues are analyzed for mutations in known PAH genes, genome-wide single nucleotide variant genotyping and expression analysis of mRNA and microRNA profiles. Similar analyses will be performed in cardiac tissues as they are accrued. Novel markers identified in the lung and heart tissues of PHBI subjects will be validated in peripheral biospecimens obtained in PVDOMICS, providing a powerful link between the primary disease sites and less invasive peripheral biomarkers. In addition, the network has entered into agreement with the Pulmonary Hypertension Association (PHA) for advancing the goals of PVDOMICS. PHA has committed support for expansion of the network to include 6 sites. PHA is in the process of accrediting PH Care Centers (PHCCs) and registry data through the PHCCs' participation in the PHA Registry (PHAR) (21). The PVDOMICS DCC will plan to harmonize

data with PHAR, so that data may potentially be evaluated collectively for similar data. PHA representatives will be invited to all PVDOMICS Steering Committee meetings to optimize interactions and patient voice to the network.

3. Study Participants

3.1 Patient Recruitment

Patients, at risk cohort comparators and true controls will be recruited amongst the centers. We will recruit patients who present for evaluation of PH, heart failure, lung disease, dyspnea and/or exercise intolerance from the various PH, Heart Failure, Advanced Lung Disease clinics. Following the catheterization, patients will be assigned to "buckets" to assure an appropriate enrollment distribution across the traditional WHO PH Groups 1-5 or WHO 1-4 comparator groups at risk for PVD associated with similar underlying diseases.

Each of the six centers will recruit patients across the spectrum of WHO PH Groups (22)

Group 1 – Pulmonary arterial hypertension (PAH)
Group 2 – PH associated with left heart disease
Group 3 – PH associated with lung diseases and/or hypoxemia
Group 4 – PH attributed to chronic thromboembolic disease (CTEPH)
Group 5 – Miscellaneous

Ideally, each center will recruit patients with the following targets, but there may be center variation based upon programmatic strengths:

Group 1 PH = 50; Group 2 PH = 50; Group 3 PH = 50; Group 4 PH = 8-9; Group 5 PH = 8-9.

WHO 1 comparators = 20: inclusive of more mild PH, exercise induced PH (ePAH), relatives of patients with heritable PAH, CTD patients with mild or no PH.

WHO 2 comparators = 20-21 with 50% moderate PVD and 50% mild to no PVD.

WHO 3 comparators = 20-21 with 50% moderate PVD risk and 50% mild to no PVD.

WHO 4 comparators = 5 with chronic PE's without associated PH.

Totals across all centers are:

Group 1 PH = 300; Group 2 PH = 300; Group 3 PH = 300; Group 4 PH = 50; Group 5 PH = 50. For comparators: WHO 1 = 120; WHO 2 = 125; WHO 3 = 125; WHO 4 = 30; and 100 "healthy" controls.

We anticipate at least 25% incident disease enrollment.

Partners or spouses or accompanying friends of patients participating in the study will be recruited to serve as true healthy controls. An effort will be made to maintain parity with regard to race, ethnicity, age and BMI with the patient population being recruited. Obesity will not be an exclusion criterion.

Recruitment via advertisement may be employed to complement the recruitment of accompanying subjects and to ensure appropriately matching cohorts.

3.2 Inclusion/Exclusion Criteria

PVD Cohort (patients and comparators) Inclusion Criteria:

- Patients ages ≥18 years of age referred for right heart catheterization for further evaluation of known PVD or to be at risk for PVD due to established cardiac disease or pulmonary disease

- Able to perform complete diagnostic testing listed subsequently (catheterization, echo, exercise test, PFT's, chest CT, QOL, ventilation/perfusion scan and ideally cardiac MRI)

- Subject signs informed consent to perform required testing for the protocol

Exclusion Criteria:

Dialysis dependent renal function

In the clinician's opinion, too ill to perform the protocol testing

Pregnant or nursing

3.2.1 Control Group

"Healthy" Volunteers without end organ disease
N = 100 (approximately 16-17 per center)

Inclusion criteria:
Age 18 or above
Informed consent obtained
Normal cardiopulmonary screening by history, exam

Subcategories permitted:
a) Obesity
b) Diabetes without end organ disease
c) Hypertension without end organ disease
d) Hyperlipidemia

e) Sleep apnea if being treated

Exclusion criteria:
Note will be made of all prescription and over the counter medications, vitamins, supplements, contraception
Active malignancy other than localized non-melanoma skin cancer
Pregnant or nursing

3.2.2 Comparator Groups

I. WHO 1 comparators
   a. Borderline PH mPAP 21 to < 25 (when presenting in symptomatic or at risk patient population including SSc, Heritable PH)
   b. Exercise induced (ePAH): Ex mPAP ≥ 30, flow < 10 L/min and mPAP-Q slope > 3 (mmHg*min/L)

II. WHO 2 comparators (systolic heart failure (HFrEF), heart failure with preserved ejection fraction (HFpEF), restrictive cardiomyopathy (RCM), hypertrophic cardiomyopathy (HCM), valvular heart disease (VHD)). Exclusion: Takotsubo cardiomyopathy, apical ballooning, acute myocarditis, must be > 6 months post-surgical or catheter based valvular intervention.

   a. Mild PVD risk associated with LHD
      Left Heart Disease (LHD) with mPAP < 25
   b. Moderate PVD risk associated with LHD
      1. Isolated post-capillary pulmonary hypertension (Ipc-PH) mPAP ≥ 25, PVR < 3, diastolic pressure gradient (DPG) < 7
      2. Provocable mPCW > 18 or mPCW > 15 with large v waves following challenge

III. WHO 3 comparators (parenchymal or non-parenchymal lung disease without resting PH)
   a. Chronic Obstructive Pulmonary Disease (COPD) as defined by ATS criteria (23) (see Appendix 1)
   b. Idiopathic Pulmonary Fibrosis (IPF) as defined by ATS criteria (see Appendix 1)
   c. Other Interstitial Lung Diseases (ILD), including combined pulmonary fibrosis emphysema (CPFE) and scleroderma-related ILD (26) (see Appendix 1)
   d. Obstructive Sleep Apnea (OSA) as defined by AASM criteria (see Appendix 1)
   e. Obesity Hypoventilation Syndrome (OHS) as defined by AASM criteria (28) (see Appendix 1)
   f. Sarcoidosis as defined by ATS criteria (29) (see Appendix 1)

g. For all categories
    1. Mild to no associated PVD risk: mPAP < 21
    2. Moderate associated PVD mPAP 21 to < 25

IV. WHO 4 comparators (chronic thromboembolic (CTE) disease with mPAP < 25)

3.2.3 PH Groups for Enrollment

A more inclusive PVD classification will include current WHO Groups 1-5 definitions and utilization of a primary and secondary group classification where appropriate. Those with more mild disease and/or considered to be at risk for development of PVD are outlined in the previous comparator Section 3.2.2.

For the purposes of meeting the RFA enrollment criteria, PVD Groups will be classified with a primary and where appropriate, secondary diagnosis according to the 5th World Symposium on Pulmonary Hypertension (WSPH) (22) and to include the extended PVD risk cohort comparisons. At regular intervals Adjudication Committee will review enrollment to be sure that we are meeting the recruitment goals with a distribution across traditional and expanded PVD criteria including severity of disease and ensuring that we meet the targets for traditional WHO Group classification distribution. The committee will notify centers if we have to target specific deficient enrollments. The committee will also review the mixed pathologies, in particular the combined WHO 2, 3 and WHO 1, 3 with hypoxemia to be sure that similar criteria are being used across centers. How well our present clinical phenotyping of the traditional groups correlate with what we hope will be a new physiological definition coupled with a pathobiological characterization will ultimately be addressed (Section 3.2.4).

PH Groups for the purposes of enrollment

I. **WHO Group 1**: rest mPAP ≥ 25, PVR > 3.0

a. IPAH, Heritable PAH, HIV, Portal hypertension, drug induced, CHD, Schistosomiasis, PVOD, PCH;
b. WHO 1 - CTD (SSc, SLE, MCTD, Sjogren, RA,)

II. **WHO Group 2** PVD: (HFrEF, HFpEF, VHD, RCM, HCM): mPAP ≥ 25, PCW > 15(systolic heart failure (HFrEF), heart failure with preserved ejection fraction (HFpEF), restrictive cardiomyopathy (RCM), hypertrophic cardiomyopathy (HCM), valvular heart disease (VHD).

a. Combined precapillary and postcapillary (Cpc-PH) mPAP ≥ 25

i. PVR > 3.0 if CO > 4;
ii. If CO < 4, then DPG $\geq$ 7 or TPG $\geq$ 12

III. WHO Group 3:

a. COPD/IPF/CPFE defined as in Appendix 1 with hemodynamically proven PH (mPAP $\geq$ 25 mmHg) will be further categorized as follows as per the 5th World Symposium recommendations (7):
   i. Moderate PH-COPD, PH-IPF and PH-CPFE (35> mPAP $\geq$ 25)
   ii. Severe PH-COPD, severe PH-IPF, severe PH-CPFE defined as mPAP $\geq$ 35 or $\geq$ 25 with CI < 2.0 0 L/min/m^2
b. Other ILD, non-parenchymal restrictive lung disease (RLD; neuromuscular disease or thoracic cage abnormalities), OSA, OHS defined as in Appendix 1 with hemodynamically proven PH (mPAP $\geq$ 25 mmHg) will be further categorized as in III.a, though specific recommendations for classification of PH severity do not exist for these disease states

IV. WHO Group 4:

a. High probability V/Q or low/intermediate V/Q and (+) CTA or pulmonary angiogram consistent with chronic thromboembolic (CTE) disease
b. supportive, OR positive pulmonary angiogram
c. > 3 months therapeutic anticoagulation
d. mPAP $\geq$ 25, mPCW $\leq$ 15 mmHg

V. WHO Group 5:

a. Sarcoidosis as defined in Appendix 1
b. Myeloproliferative disease
c. Hemoglobinopathy
   i. Sickle cell
   ii. Thalassemia

3.2.4 Phenotypic Characterization of PH Across WHO Groups

To better characterize patients who present for PVD evaluation in an unbiased approach we will categorize patients hemodynamically as pre vs post capillary and as mild, moderate and severe PVD across the categories. Similarly, we will score associated medical conditions across the full spectrum of patients. Specifically we will assess:

I. Hemodynamics
    a. Pre capillary
    b. Post capillary
        i. Pulmonary venous hypertension: iso-PH
        ii. Combined pre and post capillary pathology: Cpc-PH

II. Parenchymal and Non-Parenchymal lung disease
    a. COPD
    b. IPF
    c. CPFE
    d. Other parenchymal lung diseases (scleroderma-related ILD, other collagen vascular disease related ILD, hypersensitivity pneumonitis, drug-induced ILD, etc.)
    e. non-parenchymal RLD (neuromuscular disease and thoracic cage abnormalities),
    f. OSA, OHS including:
        i. Oxygenation
            1. Resting
            2. Nocturnal
            3. Exercise

III. Associated medical diseases, conditions

    a. Diabetes
    b. Metabolic, e.g., thyroid disorders, metabolic syndrome, adrenal disorders
    c. Atrial fibrillation
    d. Autoimmune dx (SLE, Sjogren, RA, MCTD, antisynthetase syndrome, ACL, LAC, autoimmune thyroid ITP, PBC)
        i. Active Rx
    e. Hepatic
        i. Venous hypertension i.e., Non cirrhotic fibrotic liver disease
        ii. Portal hypertension
        iii. Hepatitis (treated or not)
        iv. Nodular regenerative hyperplasia
    f. Renal
        i. CKD
    g. Hypercoaguable state
    h. Myeloproliferative Disease
    i. Abnormal SPEP/IPEP
        i. myeloma, amyloid
        ii. MGUS
        iii. POEMS

j. Chronic hemolysis
k. Splenectomy
l. HIV
m. Sarcoidosis

3.3 Patient Timeline

Patients will be enrolled during years 2-4 of the study. Follow-up data to be collected or measured are given in Section 4.4.

3.4 Consenting Patients

The informed consent process will afford the opportunity for the patients and their family members to ask questions and express any concerns about participating in the study. Potential study participants will also be given the opportunity to consider the requirements of participation and the option of not participating in the study. Efforts will be made at all times to confirm that patients understand the information and are making an informed voluntary decision to participate in the study. Patients will also be encouraged to have family members and other trusted individuals assist with their decision to participate in the study and address any questions or concerns about participation in the study.

A consent form, approved by the IRB of the clinical center at which a patient is enrolled, will be completed before any patient undergoes any study activity. Formal consent for the study will be obtained by an investigator or study coordinator. Patients will have the option of including family members or trusted advisors in their decision process. Patients will be given adequate time to review the consent. Consent forms in languages other than English can be used according to the policies of the clinical center's IRB. Patients who cannot read will be read the entire consent form and will sign the form in the presence of a witness.

A separate consent will be completed for healthy control subjects.

The original signed consent forms will be kept at each participating institution and maintained according to the policies of the clinical center's IRB.

3.5 Meeting Recruitment Goals

For the purposes of enrollment classification across the WHO Groups 1-5 designations, we will have a cumulative running total and it will be reviewed by the Adjudication/Enrollment Committee periodically for the 6 centers together to meet target enrollment goals based on the primary diagnosis and to address if centers and cohorts are lagging in enrollment. It is feasible that one center will have more primary lung disease patients, another center more scleroderma, CTEPH or cardiac related PH patients, and slight differences in center specific enrollment that

are compensated across the 6 centers is acceptable. We will utilize the primary PH diagnosis although it is clear and part of the aim of the study to discover overlapping biological features across traditional WHO Groups. Prior stated traditional definitions of WHO Groups 1-5 will be used, a clinical designation of primary and secondary (if it exists) diagnosis will be included and each center will use their current clinical diagnostic algorithm for categorization.

Role of the Adjudication Committee:

1) Address deficiencies in enrollment to include: specific WHO group, new vs established disease, comparator cardiac and pulmonary groups, controls, and center specific enrollment on a periodic basis.

2) Ensure consistency across centers for classification of the mixed disease patients utilizing previously described inclusion/exclusion
   a) *Not to be classified as WHO Group 1:*
      i) Resting mPCW < 15 but exercise mPCW > 18 or mPCW > 15 with large v waves following challenge *(these will be classified in the Group 2 cohort*)
      ii) SSc with moderate fibrosis on HRCT (*these will be classified Group 3*)
      iii) PFT's: TLC< 60% (*these will be classified as Group 3*)
   b) Review mixed group pathology notably:
      i) WHO 1, 3 hypoxic precapillary PVD patient
      ii) WHO 2, 3 hypoventilation, OSA, restrictive/obstructive lung disease with mPCW > 15.

3) Review difficult classifications at the request of center PI/Co-PI

4) Realign center/network target population goals based on on-going enrollment.

4. Data Collection

4.1 Study Visits

The patients (PH and non-PH comparators) and controls will undergo biospecimens collection (blood and urine) which will be processed and stored at the Biorepository Core at the Data Coordinating Center at the Cleveland Clinic. Where feasible and not contraindicated, all patients with pulmonary hypertension will undergo a uniform set of assessments that will include the following:

1. Comprehensive review of history, WHO functional class, exam, and medications
2. Vital signs, waist – hip ratio
3. Body composition by bioimpedance
4. Overnight sleep monitoring

5. Complete PFTs: spirometry, lung volumes and DLCO
6. Assessments of exercise tolerance (6 minute walk, cardiopulmonary exercise testing)
7. Complete HRQOL surveys (SF-36 v2®, MLHF, emPHasis-10)
8. Echocardiography
9. Cardiac MRI
10. Lung CT images
11. Ventilation perfusion scan
12. Cardiopulmonary exercise test
13. Right heart catheterization with inhaled nitric oxide vasodilator volume loading with 500 cc saline if not contraindicated, sampling of pulmonary artery, pulmonary capillary wedge, and systemic arterial blood (if arterial line clinically indicated). Exercise will be performed in selected centers and patients.
14. Phlebotomy for omics measurements

Table 4.1 lists all the measurements and tests that are to be collected. However, studies may be scheduled over the course of 6 weeks. Suggested order of items and visit components are given in the Manual of Operations. Test requirements are:

- Right heart catheterization (with invasive CPET at centers performing this), echocardiogram, MRI and omics blood draws should be performed within two weeks, but data can be used if it is within one month allowing for individual needs.
- The MRI can be performed either before or after the right heart catheterization. If they are scheduled for the same day, the MRI ideally should be performed either before the catheterization or usually a minimum of one hour after the right heart catheterization.

Table 4.1 PVDOMICS Measurements and Tests	
Measurements and Tests Being Performed	**Allotted Time and Subject Accommodations**
• Review and sign consent • Medical history, review medications, NYHA functional class, allergies • Vital signs • Physical exam • Waist to hip circumference, Body composition bioimpedance • HRQOL surveys • Blood tests (CBC, CMP, NT BNP, HIV, Hepatitis, Rheum panel) • ECG • Echocardiogram • Spirometry, DLCO, Body Box* then 6 MWT • Clinically appropriate pre-catheterization discussion will have occurred • Overnight sleep monitoring • Cardiac Catheterization with lab work before, during and after • CPET • Recovery • MRI • High res CT* • Ventilation-perfusion lung scan*	• Parking and lunch. • Wear comfortable clothes and shoes to walk in • Visit may require a hotel stay to get all events completed. • 1-2 hours for consent, medication, medical history and exam • 1 hour for blood tests waist to hip circumference, bioimpedance • 1 hour for echo • 2 hours for PFTs • 30 min for HRQOL surveys • 4-6 hour fast prior to some tests

* Acceptable if adequate test was performed within 1 year (3 months if have lung disease) prior to enrollment

4.2 Baseline Evaluation and Data Collection

Controls will undergo the same tests as the PH Groups (see Sections below). Healthy controls will also have all the tests listed below except for a right heart catheterization (RHC), ventilation perfusion lung scan and invasive CPET.

4.2.1 Demographics and History

Birth date, gender, race, ethnicity, zip code of most frequent domicile will be recorded.

Family history of pulmonary arterial hypertension

Occupational exposures (30-32) (for example mining, industrial solvents): duration

Infectious disease history (33-35) (HIV, Herpes virus, tuberculosis, tick-borne diseases, parasitic diseases)

4.2.2 Quality of Life Questionnaires

Quality of life questionnaires will be completed by each patient. This includes the 36-item health survey SF-36v2® (36), the Minnesota Living with Heart Failure (MLHF) Questionnaire (37) and the EmPHasis10 survey (38).

4.2.3 Comorbid Conditions

Tobacco use (None ever, prior, current, total pack years)

Alcohol use (Current, number of drinks per week), Alcohol abuse history (none, prior, current)

Diabetes Type I, Type II, year of diagnosis, therapy (diet, oral agent (type), insulin)

Sleep disordered breathing (year of diagnosis, duration of treatment, current type of treatment)

Systemic hypertension (year of diagnosis, medications)

Cardiac diagnoses (arrhythmias, valve disease, rheumatic disease, CAD, etc.)

Pulmonary diagnoses (if biopsy proven, provide pathologic subtype)

Renal insufficiency (dialysis, history of renal transplant, fistula, meds)

Liver disease

Obesity

Prior diet or methamphetamine drug exposure (name of medication and years of exposure)

Prior malignancy aside from localized non-melanoma skin cancer

Prior exposure to chemotherapeutic drugs (e.g., cyclophosphamide (39), multi-tyrosine kinase inhibitors (40, 41))

Prior chest radiation

Prior chemotherapy

High altitude exposure (duration)

Estrogen containing medication exposure (duration)

History of DVT or PE or hypercoagulability

Relevant surgical history:

- Cardiac surgery (type, date)
- Previous atrial septostomy (date)
- Pulmonary surgery (type, date)
- Gastric bypass or banding (type, date)
- Pacemaker
- Defibrillator

4.2.4 Medications

A complete list of medications including dose, and over the counter or homeopathic products should be compiled within 1 week of study entry. For intravenous or subcutaneous prostanoids provide dose range, duration and peak dose (ng/kg/min). Oxygen use should be described, including description of dose and when used (night, day, exertion, and approximate number of hours per 24 hours). Historical PH therapy should be noted. For medications of special interest (diuretics, any medications for heart or lung disease including pulmonary hypertension, use of oxygen, and anticoagulants, the approximate duration of therapy should be categorized (less than 2 weeks, between 2 weeks and 3 months, 3 months to 12 months, or greater than 1 year).

4.2.5 Physical Measurements

Vital Signs

Height, weight, Heart rate, rhythm, seated blood pressure, oxygen saturation (room air, or specify oxygen amount). For patients with CHD (PDA) provide pre and post ductal saturation (Right Arm and leg).

Waist – Hip Ratio

The waist circumference will be measured at the top of the iliac crest as illustrated in the Manual of Operations. (42)

Body Composition by Bioelectrical Impedance Analysis (43, 44)

Bioelectrical impedance analysis (BIA) will be performed utilizing the Tanita 240S single frequency device (Tanita Corporation of America, Arlington Heights, Illinois), except at Vanderbilt, which may utilize the Tanita BC418, which is capable of the same measurement technique. BIA will not be performed in patients with implantable pacemakers, defibrillators, or electrical pumps.

4.2.6 Lung Function Measurements (45-49)

Spirometry, lung volumes and DLCO will be performed in all subjects unless test data from within the past year is available. Subjects with evidence of parenchymal lung disease will be required to have PFTs performed within 3 months of enrollment. PFTs are only acceptable if done at a PVDOMICS-approved site using standardized, validated and agreed upon protocols. Total lung capacity will be done by body plethysmography. Repeat PFTs closer to time of enrollment will depend on clinical evaluation of possible change in functional status as assessed by the investigator.

4.2.7 Six Minute Walk Test (50)

Six minute walk test will be performed in accordance with American Thoracic Society guidelines. Resting O2 sat and heart rate will be obtained and again at the end of the walk test. A Borg Dyspnea score will also be acquired.

4.2.8 Overnight Sleep Monitoring (51)

Overnight sleep monitoring will be performed routinely in all enrollees to acquire standardized baseline information on sleep disordered breathing and to quantify overnight oxygen saturation patterns. Patients already receiving oxygen supplementation and/or positive airway pressure therapy on a regular, nightly basis will be studied during use of these. A portable home monitoring system (Nox T3, Carefusion) will be used, with recordings of nasal airflow, chest wall and abdominal motion as well as pulse oximetry, heart rate, snoring and position. These measures will permit scoring of central and obstructive apneas and hypopneas as well as to quantify level of oxygen desaturation. Each center will purchase the equipment necessary to perform these studies, and study personnel will be trained in the proper application of the

devices. The results of the portable sleep monitoring will be provided to physicians caring for the patients. The results of prior sleep studies as well as any additional testing or interventions obtained as a consequence of the portable monitoring will be recorded. Sleep studies will be scored using standardized methods for home sleep apnea testing and will conform to American Academy of Sleep Medicine guidelines. The summary data will be electronically transmitted to the DCC for integration into the database and for statistical analysis If this information is available on a clinical basis within 1 year prior to study entry and with no change in nighttime oxygen or sleep disorder therapy, and in the opinion of the investigator, no change in patient condition that would impact results, then the clinically available data may be considered adequate for inclusion in the case report form – if available, a copy of that study be acquired for central review, quality assessment and scoring.

4.2.9 Electrocardiogram (3)

All participants should have an electrocardiographic test (12 lead ECG) done after enrollment.

4.2.10 Imaging Studies

4.2.10.1 Echocardiography

Inclusion criteria:

All subjects considered for participation in the PVDOMICS study as either PH or non-PH comparators or controls will undergo a standardized transthoracic Echo exam (complete Echo protocol is available for review in the MOP).

Exclusion criteria:

- Clinical or hemodynamic instability requiring immediate therapy
- Inability to communicate with the sonographer/follow commands for any reason and/or provide consent (psychosis, agitation, etc.)

The PVDOMICS Echo protocol consists of the mandatory clinical protocol (based on the standard ASE views), and an optional research protocol with 3D full volume LV and RV dataset. Study subjects without known congenital systemic to pulmonary shunts, who did not have an agitated saline test on their prior Echo, will need to undergo a peripheral IV line insertion and injection of the agitated saline ("bubble study") in order to rule out intracardiac shunt.
The Echo for the study will be performed only by the sonographers certified and trained in the common and optional protocols based on the sonographer's PVDOMICS Echo manual (see the Manual of Operations).

The sites' Echo reports and images of the study subjects will be readily available for clinical decision making by the local clinical teams and study investigators.

All of the Echo studies will be collected as DICOM files and uploaded by the local study coordinators into the Echo Core Lab server for further assessment. The Echo variables for the PVDOMICS dataset inclusion will be derived solely from the Core Lab data.

Justification

The Transthoracic Echocardiography will objectively reveal: (52-66)

- Anatomy and morphology of the cardiac chambers
- Presence or absence of structural heart disease
- Hemodynamic status, volume status and functional performance (systolic and diastolic) of the RV and the LV
- Clinical features predominantly consistent with the WHO Group 2 PHT.
- Gross anatomy and morphology of great vessels (aorta; main pulmonary artery)
- Findings suggestive of intracardiac shunt
- Presence or absence of a pericardial effusion

4.2.10.2 Cardiac MRI

Inclusion criteria:

All subjects considered for participation in the PVDOMICS study as either PH or non-PH comparators or controls will undergo a standardized Cardiac MRI exam (complete MRI protocol is available for review in the MOP).

Exclusion criteria:

- Standard MRI contraindications; these may vary among the participating sites and may depend on local policies/experience (example – scanning patients with permanent pacemakers/ICDs)
- Inability to obtain reliable gating data during the scan (significant arrhythmias with highly irregular RR intervals (atrial fibrillation); significant tachycardia in NSR (HR >120 bpm)
- Clinical or hemodynamic instability requiring immediate therapy; severe dyspnea with inability to lay flat/breath hold.
- Inability to communicate with the MRI technician/follow commands for any reason (psychosis, agitation, etc.)

The PVDOMICS Cardiac MRI protocol consists of the mandatory common part and optional part (please review the MOP).

The common MRI protocol will include the following:

- Assessment of cardiac structure and function (SSFP sequences)
- Hemodynamic assessment of valvular flow (phase-contrast imaging), including Qp/Qs
- Tissue characterization, evaluation of inflammation, assessment of possible cardiac masses/thrombi (T1 and T2 imaging)
- Assessment of myocardial scar/fibrosis (delayed Gadolinium hyperenhancement imaging)
    - Non-contrast cMRI should still be performed in subjects selected with ESRD with GFR<30, or gadolinium allergy.
- Assessment of the pulmonary artery anatomy

The optional MRI protocol will include the following:

- Assessment of extracellular volume fractionation (using pre-contrast T1 mapping)
- Pulmonary artery compliance and vessel wall remodeling (high resolution sequences)

The sites' MRI reports and images of the study subjects will be available for clinical decision making and research considerations for the local clinical teams and study investigators.

All PVDOMICS MRI studies will be collected as DICOM files and uploaded by the local study coordinators into the MRI Core Lab server for further assessment. The MRI variables for the PVDOMICS dataset inclusion will be derived from the Core Lab data.

Justification

Cardiac MRI will objectively reveal: (60-66)

- Fine details of the anatomy and morphology of cardiac chambers
- Functional performance (systolic and diastolic function) of the RV and the LV; reproducible volumetric data
- Clinical features consistent with the WHO Groups 1, 2, 4 and 5.
- Anatomy and morphology of great vessels (aorta; main pulmonary artery)
- Findings suggestive of intracardiac shunt (anatomy; flow; Qp/Qs)
- Presence of scar/fibrosis of the LV and/or RV

4.2.10.3 High Resolution Chest CT (7, 67)

Non-contrast high resolution chest CT should be performed in all patients, unless the patient refuses. This can be done within one year prior to enrollment if the images are available to the DCC.

4.2.10.4 Ventilation Perfusion Lung Scan (3, 7, 67)

All patients are required to have a ventilation-perfusion lung scan for detection of possible pulmonary emboli. V/Q scans performed within one year prior to enrollment can be used provided a source document is made available to the DCC.

4.2.11 Right Heart Catheterization

Right heart catheterization (RHC) is performed to confirm the diagnosis of PH, establish the severity of disease and response to therapies, and assess prognosis. (3, 68-72) All WHO Group 1-5 patients will undergo right heart catheterization as well as individuals in the comparator groups. The study is expected to provide an assessment of right heart and pulmonary artery filling pressures, pulmonary vascular resistance, cardiac output, and compartment-specific oxygen saturations. All RHCs will be reviewed by the study's central RHC Core.

During the right heart catheterization, pulmonary vasodilator testing will be done by acute challenge with 100% oxygen, inhaled nitric oxide, and fluid loading if no contraindications. (Fluid loading will not be performed if right heart catheterization is coupled with invasive cardiopulmonary exercise testing). Blood samples will be collected for –omics testing.

A. General:

1. RHC to be performed using a minimum size 6F balloon occlusion catheter – this will prevent shearing and activation of blood cells during sample acquisition.

2. Administration of heparin should be avoided; if patient is on heparin or low molecular weight heparin, the drug should be stopped at least 30 minutes or 8 hours, respectively, prior to catheterization unless medically necessary.

B. Measurements:

The following measurements or calculations detailed below will be collected as appropriate. Each hemodynamic measurement should be performed at each stage and should be made both during spontaneous breathing and during a brief expiratory pause, taking care to avoid Valsalva:

1. Vital signs
   a. Systemic blood pressure – systolic, diastolic, mean
      recorded as close as possible to time of PA pressure recording
   b. Heart rate and rhythm – recorded at time of PA blood sample
   c. Hemoglobin

d. BSA.

2. Pressure measurements:
   a. Mean right atrial pressure (mid-A wave), peak A wave, peak V wave pressures
   b. Right ventricular systolic, minimal, and end-diastolic pressures
   c. Pulmonary artery systolic, diastolic and mean pressure
   d. Mean pulmonary capillary wedge pressure – mid a wave, peak a wave, peak V wave (73)
   e. Diastolic pressure gradient pull back (wedge to PA during quiet, held end-expiration).

3. Oxygen saturations obtained during baseline pressure measurements:
   a. Mixed venous oxygen saturation – should be obtained from the pulmonary artery
   b. Superior vena cava and pulmonary artery as screen to rule out shunt
   c. Arterial if line in place; if not, finger oximetry saturation.

4. Blood samples obtained for -OMICs: volumes/types of tubes determined from OMICs protocol.
   a. Peripheral venous (26.5 ml)
   b. Pulmonary artery (6.5 ml)
   c. Pulmonary capillary wedge (6.5 ml)
   d. Arterial – only if line already in place (6.5 ml).

5. Thermodilution/Fick cardiac output:
   a. All sites will obtain thermodilution cardiac output (TDCO). A direct Fick cardiac output will also be obtained if the study site is able to do so.
   b. Record VO_2 (either directly measured or assumed)
   c. The following will be calculated:
      Cardiac index
      Calculate stroke volume: SV = CO (TD and/or Fick)/HR
      Calculate SV/PP (stroke volume/pulse pressure)
      Pulmonary vascular resistance (PVR)
      Systemic vascular resistance (SVR)
      Right ventricular stroke work index (RVSWI)
      Diastolic pulmonary vascular gradient (DPG)
      Pulmonary to systemic flow ratio (Qp/Qs).

<u>C. Challenges (74-76)</u>

1. 100% Oxygen (77)
   a. Performed in all patients except with known CO_2 retention
      pH < 7.32 AND pCO2 > 50 mmHg – contraindicated
      pH > 7.32 AND pCO2 > 50 mmHg – at the discretion of the physician
   b. Inhale 100% O_2 for 5 minutes – through a face mask
   c. Record measurements from B1, B2, B3, and B5

d. No washout period required.

2. Inhaled NO (78-83)

a. Performed in all patients except with PCWP ≥ 25 mmHg
b. Inhale iNO 40 ppm (added on to 100% oxygen) for 5 minutes
c. Record measurements from B1, B2, B3, and B5
d. Stop iNO and 100% oxygen
e. 5 minute washout period.

3. Fluid challenge (84-86)

a. Performed in all patients except with baseline RA ≥ 15 or PCWP ≥ 18 mmHg or having an invasive CPET (selection likely based on center availability of invasive CPET)
b. Infuse 500 ml of 37°C 0.9% saline intravenously over 5 min
c. Record measurements from B1, B2, B3, and B5
d. Complete study.

Vasoresponders

Recognizing that acute vasoreactivity testing at the time of right heart catheterization has been primarily defined by relatively arbitrary criteria to individuals who have non-Group 2 PH, we will assess the phenotype of 'responder' to acute vasoreactivity testing. The response to vasoreactivity testing at the time of right heart catheterization will be collected. A priori, there will be a classification assigned as 'responder' or 'nonresponder' based on the change in the pulmonary artery pressure from select agents such as inhaled nitric oxide. In PVDOMICs, we will use current guidelines to classify the participant as a **responder** to the challenges if there is a fall in the mean pulmonary artery pressure (mPAP) from baseline of 10 mmHg or greater to a mean pulmonary artery pressure below 40 mmHg, without a fall in cardiac output. As a goal of PVDOMICs, we will collect data for evidence-based assignment to a responder phenotype class of PVD.

4.2.12 Cardiopulmonary Exercise Testing (CPET) (87-94)

Patients and age and gender matched sedentary controls absent the exclusion criteria below will undergo a maximum incremental non-invasive CPET or an invasive CPET. To ensure consistent stress and measurements, all studies should be performed in an upright cycle ergometry test.

Exclusion criteria:

- Current exercise-induced angina
- Inability to cycle (e.g., arthritis)
- Exertional syncope
- Known potentially lethal exercise induced arrhythmia (e.g., ventricular tachycardia)
- Severe symptomatic aortic stenosis
- Resting systolic blood pressure ≥ 200 mmHg

The non-invasive CPET will consist of:

- Maximum incremental cycling to exhaustion with:
    - Breath by breath metabolic cart measurements of ventilation and pulmonary gas exchange
    - Pulse oximetry
    - 12 lead ECG every minute

At some of the centers the CPET will be invasive (90), adding:

- Radial artery line (optional)
- IJ or brachial vein pulmonary artery line
- Hemodynamics (RAP, PAP, PCW and AO, if available) obtained at each workload increment. These measurements will be captured during spontaneous breathing and during a brief expiratory pause if possible.
- Cardiac output can be obtained by thermodilution (all sites) or direct Fick (if possible.
- Blood collection from the pulmonary artery and the arterial line should be drawn at rest (during the initial right heart catheterization), peak exercise, and 15 minutes post-exercise. At each time point, 6.5 ml of blood should be drawn from the pulmonary artery and, if one is already in place, the arterial line.

Justification:

- The non-invasive CPET will objectively determine the degree of exertional impairment (VO_{2peak}, AT, OUES) and rule out a pulmonary mechanical limit (e.g., Group 3). It will also estimate disease severity (ventilatory efficiency) (92) and suggest Group 1 vs. 2 during exercise ($PETCO_2$ change) (93).
- The invasive CPET will may further define the exercise phenotype (Group 1 vs. 2) and determine the role of limb skeletal muscle dysfunction (by systemic O2 extraction), and in mixed disease (i.e., combined Group 1 and 3) determine the functional limitation by each contributor. Additionally, CPET can indirectly quantify gas exchange abnormalities pertinent to pulmonary vascular disease such as dead space and A-a gradient.

4.2.13 Laboratory Measurements

Laboratory measurements and OMICS data will be integratively analyzed to understand the molecular phenotype of pulmonary hypertension. Laboratory measurements will be performed as necessary and may include (but not limited to): a complete blood count panel, metabolic panel (Glucose, Calcium, Albumin, Total Protein, Sodium, Potassium,CO2, Chloride, BUN, Creatinine, ALP, ALT, AST, Bilirubin, Insulin), Cholesterol and Lipid panel (Total cholesterol, HDL, LDL, Triglycerides), Cardiac injury panel (NT-ProBNP, Cardiac Troponin), inflammation

panel (MPO, C-reactive protein), iron metabolism panel (Iron, Ferritin, Transferrin) and rheumatoid factor. Historical tests may be provided for HIV and hepatitis.

4.3 Biospecimen Collection

Blood (cells, serum, plasma) and urine samples will be collected for the molecular omics analysis. Molecular network analysis incorporating genetics, genomics, epigenomics, proteomics, metabolomics and coagulomics may be done in an unbiased manner but may also take into account:

- endothelial and smooth muscle cell biology with BMPR2 (18, 95, 96), KCKN3 (and other ion channels) (97-101), endothelin (102-106), angiotensin (107, 108) , and serotonin (109-114) mediator networks and circulating progenitor cells (115, 116) that mark injury and repair processes
- hypoxia signaling pathways (117-122)
- coagulation: initiation of coagulation (procoagulant activity), coagulation cascade, fibrinolysis (123-132)
- markers of heart health(133-136) (BNP / ANP peptides; troponin), braveheart RNA (137)
- function of hormone receptor signaling (e.g. estrogen receptor, aldosterone receptor) (138-143)
- cancer-like processes (144-147)
- arachidonic acid signaling network (148-150)
- nitric oxide synthases (NOS1-3) vs. arginase balance and phosphodiesterase 5 - determinants of nitric oxide and guanylyl cyclase signaling (151-157)
- metabolic shift in lung, vascular, heart and immune cells with mitochondrial remodeling; metabolic syndrome, insulin resistance and type II diabetes (158-167)
- matrix remodeling of the lungs, the pulmonary blood vessels and the heart (168-171) (proteases to anti-protease imbalance; fibrosis)
- oxidative stress in the cell environment and intracellular imbalance of the redox system (172-175)
- responses by the network hubs of Interleukin (IL)– 1 (176) and Tumor necrosis factor – TNF (177-179) super-families
- (131, 180-183) immune response mediators (network hubs of IL-13 / IL-4 including resistin like molecule and the IL-33 receptor ST2 (135, 184-190); IL-17A (187, 191); IL-6 (176, 177))
- Interferon response (189, 192-197) (network hubs of type-I Interferons (IFN) and IFNγ) (198-202)

Details of amounts and types of specimens are given in the Manual of Operations.

4.4 Longitudinal Follow-up

All patients, or their designated contacts, will be contacted by telephone and/or letter at least annually after enrollment up to the end of the study (maximum of 3.5 years) if not seen at the enrolling center for follow-up. Vital status and occurrence/date of any lung, heart or heart-lung transplantation will be determined from this contact or by using center medical records. Cause of death will be ascertained by the site investigators.

5. Statistical Considerations

We propose the following plan for discovering PVD types and subtypes using phenotype and molecular measurements:

(1) When 750 subjects have been recruited, the first half of the anticipated study cohort, and their phenotypic variables have been measured, including demographic and clinical variables, we will perform model-based unsupervised clustering on this phenotypic data to cluster subjects into novel phenotype-derived groups. Akaike Information Criteria (AIC) (203) and Bayesian Information Criteria (BIC) (204) will be used to select the number of clusters. Based on a preliminary analysis of 1900 subjects at the Cleveland Clinic, which have a subset of the planned PVDOMICS phenotypic variables, we expect to derive four to six phenotype groups, with the minimum group percentage being greater than 10%.

(2) 50 subjects will be chosen from each phenotype group in such a way as to maximize variability across phenotype variables within each group. A full battery of omic measures, say M variables, will be taken on biological samples from this subset, the omic discovery subject subset (ODSS), with specific choices of biological layers (e.g., RNA, DNA, metabolome) and platforms (e.g., sequencing, microarrays) to be determined.

(3) Each omic variable will be tested for differences between each pair of phenotype groups using the data from the ODSS. Power and expected false discovery rates for these comparisons assuming M=20,000 omic measures are given in Table 5.1. Omic variables will be ranked by their standardized effect sizes of differences between phenotype groups and their variability within phenotype groups. Ranks will be combined using rank aggregation methods to generate a single ranked list. The rank list will be pruned to account for correlations among omic variables. Using the filtered rank list and considerations of cost and feasibility, P1 of the M omic variables will be selected for measurement in all first-half subjects not in the ODSS, which we label NODSS1. We anticipate NODSS1 will consist of between 300 and 500 subjects and expect P1 to be much smaller than M.

(4) After measuring the selected P1 omic variables in the NODSS1, clustering of the P1 omic variables within each phenotype group, i.e., a form of supervised clustering, will be done to find omic-based subtypes using the combined ODSS+NODSS1 subject sets. In addition, step (3) above will be repeated using the combined subject sets and P1 omic variables on both the phenotype groups alone and the omic-based subtypes to validate and filter the P1 omic variables.

A smaller number of omic variables, P2, may be chosen for measurement in the remaining half of the study, labeled NODSS2, ~750 subjects.

(5) Finally, after measuring the phenotype variables and P2 omic variables on the NODSS2 subject set, step (4) will be repeated on the combined ODSS+NODSS1+NODSS2 set and on the NODSS2 set alone. Adjusted Rand Index will be used to measure cluster stability of ODSS+NODSS1 derived types and subtypes in the NODSS2 set.

Biological validity of the derived types and subtypes will be assessed by associating cluster memberships with clinical outcomes, including survival times and event rates. We decided to wait to form phenotype-based clusters until half of the proposed study size (1500) has been enrolled to have a high probability (estimated from our preliminary data) of getting at least 50 subjects in each cluster. Our power calculations in Table 5.1 show that power and FDR for discriminating omic variables between clusters fall below tolerable levels when there were less than 50 subjects in a group. In those calculations, we assumed 20,000 omics variables were measured in Step (2). While global DNA and mRNA measurements might be considered at this step, leading to many more than 20,000 measures, we expect due to cost and information considerations to measure a focused subset of these variables potentially combined with miRNA and other lower dimensional omics platforms (e.g., protein arrays, metabolomics, coagulomics) (205, 206). For example, if 10% of the 20,000 omic variables differ between 2 groups, with 50% of these variables having a large effect, when performing testing at the 0.01 alpha level we have 76% power to detect a medium to large effect and overall FDR of 13% with 50 subjects per group compared to just 44% power and overall FDR of 21% with 25 subjects per group. Measuring all M omic variables on more than 50 subjects per group can substantially increase power as shown in Table 5.1, but there may be insufficient funding to do complete measurement in more than 50 samples per cluster assuming we find 4-6 phenotype clusters. If that situation changes, either due to increased funding or decreased costs, we will consider increasing the omic sample size per group. We propose to do multiple filtering steps on the omic variables to help maximize the information content while minimizing costs. The extent of the filtering will depend again on funding and costs per measure.

Our primary strategy is to first cluster/group by phenotypes in a larger subject set, determine the most informative omic variables in a subset, and then determine molecular subtypes in the larger set using the informative markers. This strategy fits well with the study's primary Aims 1 and 2, as Aim 1 is focused on understanding phenotype measures while Aim 2 is focused on exploiting molecular measures. We will also perform cluster analyses where we cluster first with the omic variables (in Steps (2), (4) & (5)), then either associate phenotypes with these molecular clusters or attempt to derive phenotypic subtypes within them. We will compare the phenotype-then-omic to the omic-then-phenotype approaches based on biological validity, interpretability and cluster overlap.

5.1 Statistical Power for Specific Hypotheses

Table 5.1 gives power and expected false discovery rates (FDR) for two-group comparisons for a mix of effect sizes, group sizes, and alpha levels assuming 20,000 variables are measured. The R package pwr was used to make the calculations. The nominal per-test alpha levels do not account for multiple testing among cluster groups but since we are primarily interested in filtering omic features and wish to include features that may only separate two of the groups, we are less concerned about Type 1 errors. This table is useful for justifying our choice of 50 subjects to measure complete omics in Step (2) as discussed above.

Table 5.2 gives minimum detectable fold changes (MDFC) between two independent groups for various number of features tested, levels of variability (as measured by a common coefficient of variation), feature intensity (as measured by average number of sequencing reads per feature) and sample sizes. A desired power level of 80% and a conservative multiple test level per feature of 0.10/# of assumed features were used in the calculations. The R package RNASeqPower, which assumed that features followed a conditional negative binomial distribution and accounted for sequencing based measurement error, was used to estimate the power of the designs. This table can be used to assess our ability to detect meaningful differences in phenotype variables at Steps (1)-(5) across phenotype clusters, assuming 50-500 phenotype variables. For example, if a set of 50 phenotype variables are tested between phenotype-derived clusters (or across *a priori* defined groups like WHO categories), we have 80% power to detect a fold change as small as 1.32 between groups assuming 100 subjects per group (cluster), phenotype variables are measured with very little error (Feature Intensity=High) and variability across subjects is medium (cv=0.5). This table can also be used to assess the MDFCs for omic variables measured in Steps (2)-(5). For example, if after filtering in Step (3), we select P1=5000 omic variables to measure in Step (4), we have a MDFC of 1.43 between two groups for say a gene expression measurement assuming 40 subjects per group (subtype), gene expression is medium and variability across subjects is small. Most of the MDFCs in Table 5.2 were below 2 for low to medium variability scenarios, indicating that our designs in these scenarios are reasonably well powered to find realistic fold changes from human samples.

Table 5.1. Average power and expected false discovery rate for two-group t-tests by percentage of true effects, size of true effects, alpha-level per test and sample size per group assuming 20,000 tested variables and the use of two-sample t-tests per variable with pooled standard deviation.

			Sample Size Per Group (N)								
			N=25			**N=50**			N=75		
% of Assoc Vars	Effect Size Dist	Alpha Per Test	FDR (%)	Ave Power (%)	Med/Large Power (%)	FDR (%)	Ave Power (%)	Med/Large Power (%)	FDR (%)	Ave Power (%)	Med/Large Power (%)
5	25/50/25	**0.01**	44	24	32	**29**	**47**	**61**	24	61	78
5	25/50/25	0.05	69	43	54	60	63	79	56	74	91
5	25/50/25	0.10	78	53	65	73	71	86	71	79	95
5	25/25/50	**0.01**	36	34	44	**25**	**58**	**76**	22	68	88
5	25/25/50	0.05	64	52	66	57	70	88	55	77	95
5	25/25/50	0.10	78	53	65	73	71	86	70	81	97
10	25/50/25	**0.01**	27	24	32	**16**	**47**	**61**	13	61	78
10	25/50/25	0.05	51	43	54	42	63	79	38	74	91
10	25/50/25	0.10	78	53	65	73	71	86	71	79	95
10	25/25/50	**0.01**	21	34	44	**13**	**58**	**76**	22	68	88
10	25/25/50	0.05	46	52	66	39	70	88	37	77	95
10	25/25/50	0.10	59	62	76	54	76	93	53	81	97

% of Assoc Vars = percentage of tested variables with non-zero effect. **Effect Size Dist** = percentage of non-zero effects in each of 3 Cohen effect size groups: small, medium & large, given in the format small %/medium %/large %. **Alpha Per Test** = type-I error rate for 2-sample t-test per variable. **FDR** = expected percentage of tested variables that are rejected at the specified type-I error rate Alpha but have zero effect among all rejected tests. **Ave Power** = power to reject a null hypothesis when the variable has a non-zero effect averaged across the entire effect size distribution. **Med/High Power** = power to reject a null hypothesis when the variable has a non-zero effect average across the medium and large effect size groups.

Table 5.2. Minimum detectable fold change by subgroup size, feature intensity, feature variability and number of features assuming desired 80% power, alpha level per test of 0.10/# of features.

		Large Subgroups: 100 vs 100			Medium Subgroups: 40 vs 40			Small Subgroups: 10 vs 10		
	Feature Intensity	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
# Features	**Variability**									
20000	Low: cv=0.3	1.40	1.27	1.26	1.69	1.47	1.44	2.87	2.15	2.07
20000	Medium: cv=0.5	1.57	1.48	1.47	2.04	1.85	1.83	4.18	3.43	3.36
20000	High: cv=1	2.23	2.16	2.15	3.55	3.37	3.35	12.63	11.36	11.23
5000	Low: cv=0.3	1.37	1.26	1.24	1.64	1.43	1.41	2.71	2.06	1.99
5000	Medium: cv=0.5	1.53	1.45	1.44	1.97	1.79	1.77	3.86	3.20	3.14
5000	High: cv=1	2.13	2.07	2.06	3.31	3.15	3.13	10.97	9.93	9.82
500	Low: cv=0.3	1.32	1.23	1.21	1.56	1.38	1.36	2.43	1.91	1.85
500	Medium: cv=0.5	1.46	1.39	1.38	1.83	1.68	1.67	3.34	2.83	2.78
500	High: cv=1	1.97	1.91	1.91	2.91	2.79	2.77	8.49	7.77	7.70
50	Low: cv=0.3	1.27	1.19	1.18	1.47	1.32	1.30	2.15	1.74	1.70
50	Medium: cv=0.5	1.39	1.33	1.32	1.68	1.57	1.55	2.83	2.45	2.41
50	High: cv=1	1.79	1.75	1.74	2.51	2.42	2.41	6.32	5.85	5.81

Feature Intensity Low=10 reads per feature
Medium=100 reads per feature
High=1000 reads per feature

Variability cv=coefficient of variation

5.2 General Methods

In general, all quantitative variables will be summarized with standard descriptive statistics and represented graphically with displays such as box plots, histograms and density function plots.

5.3 Methods by Aims

Aims 1 and 2a analyses will focus on the comparison of quantitative variables between current WHO classifications, including comparisons to non-PH comparators and controls. The first part will be comparing demographical, lab and other clinical variables. ANOVA will be used to compare continuous variables and the chi-squared test will be applied to categorical variables. Highly skewed variables may be transformed appropriately. In addition to comparisons across existing classifications, we will also build prediction models for WHO classes plus non-PH comparators using sparse regression methods (such as Elastic Net), random forests and multiple fractional polynomials (207) in an effort to gain understanding of the predictability and drivers of the existing systems. The second part will mainly be comparing various omic data, including differential analysis, test for known PH mutations and new genetic variants, etc. Large amounts of omic data will be generated by the Omic Cores. Much of the data will be high dimensional, on multiple biological layers, *e.g.,* transcriptome and metabolome, measured on tissues, *e.g.,* blood, from the same set of subjects. For example, we may receive mRNA-seq and Nanostring nCounter miRNA measures of human blood transcriptome or mass spectrometry based measures of the proteome and metabolome. Each layer presents challenges, sometimes unique, to obtaining meaningful and reproducible inferences from relevant measures of that layer. However, there is a common set of four broad steps we will apply to data from each layer to address these challenges: (1) data acquisition, (2) quality checking and preprocessing, (3) summarization and annotation and (4) statistical analysis. For example, with mRNA-seq data, the four steps may involve (1) acquisition of fastq files generated by the sequencer, (2) trimming of poor reads and alignment to the human reference genome or transcriptome to obtain bam files, (3) counting aligned reads by Ensembl annotated genes or exons and (4) differential expression analysis of gene or exon counts. We will attempt to incorporate the omic data into the prediction models built using the clinical data.

Aim 2b analyses will aim to develop a new and more accurate classification of PH. To discover molecular-based subtypes of PH, we will apply appropriate clustering or biclustering methods to each layer, including methods that give estimates of the optimal number of subtypes such as Bayesian mixture models, followed by feature reduction/selection to aid in interpretability and reproducibility. Development of molecular-based subtypes and identification of features driving the subtypes will aid in the development of a controlled vocabulary and new PH ontologies. When feasible, we will attempt to build layer-specific network representations (208), *e.g.,* co-expression or mutual information networks from transcriptome data, to aid in understanding the layer complexity and degree. Results from each layer will be compared and contrasted both analytically and visually (209), *e.g.,* by overlaying networks using Cytoscape. In addition, data

from each layer will be associated with clinical outcomes and endophenotypes using sparse regression methods to gain insight into the predictability of important downstream measures. Because inferences from high-dimensional data are particularly prone to false discoveries and non-reproducibility, and because we expect to get data from a large number of subjects over multiple years (with some data not being produced for all subjects due to cost or tissue availability), we will *partition the work for each layer into two phases: discovery and validation*. In the discovery phase we will analyze data from subjects obtained in the early part of enrollment, with the goal of finding subtypes, biomarkers and endophenotypes for later validation, while in the validation phase we will analyze data from later subjects and measures obtained at follow up(s). For example, we may build PH subtypes from mRNA-seq expression profiles of for example, 300, early-recruited subjects, and then later validate the consistency and relevance to clinical follow-up outcomes, such as death, of these profiles in the remaining subjects with lower dimensional/less expensive measures.

While per-layer analysis will provide insight into PH/PVD pathobiology, the full power of the proposed study comes from integrative analysis of the multiple biological layers, including the clinical data layer. For example, while important changes in activity of some genes may be reflected in their mRNA levels, other genes may show changes in protein levels or states but no changes in mRNA level, perhaps through protein degradation/instability rather than protein production. Integrative analysis is often more challenging than analysis of individual layers. Recent methodological developments, mainly arising from similar desires of cancer and cardiovascular researchers, have shown the feasibility and power of an integrative approach. We will use the latest methods including Bayesian machine learning methods, multivariate partitioning methods, sparse integrated clustering methods, and graphical models (210) to maximize our ability to find meaningful PH subtypes, biomarkers and endophenotypes that use the full spectrum of information available from multiple layers across samples. We will closely monitor this fast moving area to ensure the best methods are chosen. For example, a recent method that seems well suited to discovery of disease subtypes using multiple omic datasets involves sparse integrative clustering.(211)

Aim 3 will examine the associations between various molecular biomarkers and longitudinal outcomes. The mixed-effects models will be applied to repeated-measurement type of outcomes, which include random effects to account for correlations of measurements within the same subject. According to the variable type of the outcome, linear or generalized mixed models will be used appropriate. The Cox models will be applied to time-to-event outcomes, e.g., death, responses to therapy, etc. The proportional hazard assumption will be tested be checking the Schoenfeld residuals.

Aim 4 to compare PVDOMIC genetic data with that of the Nichols R24 and other available databases. These analyses will be mainly descriptive by appropriately summarizing and

annotating the genetic variants identified from the PVODOMIC database and the Nichols R24 and other available database.

5.4 Missing Data

Maximum efforts will be made to minimize missing data. If necessary, the chained-equation approach (212) will be used to impute missing values in all variables included in the analyses.

5.5 Software

All analyses will be performed with the open source R statistical and bioinformatic software. The R environment includes cutting edge packages, many from the Bioconductor project, to perform the bioinformatics analysis for every combination of biological layer, data type and analytical goal we expect to see. In addition, R has strong interconnections to other important bioinformatic and statistical tools and data sources, including Cytoscape for visualization, SAS for statistical analysis, Oracle for database management and Ensemble for biological annotation.

6. Regulatory Considerations

6.1 Institutional Review Boards (IRBs)

The protocol will be submitted to the IRB of each clinical center for review and approval. Centers may not recruit patients into the study until they have been documented as being ready to enroll and their protocol has been approved by their IRB. Protocol amendments and changes will be submitted to the IRB and approval must be received before implementation. All patients enrolled in the study must sign and date an IRB-approved consent form and medical records release form before any study related procedures are undertaken. Study personnel will explain the study and answer all of the patient's questions before asking the patient to sign and date the consent form.

The Investigator will provide the Institutional Review Board (IRB) with all requisite material, including a copy of the protocol, the informed consent and assent documents. The study will not be initiated until the IRB provides written approval of the protocol, the informed consent form, and the subject assent form, and until approved documents have been obtained by the Investigator and copies received by the Sponsor. Appropriate reports on the progress of this study by the Investigator will be made to the Institutional Review Board (IRB) and the Sponsor in accordance with the applicable government regulations and in agreement with policy established by the Sponsor.

6.2 Confidentiality and Ownership of Data and Biospecimens

All data and biospecimens will be maintained in secure locations. Data and biospecimens collected from study evaluations will be identified by study identification codes. Identifying

features including names and addresses will be known to the clinical center, but will not be provided to the Data Coordinating Center (DCC) at the Cleveland Clinic.

The study will be conducted in compliance with applicable ICH guidelines, the ICH E6 GCP guideline, and regulations, guidelines, and applicable laws of the local site where the study is conducted. The study will be conducted with the approval of a duly constituted IRB/EC in accordance with the requirement of US regulation Title 21 CFR Part 56-Institutional Reviews Boards. The nature and risks of the study will be fully explained to each subject and written consent obtained in accordance with the requirements of 21 CFR 50-Protectionof Human Subjects. Subjects will be informed of their rights, including the right to withdraw from the study at any time.

Use of study data and biospecimens, and resulting findings, during the PVDOMICS study are subject to approval of the PVDOMICS Steering Committee and follow guidelines of the Publications and Ancillary Study Committee. The data and biospecimens from subjects at a clinical center are the property of the PVDOMICS study and are under the custody of that center. The data and biospecimens are transmitted to the PVDOMICS DCC, but cannot be moved to another institution unless approved by the PVDOMICS Steering Committee; for example, if the Principal Investigator of the center moves to another place. At the conclusion of the PVDOMICS study, the data and remaining biospecimens will be transferred to a PVDOMICS specified biobank and/or to the National Heart, Lung and Blood Institute (NHLBI) Biological Specimen and Data Repository Information Coordinating Center (BioLINCC) biobank and become the property of one of these organizations. Future researchers may request data or biospecimens from a biobank with approval following the guidelines of the biobank.

6.3 Subject Information and Consent

A properly executed, written informed consent in compliance with national and local regulation and Good Clinical Practice (GCP) guidelines will be obtained from each prior to entering the study or performing any study-related procedures that are not part of the subject's standard care. The Investigator will submit a copy of the informed consent document to the Institutional Review Board (IRB) for review and approval before research subjects are enrolled. The Investigator will provide a copy of the signed informed consent form to the subject and the originals will be maintained in the subject's study binder.

6.4 Adverse Events

A. Definition of an Adverse Event

Adverse event. An adverse event (AE) shall be considered any detrimental change in the patient's condition.

Anticipated adverse event. Anticipated adverse events are defined for each of the protocol procedures in the sections specifically written for those procedures.

Unanticipated adverse event. Any adverse event that results in risk or harm to the subject or others that differs from the known, predicted possible effects of the research protocol. An unanticipated adverse event is one that varies in nature, intensity, or frequency from information in the informed consent document.
Serious adverse event. Any event that results in death, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly or birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

B. Monitoring of adverse events related to the study

The occurrence of an AE may come to the attention of study personnel during study visits or telephone interviews or by a patient presenting for medical care. The AEs that are expected from participation in the study are given in the consent form.

6.5 Incidental Findings

In this study, research subjects, at risk cohort comparators, true controls and all five WHO groups of pulmonary hypertension patients will be recruited. Subjects will undergo many tests as part of the study protocol, including but not limited to, overnight sleep monitoring, complete PFTs, echocardiography, cardiac MRI, chest CT, blood work and ventilation perfusion scan. Each subject will identify a primary care provider and their contact information upon entrance into the study. This will be kept in the research file at each site by the research coordinator. Although these research tests are part of a research protocol, they may reveal incidental findings (abnormalities) in study subjects that do not pertain to pulmonary hypertension.

If there is a problem or abnormality found during the medical tests done at their site, the subject will be notified about it by the research investigator there. If the subject would like, their primary care provider can also be notified. If an abnormality is found by a PVDOMICS core lab during research analyses, this finding will be reported by the DCC to the Principal Investigator and the lead coordinator at the subject's site. Results from genetic testing will only be reported if the subject agrees at the start of the study to have these findings reported. Only genetic results related to pulmonary hypertension risk or other clinically actionable genes (as defined by the American College of Medical Genetics) will be reported. The central core laboratory that is doing genetic testing is a research facility and does not have the ability to provide genetic test results to the subjects. Therefore, the information will be conveyed to the site PI in general terms only. Site investigators will refer the subject to a genetic counselor to discuss options for clinical testing in a CLIA-approved laboratory. The cost of genetic counseling and clinical genetic testing is not covered under the research protocol.

The study site will provide acknowledgement to the DCC of their receipt of an incidental finding. The study site once notified by the DCC is responsible for the subject notification. We cannot forecast what incidental findings may be found on these tests and their clinical significance. That is why this information will be provided to the clinical center Principal Investigator that knows the subject at their site. Incidental findings will be given to the subject and the health care provider by the study site personnel as further delineated in the Manual of Procedures.

Many of the studies performed locally at a site will likely be placed into the subject's medical record, which means they would be available to the subject and their healthcare provider. If an abnormality is found by a PVDOMICs core lab during research analyses, the results will not be placed into the subject's medical record.

6.6 Observational Safety and Monitoring Board (OSMB)

The OSMB was established by the NHLBI in accordance with NIH policies and is responsible for monitoring of patient safety and review of study performance. The OSMB consists of a chair, clinicians with expertise in pulmonary hypertension, bioethics, and biostatistics. An NHLBI scientist other than the NHLBI's Project Scientist serves as the Executive Secretary to the OSMB. The OSMB meets once per year in person and by teleconference at other times as necessary.

The purpose of monitoring is to verify that the rights and well-being of human subjects are protected. The reported study data are accurate, complete, and verifiable from source documents. The conduct of the study is in compliance with the currently approved protocol/amendment, with GCP, and with applicable regulatory requirement(s). The OSMB will also provide oversight and monitoring of ancillary studies (see Section 7).

Monitoring of Centers

Clinical Centers will be monitored primarily by on-going reports based on data collected in the study database and by conference call discussions. Reports on recruitment, retention, adherence, missing visits, missing procedures, missing data items, and procedural quality control will be generated regularly (e.g., weekly) and posted on the study website or sent via email to investigators and the NHLBI Project Officer, as appropriate.

Clinical centers will be site visited as part of the quality review of the performance of each site. These visits may be performed at each center every other year unless needed more frequently. Site visit reports should be provided that include the significant findings/facts, deviations and deficiencies, conclusions, actions taken or to be taken, and/or actions recommended to secure compliance.

7. Ancillary Studies

To enhance the value of the PVDOMICS Study, the Steering Committee encourages ancillary studies be done in collaboration with the PVDOMICS Study investigators. An ancillary study is one based on information, images or biospecimens from the PVDOMICS Study participants in an investigation that is relevant to, yet not part of the main goals of the PVDOMICS parent study, and derives support from non-PVDOMICS Study funds. It is anticipated that a typical ancillary study will propose the collection of additional data not collected or analyzed as part of the PVDOMICS Study parent data set.

Participation in, and approval of, an ancillary study is subject to review by the PVDOMICS Steering Committee. Also, the PVDOMICS Study Observational Study Monitoring Board (OSMB) will be notified of all ancillary studies and will provide oversight. Namely, the OSMB will determine if there are any safety concerns and if the study impacts the parent PVDOMICS study. The OSMB will monitor ancillary studies in a similar manner as the parent study.

A detailed description of ancillary study principles, approval and operational procedures are provided in Appendix 2 (which will also be in the PVDOMICS Manual of Operations).

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APPENDIX 1: Definitions of Parenchymal and Non-Parenchymal Lung Diseases

I. Diagnosis of COPD (23)

Severity	Postbronchodilator FEV_1/FVC	FEV_1 % predicted
At risk*	> 0.7	≥ 80
Mild COPD	≤ 0.7	≥ 80
Moderate COPD	≤ 0.7	50-80
Severe COPD	≤ 0.7	30-50
Very severe COPD	≤ 0.7	< 30

*At risk: history of or current use of inhaled tobacco

II. Diagnosis of IPF (24)

(a) Exclusion of other known causes of ILD (e.g. domestic and occupational environmental exposures, connective tissue disease, and drug toxicity)

(b) The presence of a UIP pattern on HRCT in patients not subjected to surgical lung biopsy

(c) Specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy (see Tables A1.1, A1.2, A1.3)

(d) Severity of IPF

(i) Mild FVC > 70% and DLCO > 60%

(ii) Moderate FVC 50-70% or DLCO 40 to 60% (if FVC and DLCO are discordant, more severely reduced value determines overall severity)

(iii) Severe FVC <50% or DLCO < 40%

III. Diagnosis of CPFE (26)

a. Radiographic evidence of centrilobular and/or paraseptal emphysema in the upper lobes and pulmonary fibrosis in the lower lobes

IV. Diagnosis of SSc-ILD (25)

a. Pulmonary fibrosis seen on high resolution CT or chest radiography, most pronounced in the basilar portions of the lungs, or occurrence of "velcro" crackles on auscultation, not due to another cause such as congestive heart failure.

V. Diagnosis of OSA (27)

a. Number of obstructive events (apneas, hypopneas + respiratory event related arousals) on PSG is greater than 15 events/hr or greater than 5/hour in a patient who reports any of the following:

Daytime sleepiness
Unrefreshing sleep
Fatigue
Insomnia
Waking up breath holding, gasping, or choking
Bed partner describing loud snoring, breath interruptions, or both

b. OSA severity :
   i. Mild for RDI ≥ 5 and < 15
   ii. Moderate for RDI ≥ 15 and < 30
   iii. Severe for RDI > 30/hr

VI. Diagnosis of OHS (28)
   a. Sleep disordered breathing (either OSA or central sleep apnea or both)
   b. BMI > 30 kg/m2
   c. Daytime hypercapnia (PCO2 > 45 mmHg) in the absence of other known causes of alveolar hypoventilation

VII. Diagnosis of Sarcoidosis (29)
   a. Histologic findings of noncaseating epitheliod cell granulomas on biopsy and exclusion of other causes

Table A1.1: HRCT Criteria for UIP Pattern

Table A1.1 HIGH-RESOLUTION COMPUTED TOMOGRAPHY CRITERIA FOR UIP PATTERN

UIP Pattern (All Four Features)	Possible UIP Pattern (All Three Features)	Inconsistent with UIP Pattern (Any of the Seven Features)
• Subpleural, basal predominance • Reticular abnormality • Honeycombing with or without traction bronchiectasis • Absence of features listed as inconsistent with UIP pattern (*see* third column)	• Subpleural, basal predominance • Reticular abnormality • Absence of features listed as inconsistent with UIP pattern (*see* third column)	• Upper or mid-lung predominance • Peribronchovascular predominance • Extensive ground glass abnormality (extent > reticular abnormality) • Profuse micronodules (bilateral, predominantly upper lobes) • Discrete cysts (multiple, bilateral, away from areas of honeycombing) • Diffuse mosaic attenuation/air-trapping (bilateral, in three or more lobes) • Consolidation in bronchopulmonary segment(s)/lobe(s)

Table A1.2: Histopathological Criteria for UIP Pattern

Table A1.2 HISTOPATHOLOGICAL CRITERIA FOR UIP PATTERN

UIP Pattern (All Four Criteria)	Probable UIP Pattern	Possible UIP Pattern (All Three Criteria)	Not UIP Pattern (Any of the Six Criteria)
• Evidence of marked fibrosis/ architectural distortion, ± honeycombing in a predominantly subpleural/ paraseptal distribution • Presence of patchy involvement of lung parenchyma by fibrosis • Presence of fibroblast foci • Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (see fourth column)	• Evidence of marked fibrosis / architectural distortion, ± honeycombing • Absence of either patchy involvement or fibroblastic foci, but not both • Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (*see* fourth column) OR • Honeycomb changes only‡	• Patchy or diffuse involvement of lung parenchyma by fibrosis, with or without interstitial inflammation • Absence of other criteria for UIP (*see* UIP PATTERN column) • Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (*see* fourth column)	• Hyaline membranes* • Organizing pneumonia*† • Granulomas† • Marked interstitial inflammatory cell infiltrate away from honeycombing • Predominant airway centered changes • Other features suggestive of an alternate diagnosis

Table A1.3: Combination of HRCT and Lung Biopsy for the Diagnosis of IPF

Table A1.3 COMBINATION OF HIGH-RESOLUTION COMPUTED TOMOGRAPHY AND SURGICAL LUNG BIOPSY FOR THE DIAGNOSIS OF IPF (REQUIRES MULTIDISCIPLINARY DISCUSSION)

HRCT Pattern*	Surgical Lung Biopsy Pattern* (When Performed)	Diagnosis of IPF?†
UIP	**UIP** **Probable UIP** **Possible UIP** **Nonclassifiable fibrosis‡**	**YES**
	Not UIP	No
Possible UIP	**UIP** **Probable UIP**	**YES**
	Possible UIP Nonclassifiable fibrosis	Probable§
	Not UIP	No
Inconsistent with UIP	UIP	Possible§
	Probable UIP Possible UIP Nonclassifiable fibrosis Not UIP	No

APPENDIX 2: PVDOMICS Ancillary Studies Policy

A2.1 Purpose

To enhance the value of the PVDOMICS Study, the Steering Committee welcomes proposals from individual investigators to carry out ancillary studies in collaboration with the PVDOMICS Study investigators.

A2.2 Definition of Ancillary Study

An ancillary study is one based on information, images or biospecimens from the PVDOMICS Study participants in an investigation that is relevant to, yet not part of the main goals of the PVDOMICS parent study, and derives support from non-PVDOMICS Study funds. It is anticipated that a typical ancillary study will propose the collection of additional data not collected or analyzed as part of the PVDOMICS Study parent data set.

A2. 3 Ancillary Study Principles

1) Participation in, and approval of, an ancillary study is subject to review by the PVDOMICS Steering Committee. Also, the PVDOMICS Study Observational Study Monitoring Board (OSMB) will be notified of all ancillary studies and will provide oversight. Namely, the OSMB will determine if there are any safety concerns and if the study impacts the parent PVDOMICS study. The OSMB will monitor ancillary studies in a similar manner as the parent study.

2) Approval by the Steering Committee will be defined as a majority of votes in favor of the proposal. In the case of an ancillary study which includes subject participation at all clinical centers, two-thirds approval of the Steering Committee membership will be required. Furthermore, the centers participating must each approve.

3) An ancillary study must receive Steering Committee approval before a grant to support it is submitted to a funding agency or to local institutional authorities (e.g., IRB), and before the study is permitted to begin.

4) All PVDOMICS ancillary study proposals initiated by a non-PVDOMICS investigator as PI must include as a Co-investigator at least one PVDOMICS Study PI or Co-investigator.

5) Ancillary studies require external (non-PVDOMICS Study) funding. Any ancillary study must have sufficient funding to cover the costs incurred by the PVDOMICS Study Clinical Centers and Cores (e.g., to process or ship samples), and the Data Coordinating Center (DCC) (for tasks such as sample selection, data management, preparing and documenting analysis files, participating in statistical analysis, and integrating the new ancillary data into the combined PVDOMICS Study database). Also, studies using Repository biospecimens

must have adequate support for handling and using the specimens. Special consideration will be given to requests for ancillary studies to be funded through training grants or career development awards through the NIH or other peer-reviewed funding sources.

6) Considerations for approval of ancillary studies
   The proposed study:

   A) must meet requirements of the highest scientific merit.
   B) must not, or minimally, interfere with the completion of the main objectives of PVDOMICS Study.
   C) must not, or minimally, adversely affect participant cooperation or compliance with PVDOMICS Study.
   D) must not create a serious diversion of PVDOMICS Study resources.
   E) must put minimal demand on scarce PVDOMICS Study resources, such as blood samples.
   F) must require the unique characteristics of the PVDOMICS Study patient data to accomplish its goals.
   G) must have adequate resources to effectively complete the project.
   H) must agree to provide the ancillary data to the PVDOMICS Study (also see 10 and 13).
   I) must not jeopardize the public image of the PVDOMICS Study.
   J) consider using the entire cohort for testing, rather than individual centers or isolated subgroups when appropriate.

7) Once an ancillary study is approved, if a change occurs in the structure or concept of the study (for example as a result of the NIH review process), including any change in data elements to be collected or analyzed, or any change to study aims, such changes must be disclosed to the PVDOMICS Study Steering Committee, for review and approval before the proposal is (re-)submitted to a funding agency.

8) A written progress report on ancillary studies must be made periodically (e.g., at time of Steering Committee meetings) to the Steering Committee.

9) All data collected under the auspices of an ancillary study is expected to adhere to the same high standards of quality applied to data collected in the core PVDOMICS Study. A plan for quality control must be submitted to the DCC for funded studies. In addition, once the ancillary study is initiated, periodic quality control reports must be sent to the DCC.

10) Data from ancillary studies will be made available to the DCC either on a real time basis using direct data entry into the DCC's computer server or through periodic transfers of ancillary data to the DCC.

11) Images, tracings and biosamples may usually be kept by the ancillary study investigators. During the PVDOMICS Study operation, further use of the samples beyond the objectives that have been approved by the PVDOMICS Steering Committee is prohibited without additional consent from the Steering Committee. The restrictions on specimens imposed by

the Veterans Affairs for specimens collected at any VA sites must be observed. Also, the NHLBI has the option to require that the images, tracings (or copies of) and remaining biosamples be transferred to the NHLBI Biological Specimen and Data Repository Information Coordinating Center (BioLINCC) Repository at the end of the PVDOMICS Study.

12) Unless specifically arranged, all analyses will take place at the DCC and be conducted under the supervision of its biostatistician-investigators in collaboration with the ancillary study investigators. Under specifically approved circumstances, datasets will be released to external investigators for local analysis.

13) Proposals for abstracts and manuscripts resulting from all ancillary studies shall be submitted to the Steering Committee for review and approval before establishment of a writing committee or a submission for publication or presentation. It is anticipated that principal investigators of approved ancillary studies will lead at least one scientific paper emerging from the ancillary study analyses.

14) An archival copy of the collected data and/or laboratory results not already held at the DCC will be sent to the PVDOMICS Study Data Coordinating Center at the conclusion of the data analysis and publication of the main ancillary study results. This transfer is the responsibility of the ancillary study PVDOMICS Study collaborator(s). Once transferred back to the PVDOMICS, these ancillary data will become part of the aggregate PVDOMICS Study data.

15) Information about proposed ancillary studies, and progress and results from approved ancillary studies are considered to be confidential and are not to be shared with others outside of the PVDOMICS Study except as provided for by the PVDOMICS Study Publications and Ancillary Studies Policy. Ancillary study investigators can share information among their co-investigators and with PVDOMICS Study investigators.

A2.4 Funding of Ancillary Studies

Ancillary studies will not be funded by the PVDOMICS Study, but will require an independent source of funding.

A2.5 Approval Procedures

1. Proposals may be generated by a participating clinical center or by other interested investigators providing at least one PVDOMICS Study PI or Co-investigator is included as a co-investigator. These applications are submitted to the Data Coordinating Center for review by the PVDOMICS Steering Committee.

2. There will be a two-step review by the Steering Committee. The first step is to have the proposal reviewed for its concept and general acceptability. This will be done in 2-4 weeks after submission. A short description of the study including the following information should be submitted.

a. Hypotheses to be tested.
Specific outcome variables that will be assessed.

Need for data and specimens from the DCC or Repositories.

b. Significance of the proposed ancillary study.

c. How will performance of this ancillary study affect the PVDOMICS Study? Specifically:

i. Will there be any data/specimen/image collection beyond that specified in the PVDOMICS Study protocol? If so, what additional information/samples will be obtained? What, if any, impact will this additional information/sample have on the main study?

ii. How much additional participant burden and time will be required to complete this ancillary study?

iii. Will additional funds be requested for the study and what will their source be?

3. If this proposal is acceptable in concept to the Steering Committee, a more detailed proposal should be written and submitted for review. This proposal should include detailed information on:

a. Hypotheses to be tested.
b. Background and significance of the study.
c. Conduct and performance of the study including specifying the study population and the data to be collected.
d. PVDOMICS Study staff and DCC burden. Costs for this work need to be included in the project's support.
e. Sample size justification.
f. Quality control of the data.
g. Data analysis methods.

4. The Steering Committee will review the proposal within 2-4 weeks. The decision can be for approval, modifications with further review, or disapproval.

A2.6 Publication of Ancillary Study Results

The policies regarding publications and presentations of the result of ancillary studies are the same as those governing the publications and presentations of results of the main study (see Manual of Operations). These policies are designed to:

1. Assure timely publication of the results to the appropriate professional audiences.

2. Avoid premature publications of results that might compromise the performance of the main study or that might compromise the ability to publish the results in high quality peer reviewed journals.

3. Maintain high standards of the published material.

4. To guard against duplicate publication of results, unless in review articles after the results have been published in a peer-reviewed article.

5. Assure equitable attribution of credit to all of the professionals participating in the ancillary study and the PVDOMICS Study.

APPENDIX 3: Glossary of Terms

Abbreviation and Term	Definition
ALP- Alkaline phosphatase	is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids.
ALT- Alanine Aminotransferase	Blood test measures the amount of this enzyme in the blood. ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas
ANOVA-Analysis of Variance	is a collection of statistical models used in order to analyze the differences of means between groups
ANP-Atrial Natriuretic Peptide	is a powerful vasodilator, and a protein (polypeptide) hormone secreted by heart muscle cells
Antisynthetase Syndrome	is a chronic autoimmune condition that affects the muscles and various other parts of the body. The signs and symptoms can vary but may include muscle inflammation (myositis), polyarthritis (inflammation of many joints), interstitial lung disease and Raynaud phenomenon
AO-Aortic pressure	Or central aortic pressure is the blood pressure at the root of the aorta
ASE-American Society of Echocardiography	American Society of Echocardiography is an organization of professionals committed to excellence in cardiovascular ultrasound and its application to patient care through education, advocacy, research, innovation and service to our members and the public.
AST- Aspartate Aminotransferase	It is an enzyme found in various cells, including liver cells, and elevated levels may indicate liver damage
AT-Anaerobic Threshold	The point at which you begin working your muscles without oxygen, from an aerobic level, believed to be at about 87% of your Maximum Heart Rate.
BIA-Bioelectrical Impedance Analysis	is a commonly used method for estimating body composition. It actually determines the electrical impedance, or opposition to the flow of an electric current through body tissues which can then be used to calculate an estimate of total body water
BMI-Body Mass Index	a measure of body fat based on height and weight that applies to adult men and women
BMPR2-Bone Morphogenetic Protein Receptor-II	A gene on chromosome 2q33-q34 that encodes a member of the bone morphogenetic protein (BMP)-receptor family of transmembrane serine/threonine kinases, which binds BMPs and plays a central role in endochondral bone formation and embryogenesis
BSA-Body Surface Area	The total surface area of the human body

BUN-Blood Urea Nitrogen	A blood test that measures the amount of nitrogen in your blood that comes from the waste product urea
CAD-Coronary Artery Disease	is a narrowing of the blood vessels (coronary arteries) that supply oxygen and blood to the heart.
CBC-Complete Blood Count	complete blood count measures the quantity of all the different types of cells in the blood
CFR-Code of Federal Regulations	is the codification of the general and permanent rules and regulations (sometimes called administrative law) published in the *Federal Register* by the executive departments and agencies of the federal government of the United States
CHD-Congenital Heart Disease	heart condition or defect that develops in the womb before a baby is born
CI-Cardiac Index	is a hemodynamic parameter that relates the cardiac output from left ventricle in one minute to body surface area
CKD-Chronic Kidney Disease	Kidney disease occurs when the kidneys are damaged and cannot function properly
CMP-Comprehensive Metabolic Panel	is used as a broad screening tool to evaluate organ function and electrolytes.
CO-Cardiac Output	describes the volume of blood being pumped by the heart, in particular by a left or right ventricle, per unit time
CO2-Carbon Dioxide	colorless, odorless gas produced by burning carbon and organic compounds and by respiration.
COPD-Chronic Obstructive Pulmonary Disease	group of lung diseases that block airflow and make breathing difficult
CPC-PH Combined precapillary post capillary pulmonary hypertension	Pulmonary hypertension with both pre and post capillary component
CPET-Cardiopulmonary Exercise Testing	method used to assess the performance of the heart and lungs at rest and during exercise
CPFE-Combine Pulmonary Fibrosis and Emphysema	Individuals with combined pulmonary fibrosis and emphysemagroup of lung diseases that block airflow and make breathing difficult
CRF-Clinical Research Form	Forms used in a research study to record data
CT/CTA-Computed tomography/Computed tomography angiography	is a type of medical exam that combines a computed tomography scan with an injection of a special dye called contrast material to produce pictures of blood vessels and tissues in a part of your body
CTD-Connective Tissue Disease	A connective tissue disease is any disease that has the connective tissues of the body as a primary target of pathology
CTE disease	Chronic thromboembolic disease
CTEPH-Chronic Thromboembolic Pulmonary Hypertension	Chronic thromboembolic pulmonary hypertension is a form of pulmonary hypertension caused by blood clots in the lungs.

CXR-Chest X-ray	is a projection radiograph of the chest used to diagnose conditions affecting the chest, its contents, and nearby structures
DICOM-Digital Imaging and Communications in Medicine	is a standard for handling, storing, printing, and transmitting information in medical imaging
DLCO-Diffusion Capacity	This test measures how well gases move through the lung and into the bloodstream
DNA- Deoxyribonucleic acid	encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses
DPG-Diastolic Pulmonary Vascular Pressure Gradient	the difference between invasive diastolic pulmonary artery pressure and mean pulmonary capillary wedge pressure
DVT-Deep Vein Thrombosis	is a blood clot in a deep vein
ECG-Electrocardiogram	is a diagnostic tool that measures and records the electrical activity of the heart
E-PAH-Exercise-Induced Pulmonary Hypertension	is actually high blood pressure in the lungs which is unmasked by exercise.
ESRD-End Stage Renal Disease	Chronic renal disease or Stage 5 kidney disease
FEV1-Forced Expiratory Volume at one second	is defined as the amount of air which can be forcibly exhaled from the lungs in the first second of a forced exhalation
FFM-Fat Free Mass	Fat free tissue
FM-Fat Mass	Amount of fat in tissue
GFR-Glomerular Filtration Rate	test to measure level of kidney function and determine the stage of kidney disease. It can be calculated from the results of blood creatinine test, age, body size and gender.
HCM-Hypertrophic Cardiomyopathy	is a disease of the muscle of the heart in which a portion of the myocardium is thickened, creating functional impairment of the cardiac muscle
HDL-High Density Lipoprotein	Is a lipoprotein that removes fat molecules from cells which want to export fat molecules
HFpEF-Heart Failure with preserved ejection fraction	The amount of blood pumped from the left ventricle with each heartbeat is within normal limits but the heart is unable to pump sufficiently to maintain blood flow to meet the body's needs
HfrEF-Heart Failure with reduced ejection	The amount of blood pumped from the left ventricle is reduced with each heart beat and the heart is unable to pump sufficiently to maintain blood flow to meet the body's needs.
HRCT- High Resolution CT	Is computed tomography (CT) with high resolution
HIV-Human Immunodeficiency Disease	It is the virus that can lead to acquired immunodeficiency syndrome
HR-Heart rate	Number of times the heart beats per minute
HRQOL-Health-Related Quality of Life	Questionnaire relating to how one's health affects their quality of life

ICD- implantable cardioverter-defibrillator	is a device implantable inside the body, able to perform both cardioversion, defibrillation and pacing of the heart.It is the virus that can lead to acquired immunodeficiency syndrome
ILD-Interstitial Lung Disease	is a large group of lung disorders associated with inflammation and scarring of lung tissue
IPAH-Idiopathic Pulmonary Arterial Hypertension	An increase of blood pressure in the pulmonary artery, pulmonary vein, or pulmonary capillaries without a known cause
IPC-PH-Isolated Post Capillary Pulmonary Hypertension	Post capillary pulmonary hypertension
IPEP-Immunoglobin Protein Electrophoresis	Are tests looking for abnormal proteins in the bloodis a large group of lung disorders associated with inflammation and scarring of lung tissue
IPF-Idiopathic Pulmonary Fibrosis	is a lung progressive disease. In IPF, lung tissue becomes scarred. The scarring typically starts at the edges of the lungs and progresses towards the center of the lungs.
IRB-Institutional Review Board	is a committee that has been formally designated to approve, monitor, and review biomedical and behavioral research involving humans
ITP-Idiopathic Thrombocytopenic Purpura	is a bleeding disorder caused by an abnormally low level of platelets in the patient's blood
LA-Left Atrium	is one of four chambers in the heart. It receives oxygenated blood from the pulmonary veins, and pumps it into the left ventricle, via the mitral valve
LAC-Lupus Anticoagulant	is an immunoglobulin that binds to phospholipids and proteins associated with the cell membrane
LDL-Low Density Lipoprotein	LDL is one of the five major groups of lipoproteins. LDL particles are sometimes referred to as *bad cholesterol* because they can transport their content of fat molecules into artery walls, attract macrophages, and thus drive atherosclerosis
LHD-Left Heart Disease	Disease on the left side of the heart
LV-Left Ventricle	is one of four chambers of the heart. It is located in the bottom left portion of the heart below the left atrium
LVEF-Left Ventricular Ejection Fraction	is the fraction of outbound blood pumped from the left ventricle with each heartbeat
LVEDP-Left Ventricular end diastolic pressure LV-Left Ventricle	is the volume of blood in the left ventricle at end load or filling is one of four chambers of the heart.
MCTD-Mixed Connective Tissue Disease	is a rare autoimmune disorder that is characterized by features commonly seen in three different connective tissue disorders: systemic lupus erythematous, scleroderma, and polymyositis
MGUS- Monoclonal gammopathy of undetermined significance	is a condition in which an abnormal protein (monoclonal protein, or M protein)

MLHF-Minnesota Living with Heart Failure Questionnaire	It is a questionnaire for patients to answer on how heart failure is affecting their lives.
MOP-Manual of Procedures	It is a "how to" guide for a study or business so that procedures are done uniformly and with consistency.
mPAP-Mean Pulmonary Artery Pressure	is a measure of the blood pressure found in the pulmonary artery. This is measured by inserting a catheter into the pulmonary artery. The mean pressure is typically 9 – 18 mmHg
MRA-Magnetic Resonance Angiogram	is a type of MRI that looks specifically at the body's blood vessels
MRI-Magnetic Resonance Imaging	Magnetic resonance imaging is a test that uses a magnetic field and pulses of radio wave energy to make pictures of organs and structures inside the body
MWT-Minute Walk Test	A test that determines how far a person can walk during a specific period of time
NHLBI- National Heart Lung Blood Institute	The primary responsibility this division of the National Institute of Health is the scientific investigation of heart, blood vessel, lung, and blood diseases. NHLBI oversees resources and research, demonstration, prevention, education, control, and sleep disorders and training activities in these fields.
iNO-inhaled Nitric Oxide	is a pulmonary vasodilator that plays a major role in regulating vascular muscle tone.
Non parenchymal RLD-Restrictive Lung Disease	a category of extrapulmonary, pleural, or parenchymal respiratory diseases that restrict lung expansion.
NT BNP-N-terminal pro b-type natriuretic peptide	A blood test that measures a certain enzyme that may be used to help detect, diagnose, and evaluate the severity of heart failure.
NYHA-New York Heart Association classification	provides a classification of the extent of heart failure. Patients are placed in one of four categories based on how much they are limited during physical activity; the limitations/symptoms are in regard to normal breathing and varying degrees in shortness of breath and/or angina pain
O2-Oxygen	a colorless, odorless reactive gas, the chemical element of atomic number 8 and the life-supporting component of the air
OHS-Obesity Hypoventilation Syndrome	is a condition in some obese people in which poor breathing leads to lower oxygen and higher carbon dioxide levels in the blood.
OSA-Obstructive Sleep Apnea	is a common and serious disorder in which breathing repeatedly stops for 10 seconds or more during sleep
OTC-Over-the-Counter	Medications or supplements that can be purchased without a prescription.
OUES-Oxygen Uptake Efficiency Slope	has been suggested as a submaximal measurement of cardiorespiratory fitness that is independent of exercise intensity

PA-Pulmonary Artery	the artery carrying blood from the right ventricle of the heart to the lungs for oxygenation.
PAH-Pulmonary Arterial Hypertension	Disease identified by high blood pressure in the arteries of the lungs.
PBC-Primary Biliary Cirrhosis	is an autoimmune disease of the liver.disease identified by high blood pressure in the arteries of the lungs
PCH -– Pulmonary capillary emangiomatosis	A rare vascular proliferative condition characterized by tumorous capillary vessels in the lung that can lead to pulmonary hypertension
PCW-pulmonary capillary wedge pressure mPCW (mean pulmonary capillary wedge pressure	the pressure measured by wedging a pulmonary catheter with an inflated balloon into a small pulmonary arterial branch
PDA-Patent ductus arteriosus (PDA)	is a heart defect that occurs when the ductus arteriosus (the temporary fetal blood vessel that connects the aorta and the pulmonary artery) does not close at birth.
PDG-Pulmonary end-diastolic gradient	is a Doppler measure that correlates with pulmonary artery diastolic pressures
PE-Pulmonary Embolism	is a blockage of the main artery of the lung or one of its branches by a substance that has travelled from elsewhere in the body
PET-Positron emission tomography	Uses positron emitting radionuclides, or tracers, to look at the function of tissues and organs in the body
PFTs-Pulmonary Function Tests	Pulmonary function tests are a group of tests that measure how well the lungs take in and release air and how well they move gases such as oxygen from the atmosphere into the body's circulation
PH or PHT-Pulmonary Hypertension	An increase of blood pressure in the pulmonary artery, pulmonary vein, or pulmonary capillaries
POEMS-Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Gammopathy,	A condition characterized by polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes.
PP-Pulse Pressure	The variation in blood pressure occurring in an artery during the cardiac cycle
PVD-Pulmonary Vascular Disease	Disease involving the vessels of the lungs. An increase of blood pressure in the pulmonary artery, pulmonary vein, or pulmonary capillaries.
PVDOMICS- Pulmonary Vascular Disease Phenomics Program	A research protocol developed by six centers who were awarded the collaborative grant under RFA-HL-027
PVH-Pulmonary Venous Hypertension	Pulmonary Hypertension that involves the pulmonary circulation at the postcapillary level
PVOD-Pulmonary Veno-Occlusive Disease	is a rare form of pulmonary hypertension caused by progressive blockage of the small veins in the lungs
PVR-Pulmonary Vascular Resistance	Refers to the resistance that must be overcome to push blood through the vasculature of the lungs
QOL-Quality of life questionnaire	Questionnaire for patients related to how they perceive their quality of their life

Qp/Qs-Pulmonary-to-Systemic flow ratio	Pulmonary to systemic blood flow ratio requires calculation of pulmonary artery and aortic lumens and systolic velocity curves
RA-room air	Oxygen comprises 21% of the air.
RA-Rheumatoid Arthritis	is a long lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly the wrist and hands are involved with typically the same joints involved on both sides of the body. The disease may also affect other parts of the body
RAP-Right Atrial Pressure	is the pressure of blood in the thoracic vena cava, near the right atrium of the heart.
RCM-Restrictive Cardiomyopathy	The heart muscle becomes rigid and unable to relax and fill with blood
RHC-Right Heart Catheterization	is the passing of a catheter into the right side of the heart and the arteries leading to the lungs.
RLD-Restrictive Lung Disease	Category of extrapulmonary, pleural, or parenchymal respiratory diseases that restrict lung expansion resulting in a decreased lung volume, an increased work of breathing, and inadequate ventilation and/or oxygenation.
RNA- Ribonucleic acid	Biological roles in coding, decoding, regulation, and expression of genesis
RHC-Right Heart Catheterization	is the passing of a catheter into the right side of the heart and the arteries leading to the lungs. It is done to monitor the heart's function and blood flow.
RV-Right Ventricle	The muscular chamber of the heart which accepts blood from the right atrium and pumps it through the pulmonary artery
RVSWI-Right Ventricular Stroke Work Index	A measure of the work done by the right ventricle with each contraction and equal to the stroke volume multiplied by the arterial pressure and divided by the body surface
SF-36- The Short Form (36) Health Survey	is a 36-item, patient-reported survey of patient health. The SF-36 is a measure of health status and an abbreviated variant of it, the SF-6D, is commonly used in health economics as a variable in the quality-adjusted life year calculation to determine the cost-effectiveness of a health treatment.
SLE- Systemic lupus erythematosus	is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue
SPEP- Serum protein electrophoresis	are tests looking for abnormal proteins in the blood
SSc-Systemic Scleroderma	is an autoimmune or connective tissue disease. It is characterized by thickening of the skin caused by accumulation of collagen, and by injuries to the smallest arteries

SV-Stroke Volume	is the volume of blood pumped from the left ventricle of the heart per beat
SVR-Systemic Vascular Resistance	The resistance offered by the peripheral circulation
TBW-Total Body Water	is the water content of a body is contained in the tissues, the blood, the bones and elsewhere
TD-Thermodilution TDCO-Thermodilution Cardiac Output	relating to or being a method of determining cardiac output by measurement of the change in temperature in the bloodstream after injecting a measured amount of cool fluid (as saline)
TLC-Total Lung Capacity	the volume of gas contained in the lung after a full inhalation is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue
TPG-Transpulmonary Gradient	defined by the difference between mean pulmonary arterial pressure and left atrial pressure
UIP-Usual interstitial pneumonia	is a form of lung disease characterized by progressive scarring of both lungs. The scarring (fibrosis) involves the supporting framework (interstitium) of the lung.
VC-Vital Capacity	is the maximum amount of air a person can expel from the lungs after a maximum inhalation
VHD-Valvular Heart Disease	is any disease process involving one or more of the four valves of the heart
VO2-Oxygen Consumption	is a measure of the volume of oxygen that is used by your body to convert the energy from the food you eat into the energy molecules
V/Q-Ventilation perfusion scan	is a type of imaging using scintigraphy and medical isotopes to evaluate the circulation of air and blood within a patient's lungs, in order to determine the ventilation/perfusion ratio
WHO-World Health Organization	A specialized agency of the United Nations (UN) that is concerned with international public health
WHO Groups	Definitions of Pulmonary Hypertension created during the World Symposium of Pulmonary Hypertension
WSPH-World Symposium on Pulmonary Hypertension	An international symposium on pulmonary hypertension