**A Phase IB Pilot Trial of Herpesvirus Treatment in Idiopathic Pulmonary Fibrosis**

Table of Contents

Topic Section

1. Summary 1.2-1.3
2. Hypothesis
3. Specific Aims 2 and 3
4. Endpoints
	1. Primary 4.1.1
	2. Secondary 4.2.1-4.2.9
	3. Pre-specified subgroups of interest 4.3.1
5. Background and significance 5.1-5.7
6. Protocol Design
	1. Study design 6.1
	2. Rationale for placebo 6.1.1
	3. Rationale for blinding 6.1.2
7. Study population 7.0
	1. Inclusion criteria 7.1
	2. Exclusion criteria 7.2
8. Study Drug
	1. Dose justification of valganciclovir 8.1
	2. Justification of treating combined CMV/EBV 8.2
	3. Protocol for dose adjustment 8.3
9. Study Feasibility and Timeline 9.1-9.2
10. Study Visits 10.1-10.6
	1. Table of study visits 10.7
11. Data collection for specific aims
	1. Determination of clinical endpoints 11.1
	2. Impact of anti-herpesvirus treatment on biomarkers, epithelial injury

and immune responses 11.2-11.3

* 1. Bronchoscopy 11.4
	2. Biologic specimen management 11.5
1. Study drug procedures 12.1-12.3
2. Data management, statistical analysis plan
	1. Data management 13.1
	2. Sample size determination/Randomization 13.2
	3. Statistical analysis plan 13.3
3. Safety and adverse event monitoring 14
4. Recruitment and Informed Consent 15
5. Good Clinical Practice 16

 **Protocol: A Phase IB Pilot Trial of Herpesvirus Treatment in Idiopathic Pulmonary Fibrosis**

1. **Summary**
	1. Most patients with idiopathic pulmonary fibrosis (IPF) have progression to respiratory failure within a period of 3 - 5 years after diagnosis([1](#_ENREF_1)). Unfortunately, only modestly effective therapies are available for patients with IPF, and these treatments do not reduce mortality or improve quality of life. Over the last several years, our studies have focused on the concept that alveolar epithelial cells (AECs) play a critical role in the ongoing cycle of injury and repair that drives progressive fibrotic remodeling in IPF. Our preliminary data for this proposal suggest that reactivation of latent herpesviruses in AECs could be directly involved in progression of IPF. As a result, we propose to study the effectiveness of targeted anti-herpesvirus treatment to reduce the burden of herpesvirus in the lungs and limit progression of the disease.
	2. A viral etiology of IPF was first suspected more than half a century ago, but identification of an important role for viruses in IPF pathogenesis has been challenging([2](#_ENREF_2)). In 2003, our group used a PCR strategy for identification of all known herpesviruses in lung tissue samples and found that 97% of lungs from IPF patients contained herpesvirus DNA([3](#_ENREF_3)). Importantly, viral proteins were expressed in AECs lining areas of fibrosis in IPF lungs, but were not detected in AECs in normal lungs. To investigate the prevalence of herpesvirus antigens in AECs of IPF patients, we performed IHC on lung sections from 34 individuals with IPF and 10 normal control lungs for cytomegalovirus (CMV) and Epstein-Barr virus (EBV), the most common herpesviruses in the lungs of IPF patients. We identified expression of EBV and/or CMV proteins, which is indicative of chronic infection or viral reactivation, in AECs from 29/34 patients (85%) with IPF and 0/10 normals. We also showed that expression of herpesvirus antigens in IPF co-localizes with markers of endoplasmic reticulum (ER) stress, which is common in IPF and may contribute to AEC dysfunction and apoptosis([4](#_ENREF_4)). Recently, we identified DNA for CMV and EBV in cell-free fluid obtained by bronchoalveolar lavage (BAL) from IPF patients and controls. Using standard PCR to identify DNA for EBV and CMV in concentrated DNA from cell-free BAL, we found that 21/28 IPF subjects were positive for CMV and 6/28 were positive for EBV DNA (23/28 were positive for at least one herpesvirus). Taken together, these data suggest that herpesvirus infection of alveolar epithelial cells is common in IPF and could contribute to fibrosis.
	3. In addition to the considerable evidence implicating herpesviruses in human IPF, a number of animal model studies have also indicated that herpesviruses could play a pathogenic role in lung fibrosis([5](#_ENREF_5), [6](#_ENREF_6)). Given this combination of evidence for a role for herpesviruses in IPF and the fact that oral treatments for herpesviruses are already available with a proven track record, we propose this timely study evaluating the effectiveness of anti-herpesvirus treatment in IPF.
2. **Hypothesis:** Reactivation of latent herpesviruses (EBV and CMV) is common in diseased alveolar epithelial cells of IPF patients and contributes to the recurrent injury-repair cycle that drives progressive fibrosis. Targeted anti-herpesvirus therapy will decrease herpesvirus replication in the alveolar epithelium and limit progression of lung fibrosis.
3. **Specific Aims**

3.1To determine the safety and tolerability of oral valganciclovir as add-on therapy in patients with IPF tolerating standard treatment with pirfenidone. 30 individuals with a diagnosis of IPF and positive serology for EBV or CMV will be enrolled in this study at Vanderbilt University Medical Center and randomized to receive either valganciclovir or placebo in a 2:1 ratio for 12 weeks, The primary endpoint will be safety and tolerability as determined by type, frequency and duration of adverse events (AE) and serious adverse events (SAE) in the group treated with valganciclovir. Secondary clinical endpoints will include changes over time in forced vital capacity (FVC), diffusing capacity for carbon monoxide (DLCO), oxygen requirements, and exercise capacity.

3.2 To investigate the effect of anti-herpesvirus treatment on changes in viral load and immune responses against herpesvirus. A subset of study subjects will undergo bronchoscopy with BAL at the time of study entry and at 12 weeks for measurement of: 1) immune response to herpesviruses in lung lymphocytes and 2) herpesvirus DNA in cell-free BAL fluid. Additional studies to investigate changes in the immune response in peripheral blood lymphocytes to herpesviruses will include: 1) analyzing PD-1 expression by T lymphocytes and 2) quantifying CD8+ T cell immune responses to herpesvirus antigens at study entry and after 12 weeks of therapy. These parameters will be reassessed at 6 months and 1 year after study entry to determine whether changes in anti-herpesvirus immune responses persist or revert to pre-treatment levels, thus shedding light on the duration of therapy needed to potentially alter disease progression.

1. **Endpoints**
	1. **Primary Endpoint**
		1. The primary outcome will be safety and tolerability will be determined by type, frequency and duration of adverse events (AE) and serious adverse events (SAE) in subjects receiving valganciclovir versus placebo.
	2. **Secondary Endpoints:** We will evaluate for trends in other clinical endpoints, as well as viral and immune endpoints over time in the valganciclovir group compared to the placebo group.
		1. We will determine the change in pulmonary function tests including forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) in the valganciclovir treated group compared to placebo during active treatment and post-treatment follow-up. PFTs will be measured at baseline, 3 months (12 weeks), 6, 9 and 12 months after randomization to determine the FVC slope (change in FVC over time) and change in diffusion capacity (DLCO).
		2. We will determine the effect of valganciclovir therapy on viral load in cell-free BAL fluid.
			1. We will evaluate the change from baseline in daytime oxygen requirements. Oxygen requirements will be measured at study visits baseline, 3, 6, 9, 12 months. At each study visit, while at rest, the subject will be placed on the amount of supplemental O2 required at the enrollment visit. After 5 minutes, the SpO2 on this supplemental flow rate will then be recorded and evaluated in comparison to baseline. In addition, the patient will have his/her supplemental oxygen requirement determined to maintain oxygen saturation ≥ 88% at rest. The change in liters per minute flow rate in oxygen compared to baseline will be recorded.
		3. Exercise capacity will be determined on the basis of the 6 minute walk test (6MWT). The principal exercise capacity measurement will be the change in distance until SpO2 reaches 80% compared to baseline. 6 MWT will be measured at study visits baseline, 3, 6, 9, 12 months. The 6MWT will be performed using a standardized protocol while the subject is breathing room air (i.e. without supplemental oxygen).
		4. Additional secondary endpoints will analyze the immune response to herpesviruses by analyzing PD-1 expression by T lymphocytes and quantifying CMV- and EBV-antigen specific CD8+ T cell immune responses.
2. **Background and Significance**
	1. *Idiopathic pulmonary fibrosis - current status:* IPF is a chronic progressive lung disease characterized by the insidious onset of interstitial infiltrates in the lung parenchyma associated with progressive dyspnea and impaired pulmonary function. Estimates for prevalence suggest that approximately 20 per 100,000 males and 13 per 100,000 females have the disease, and depending on the criteria used to define IPF, the disease may have a higher prevalence([1](#_ENREF_1)). The clinical definition of IPF requires exclusion of other known causes of interstitial lung disease, and its diagnosis requires characteristic changes on high-resolution computed tomography (HRCT), restriction on pulmonary function testing, impaired gas exchange, and absence of an alternative diagnosis on transbronchial biopsy or bronchoalveolar lavage (BAL)([11](#_ENREF_11)). Absolute confirmation of IPF by histologic diagnosis requires a surgical lung biopsy. The pathological equivalent of IPF is described as usual interstitial pneumonia (IPF/UIP). Of the Idiopathic Interstitial Pneumonias, IPF/UIP is the most common (70% of total) and most severe, so it is the form targeted in treatment trials([12](#_ENREF_12)). RCTs of interferon β and γ, bosentan, and sildenafil in IPF patients have been either negative or have shown inconsistent results, and there were no FDA approved therapies([13-17](#_ENREF_13)) until the recent approval of pirfenidone and nintedanib ([18](#_ENREF_18), [19](#_ENREF_19)). To date, clinical trials in IPF have been based on the premise that inhibition of inflammation or collagen deposition would be of benefit, or that treatment directed to the pulmonary vasculature would limit disease progression. In contrast, we suggest that therapy aimed at decreasing AEC injury and preserving alveolar epithelial function could be an effective approach.
	2. *Environmental factors in IPF:* There are several lines of evidence that environmental factors are important in IPF. The lungs are continuously exposed to the environment and are at constant risk of microscopic and macroscopic environmental lung injury. A pathologic hallmark of IPF/UIP is spatial and temporal heterogeneity of fibrotic remodeling. This feature is consistent with multiple episodes of microscopic lung injury by environmental stressors. Environmental risk factors linked to IPF in epidemiological studies include cigarette smoking([21](#_ENREF_21)), inhaled particulates([22](#_ENREF_22)), metal dust([23](#_ENREF_23), [24](#_ENREF_24)), wood dust([23](#_ENREF_23)), and viral infections. Cigarette smoking has consistently been associated with increased risk for IPF with odds ratio from case-control studies ranging from 1.11-3.23. In addition to inhaled irritants, several viruses including adenovirus and herpesviruses have been associated with IPF([2](#_ENREF_2), [3](#_ENREF_3), [25](#_ENREF_25), [26](#_ENREF_26)). A common feature of all these environmental factors is the ability to injure the alveolar epithelium. Interestingly, cigarette smoke([27](#_ENREF_27), [28](#_ENREF_28)), inhaled particulates([29](#_ENREF_29)), and herpesviruses([30](#_ENREF_30), [31](#_ENREF_31)) have all been shown to induce endoplasmic reticulum (ER) stress in lung epithelium, which we believe is an important underlying abnormality in IPF that predisposes to injury and limits recovery of the epithelial barrier.
	3. *Alveolar Epithelium in IPF:*Our current concept for the pathogenesis of IPF is repeated injury of the alveolar epithelium followed by incomplete epithelial repair that results in an aberrant wound healing response, leading to pulmonary fibrosis. A variety of studies have emphasized the role of the alveolar epithelium in both clinical IPF and animal models of lung fibrosis. In lung biopsies from patients with IPF, epithelial abnormalities are common and include hyperplastic type II AECs and bronchiolar-like epithelial cells lining areas of honeycombing([32](#_ENREF_32)). The importance of AECs in IPF is highlighted by the identification of mutations in the epithelial-restricted genes, surfactant protein C (*SFTPC*) and *SFTPA2*, that are associated with Familial Interstitial Pneumonia (FIP), the familial form of IPF([33](#_ENREF_33), [34](#_ENREF_34" \o "Wang, 2009 #1127)). AECs engineered to express mutant forms of these genes result in protein products that are misfolded and accumulate in the ER, induce ER stress, and alter cell survival([35](#_ENREF_35), [36](#_ENREF_36)). In 2008, we evaluated human lung tissues samples from FIP patients with a mutation in *SFTPC* (L188Q), FIP patients without *SFTPC* mutations, and sporadic IPF patients for expression of ER stress markers([4](#_ENREF_4)). These ER stress markers were prominently expressed in AECs lining areas of fibrosis in patients with *SFTPC* mutations as well as patients with lung fibrosis in the absence of *SFTPC* mutations. Subsequently, Korfei et al reported that AECs lining areas of fibrosis in IPF lung tissue sections not only had expression of ER stress markers, but these same cells also had activation of pro-apoptotic pathways([37](#_ENREF_37)). Recently, we developed a mouse model in which a mutant form of *SFTPC* is inducibly expressed in type II AECs([38](#_ENREF_38)). Such expression leads to ER stress in the type II AEC population, but these cells are able to maintain homeostasis in the setting of ER stress without the development of lung fibrosis. However, following low dose bleomycin, these mice develop excessive lung fibrosis. In a separate model, we induced ER stress in the lungs of wild type mice by intratracheal administration of tunicamycin([38](#_ENREF_38)). As with mutant *SFTPC* expression, ER stress alone did not cause lung fibrosis, but did predispose to greater bleomycin induced fibrosis. Taken together, these studies suggest that underlying ER stress leads to a vulnerable AEC population with predisposition to fibrosis after injury; however, a ‘second hit’ is necessary for development of lung fibrosis. We suggest that genetic predisposition and/or recurrent environmental stimuli render AECs vulnerable to a ‘second hit’ such as re-activation of herpesvirus that result in a pro-fibrotic cascade in the lung parenchyma.
	4. *Herpesvirus infection in IPF:* Reports from several laboratories have demonstrated a striking association between herpesvirus infection in the lung and IPF. Increased titers of antibodies to cytomegalovirus (CMV) and Epstein Barr viral capsid antigen (EBV VCA) have been reported in patients with IPF and collagen vascular disease-related interstitial pneumonitis compared with controls([2](#_ENREF_2)). Using PCR for EBV DNA and immunohistochemistry (IHC) for EBV proteins, EBV VCA and LMP-1 (latent membrane protein 1) have been identified more frequently in biopsies from IPF patients than controls and only biopsies from IPF patients were positive by both IHC and PCR([26](#_ENREF_26)). Our group extended these investigations to identify all known herpesviruses by PCR for viral DNA. CMV, EBV, and Kaposi’s sarcoma associated herpesvirus (KSHV, HHV-8) were present significantly more frequently in IPF lungs than controls, with 97% of IPF patients positive, whereas two other viruses reactivated by immunosuppression were not found([3](#_ENREF_3)). Furthermore, only IPF patients demonstrated co-infection with two or more herpesviruses. An important finding in these studies was that viral proteins were highly expressed in the abnormal airway epithelial cells lining distended airspaces in areas of honeycombing.
3. **Protocol Design and Methods**
	1. *****Study design:* We propose to conduct a single-center, prospective, randomized, placebo-controlled, double-blind pilot study of anti-herpesvirus therapy in patients with IPF. Patients with mild, moderate or severe IPF will be considered for study inclusion. Randomization will be to pirfenidone plus placebo or pirfenidone plus valganciclovir. We prefer to have all subjects on pirfenidone as the standard of care for this study since the majority of patients elect to be treated with pirfenidone in our clinic. In addition, we anticipate valganciclovir will be better tolerated in combination with pirfenidone. We propose to enroll and randomize all eligible subjects with evidence of current or past EBV or CMV infection as determined by serologic testing for anti-EBV and CMV antibodies (total n=30) to treatment with pirfenidone plus valganciclovir (20 patients) or pirfenidone plus placebo (10 patients) for 12 weeks (**Fig. 1**). In order to maximize exposure to active treatment and assess for safety and tolerability, randomization will be 2:1 valganciclovir vs. placebo. The primary outcome will be safety and tolerability will be determined by type, frequency and duration of AE and SAEs. All study subjects will be offered bronchoscopy with BAL at study initiation and upon completion of treatment (12 weeks). Subjects will then be followed up at routine clinic visits at 6, 9 and 12 months for data collection.

Figure 1. Study flow chart.

* 1. Rationale for Blinding and Placebo**:**
		1. A RCT is necessary given the clinical heterogeneity of IPF, and the inability to have two similar arms without randomization. A placebo arm will minimize bias since GI upset is a side effect of valganciclovir and standard treatment, pirfenidone. All subjects will be provided standard of care treatment to be applied equally across study groups as much as feasible and blinding will help avoid bias in the care provided.
		2. Blinding is necessary to have optimal scientific validity, to make objective assessments of treatment effects, and to minimize dropout rates across study arms. In this study, there is particular concern that without blinding, subjects may opt to obtain off-label anti-viral therapy from non-study physicians if they become aware of the presence of herpesviruses in their lungs.
1. **Study population:** The study population will consist of male and female patients diagnosed with IPF, ages 21 and above, who are followed in the outpatient clinic setting.
	1. Inclusion criteria will be:
		1. age >21 and <80 years
		2. ability to provided informed consent
		3. diagnosis of probable or definite IPF according to ATS criteria([11](#_ENREF_11))
		4. tolerance of full-dose (2403 mg/day) pirfenidone
		5. Positive serology for EBV or CMV
	2. Exclusion criteria will be:
		1. FVC < 40% predicted
		2. DLCO < 35% predicted (Crapo)
		3. FEV1/FVC <0.7
		4. Significant centrilobular emphysema (>40% by HRCT)
		5. Active tobacco use (cigarette or cigar smoking)
		6. Resting SpO2 on room air <89%
		7. listed for lung transplantation defined as being assigned a lung allocation score
		8. environmental exposure (occupational, environmental, drug, etc.) felt by the principal investigator (PI) to be the etiology of the interstitial disease
		9. diagnosis of collagen-vascular conditions (according to the published American College of Rheumatology criteria)
		10. history of unstable or deteriorating cardiac disease
		11. acute coronary syndrome, coronary artery bypass, or angioplasty within 3 months of screening
		12. uncontrolled arrhythmia
		13. uncontrolled hypertension
		14. known HIV or hepatitis C
		15. known cirrhosis or chronic active hepatitis
		16. active substance or alcohol abuse
		17. pregnancy or lactation
		18. Women of childbearing potential who are not using a medically approved means of contraception. Subjects will be considered of childbearing potential if they are not surgically sterile or have not been postmenopausal for at least 2 years [any subject who is postmenopausal for < 2 years will be required to have a follicle-stimulating hormone (FSH) level to assess her potential to become pregnant
		19. clinically relevant lab abnormalities (obtained within 30 days before enrollment), including:
			1. creatinine > 2 x upper limit of normal (ULN)
			2. hematology outside of specified limits: white blood cells (WBCs) < 3,500/mm3; hematocrit < 25% or > 59%; platelets < 100,000/mm3;
			3. total bilirubin > 2 x ULN
			4. Aspartate (AST) or alanine aminotransferases (ALT) (serum glutamic-oxaloacetic; transaminase [SGOT], or serum glutamic pyruvic transaminase [SGPT]) > 2.0 x ULN
			5. alkaline phosphatase > 3 x ULN
			6. albumin < 3.0 mg/dL at screening
		20. known hypersensitivity to study medication
		21. any condition that, in the judgment of the PI, might cause participation in this study to be detrimental to the subject or that the PI deems makes the subject a poor candidate
		22. any therapy with immunosuppressants such as prednisone, azathioprine, or mycophenalate currently or anticipated to be needed during the study period (subjects on these drugs prior to the study will require a 30-day washout period before randomization)
		23. participation in another IPF clinical treatment trial during the study period (if completing another IPF clinical treatment trial, then a 30-day washout period is required before randomization)
		24. requirement for chronic suppressive therapy with valacyclovir for recurrent herpes virus infection
		25. History of myelodysplasia, aplastic anemia, refractory anemia, or multiple myeloma.
2. **Study Drug**
	1. *Dose justification of valganciclovir:* The standard dosing of valganciclovir for CMV disease when there is active pneumonitis with histopathologic changes such as intracellular inclusions, positive IHC, and acute inflammation is 900 mg twice daily([42](#_ENREF_42)). Typically, this occurs in solid organ or bone marrow transplant recipients, and is associated with viremia with viral loads in blood in the range of 10,000-100,000 viral genomes/ml([43](#_ENREF_43)). This dose is typically given for 21 days, and can be reduced 900 mg daily to further reduce low-grade viremia (200-2,000 copies/ml), and 900 mg/day can be given long term to prevent relapse or recurrent CMV disease([42](#_ENREF_42)). In IPF patients, CMV viral load in BAL is below 10,000 genomes/ml. Hence, the overall viral load is much lower in IPF patients compared to solid organ transplant recipients. Consequently, we believe a dose of 900 mg/day comparable to that used for chronic suppressive therapy in solid organ transplant recipients is most appropriate. A dose of 900 mg/day is generally well tolerated when administered for several months([42](#_ENREF_42)), and avoids the bone marrow suppression effects of long-term 900 mg twice daily dosing. Standard dosing of this regimen will be 900 mg once daily.
	2. *Justification of treating concomitant EBV and CMV with valganciclovir:* Valganciclovir has activity against EBV and CMV. The 50% inhibitory concentration of valganciclovir against EBV is 1.5 mcg/ml([44](#_ENREF_44)), and the median area under the concentration versus time curve for 900 mg valganciclovir over 24 hrs (AUC 0-24) is 29 μg/ml with a peak concentration of 4.4 μg/ml([45](#_ENREF_45)). Hence the average concentration of valganciclovir is above the IC50 of EBV. We believe that monotherapy with valganciclovir should be effective and safe for subjects with CMV and EBV.
	3. *Protocol for dosage adjustment*: In subjects who develop a treatment-emergent, nonserious adverse events (i.e. nausea), valganciclovir will be administered in divided doses (450 mg twice daily). In subjects who develop treatment emergent clinically significant laboratory abnormalities possibly related to the study drug, study drug will be held until lab abnormalities have resolved. Current and new medication review will be conducted to assess for alternative contributors to lab abnormalities. After the treatment-emergent laboratory abnormality resolves, subjects will then resume study drug with repeat laboratory monitoring within 1 week after resumption of treatment, or earlier if symptoms recur in an earlier time period. If the treatment emergent laboratory abnormality recurs, study drug will be reduced to 450mg QD with close monitoring. If symptoms or lab abnormalities occur with 450mg QD, the study drug will be terminated.
3. **Study Feasibility and Timeline**

All subjects will be enrolled through the Vanderbilt Interstitial Lung Disease (ILD) clinics have been top enrolling sites for several multi-center clinical trials in IPF, and given this proven track record, we are confident we will meet enrollment targets. We will place priority on enrollment in this VICTR support pilot trial over industry-sponsored trials. We currently evaluate >200 new IPF cases per year in our clinics. At the present time, approximately 125 IPF patients are being treated with pirfenidone in the Vanderbilt ILD clinic, and the majority of these patients should be eligible for this study. We expect to begin recruiting patients immediately upon approval of this study and complete enrollment within 9 months such that subjects will reach the primary study endpoint by the end of Year 1. Ongoing ascertainment of data collected during routine clinical follow-up of these study subjects will then be completed in Year 2.

1. **Study Visits**
	1. *Screening visit:* Once informed consent is obtained, subjects may begin the screening process. If a study subject has been evaluated at VUMC by a study physician and has performed clinical testing that meets study guidelines within 30 days, then this testing maybe used to qualify for study entry. This will facilitate entry into the study, avoid repeated testing, and reduce costs. The following procedures will be performed at screening: 1) medical history and physical examination; 2) vital signs including oximetry; 3) blood chemistries, hematology counts; EBV and CMV serology 4) PFTs including spirometry, lung volumes, and diffusing capacity; 5) HRCT review; 6) surgical lung biopsy review; and 7) recording of current medications. If patients are not taking pirfenidone at the time of screening, then patients will be placed on pirfenidone and will be re-assessed to determine study eligibility 4 weeks later to allow ample time to determine tolerance to pirfenidone.
	2. *Enrollment visit:* For subjects who meet eligibility criteria, enrollment may occur the same day as the screening visit. For subjects who require blood tests to be drawn at the screening visit, they will be notified by telephone of their eligibility for the study and study drug will be mailed to subjects by the investigational drug pharmacy. Randomization will be 2:1 valganciclovir versus placebo. Subjects will be offered the opportunity to undergo an optional research bronchoscopy. For subjects who undergo bronchoscopy, study drug will begin immediately following the bronchoscopy. Subjects who do not undergo bronchoscopy will begin study drug immediately. Additional tests that will be obtained are the following: 1) six minute walk test (6 MWT); 2) baseline blood chemistries and blood counts if >30 days after the screening visit; 3) baseline blood draws for biomarker and immunology studies; 4) teaching subjects self-monitoring and diary recording; and 5) optional bronchoscopy with BAL. Bronchoscopy will be the last test performed at enrollment.
	3. *Week 4 and 8 study visits:* The primary purpose of these visits is to establish safety of study treatment and adherence to study treatment. Subject’s diaries will be reviewed, medical history and physical examination will be performed, a pill count will be performed, current medications will be recorded, and chemistries and hematology counts will be obtained.
	4. *Week 12 study visits:* This visit will conclude active treatment in the study and will be the primary endpoint for safety and tolerability. In addition, 6 MWT, and oximetry will be performed at this study visit. Subject’s diaries will be reviewed, medical history and physical examination will be performed, PFTs will be obtained according to usual clinical care protocol, a pill count will be performed, current medications will be recorded, chemistries/hematology counts will be obtained, and blood will be obtained for biomarker and immunology studies. Subjects who consent to research bronchoscopy will undergo a second research bronchoscopy coordinated with the week 12 study visit.
	5. *6, 9, and 12 month visits:*These visits will coincide with routine clinical care visits. Medical history and physical examination will be performed, and PFTs, 6MWT and oximetry will be performed according to usual clinical care protocol studies. A blood sample will be obtained for immunology studies.
	6. *Unscheduled disease progression visits:*If a subject is found to have new symptoms or worsening of dyspnea or oxygen utilization, the subject will be scheduled for a clinic visit as soon as feasible to reassess secondary endpoints.
	7. *Table of Study Visits:*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Screening/ Enrollment Visit**  | **Bronchoscopy Visit (optional)** | **Week 4****(study visit)** | **Week 8****(study visit)** | **Week 12****(study visit)** | **Month 6****(with clinical visit)** |  | **Month 9****(with clinical visit)** | **Month 12****(with clinical visit)** |
| Consent  | **X** |  |  |  |  |  |  |  |  |
| Randomization | **X** |  |  |  |  |  |  |  |  |
| Medical History | **X** |  |  |  | **X** |  |  |  |  |
| Physical Exam | **X** |  |  |  | **X** |  |  |  |  |
| Blood Chemistry (CMP; CBC) | **X** |  | **X** | **X** | **X** |  |  |  |  |
| Dispense study drug | **X** | **(X)** |  |  |  |  |  |  |  |
| T cell studies from peripheral blood | **X** | **(X)** |  |  | **X** | **X** |  | **X** | **X** |
| Spirometry Test/DLCO | **X** |  |  |  | **X** | **X** |  | **X** | **X** |
| 6 MWT | **X** |  |  |  | **X** | **X** |  | **X** | **X** |
| Bronchoscopy (if patient consents) |  | **X** |  |  | **X** |  |  |  |  |
| Dispense Study/Review Study Diary for compliance evaluation | **X** |  | **X** | **X** | **X** |  |  |  |  |
| Adverse Events |  |  | **X** | **X** | **X** | **X** |  | **X** | **X** |

1. **Data Collection for Specific Aims**
	1. Aim 1: To determine the safety and tolerability of oral valganciclovir as add-on therapy in patients with IPF tolerating standard treatment with pirfenidone..
		1. *Rationale:* This study is designed to assess for safety and tolerability of valganciclovir in IPF patients who are taking standard therapy for IPF (pirfenidone) as assessed by type, frequency and duration of AE and SAEs. Exploratory secondary analysis to evaluate for clinical efficacy will include changes in FVC slope of patients with IPF, oxygenation, and exercise capacity. In addition to identifying whether anti-viral therapy could positively impact any of these parameters, collecting these data will ensure that any detrimental effects of the drugs used to treat herpesviruses on clinically relevant endpoints will be identified. Information will be collected as outlined below. For analysis of all these variables, the standard treatment arm with pirfenidone and placebo will be compared to valganciclovir and pirfenidone.
		2. Evaluation for safety of valganciclovir. Assessment of safety will be determined by ongoing review of clinical laboratory tests (blood sampling for clinical laboratory parameters-see schedule of events), physical examination including vital sign measurements, functional status assessments and adverse events. Women of childbearing potential will undergo a urine pregnancy test at each study visit.
		3. Evaluation for treatment emergent adverse events. A treatment-emergent adverse event (TEAE) is defined as a sign or symptom that emerges during treatment or within the 12 weeks following the last dose of the study drug, having been absent pretreatment or that has worsened relative to the pre-treatment state. Any adverse event deemed related to study drug will also be considered a TEAE regardless of elapsed time since last study drug dose. Adverse events will be graded using National Cancer Institute [NCI] CTCAE Version 3.0 during the study evaluation. Study drug-related adverse events will be listed individually.
		4. Evaluation for change in pulmonary function. PFTs will be captured for all subjects using a CareFusion Sensormedics pulmonary function testing equipment (CareFusion, San Diego, CA), located in the VUMC pulmonary clinic. Measurements will be performed according to current ATS/ERS guidelines([46](#_ENREF_46)), and include spirometry (FEV1 and FVC) by NHANES, total lung capacity by body box plethysmography, and diffusing capacity of the lung for carbon monoxide (DLCO) all by %predicted according to Crapo([47](#_ENREF_47)). PFTs will be performed by registered respiratory technicians who are experienced in clinical trials. All registered respiratory technicians performing these measurements will be trained in this protocol All subjects will perform measurements on one machine dedicated to the study to reduce variance.
		5. Change from baseline in daytime resting oxygen requirements. Two measurements will be performed to determine if the research subject has a change in SpO2 or oxygen requirement at rest on each study visit. At each study visit, any supplemental oxygen will be removed for 5 minutes and SpO2 breathing ambient air will then be recorded and evaluated in comparison to baseline. In addition, the patient will have his/her supplemental oxygen requirement determined to maintain oxygen saturation ≥ 88%. The change in liters per minute flow rate in oxygen compared to baseline will be recorded.
		6. Evaluation of exercise capacity. Exercise capacity will be determined on the basis of the 6 minute walk test (6MWT). The principal exercise capacity measurement will be the change in distance until SaO2 reaches 80% from the 6MWT compared to baseline. The 6MWT will be performed using current guidelines([48](#_ENREF_48)). The walk is performed on room air throughout the study. Patients on supplemental oxygen will be off oxygen for a period of 10 minutes prior to initiation of the walk. Continuous finger pulse oximetry is performed during the test and the patient will walk at their own pace for as far as he/she can in the 6 minute duration. Measurements that will be collected include: 1) distance walked until SpO2 reaches 88%; 2) time until SpO2 reaches 88%; 3) distance walked to SpO2 reaches 80%; 4) time until SpO2 reaches 80%; 5) pre-test SpO2; 6) post-test SpO2; 7) lowest SpO2 during test; and 8) total distance walked in 6 minutes; and 9) average velocity calculated as distance/time. In subjects who desaturate to <88%, an oxygen titration study will then be performed to determine exertional oxygen requirement. This value will be recorded and compared to baseline.

* 1. Aim 2: To investigate the effect of anti-herpesvirus treatment on changes in viral load and immune responses against herpesvirus.
		1. *Experimental design:* Determination of herpesvirus DNA in BAL. BAL will be collected at bronchoscopy and the cell free portion will be used for determination of herpesvirus DNA. Total DNA will be isolated by the following procedure. After 10-fold concentration of cell free BAL fluid (10 ml), protein is digested overnight at 55°C in the presence of 500 μg/ml of proteinase K. DNA is then extracted with phenol/chloroform, followed by addition of 3 M sodium acetic acid (pH 6.5), precipitation in 100% isopropanol, and reconstitution in TE buffer. Real time qPCR amplification is performed on a StepOne Plus Real Time PCR machine (Applied Biosystems) to determine viral load based on a standard curve derived from a plasmid vector containing CMV or EBV DNA. Primer sequences used for CMV detection are as follows: (forward) 5’-TGA AGC GCC GCA TTG A-3’, (reverse) 5’-TGG CCC GTA GGT CAT CCA-3’. Primer sequences used for EBV detection are as follows: (forward) 5’-CCC AAC ACT CCA CCA CAC C-3’, (reverse) 5’-TCT TAG GAG CTG TCC GAG GG-3’. Results are reported as herpesvirus genomes per ml of BAL fluid.
		2. Measurement of the anti-herpesvirus immune response. All patients will be HLA typed at the beginning of the study. Peripheral blood mononuclear cells (PBMCs) will be separated using standard Ficoll-Paque™ density centrifugation. Lymphocytes from peripheral blood obtained at baseline, 12 weeks (3 months), 6 months, 9 months, and 12 months and BAL obtained at baseline and 12 weeks will be studied. The following assays will be performed to quantify herpesviruses specific T-cell responses in subjects randomized to antiviral therapy or placebo:
			1. As a marker of chronic immune activation in response to herpesvirus infection, we will evaluate PD-1 expression on naive, central memory (TCM), effector memory (TEM), and terminal effector memory (TEMRA) populations using CCR7 and CD45RO to distinguish these memory T cell subsets. In addition, we will directly evaluate CD8+ T cell immune responses to herpesvirus antigens using MHC Class I tetramer assays. HLA-specific MHC-I CMV and EBV tetramers are available for individuals with HLA-A2, HLA-B7, HLA-B35, and HLA-B44 serotypes (Glycotope Biotechnology, Heidelberg, Germany), which comprises ~80% of the U.S. Caucasian population. Lymphocyte subsets will be separated by flow cytometry with anti-CD3 PerCPCy5, anti-CD8 FITC, anti-PD-1 Brilliant Violet 421, anti-CD45RO PECy5, and anti-CCR7 PE-CF594. CMV or EBV HLA class I tetramers conjugated with APC or Cy7 will be used for these studies (for example, HLA-A2/pp65 tetramers for CMV and HLA-A2/LMP1 tetramers; HLA-A2/LMP2 tetramers for EBV will be used for subjects with the HLA-A2 genotype). Live cells will be gated based on forward- and side-scatter properties, and analysis performed using FlowJo software (Tree Star, Ashland, OR), as previously described. All experiments will be acquired with an LSR-II flow cytometer (BD Biosciences), with a minimum of 30,000 events per sample. Detection of tetramer binding by flow cytometry will provide a specific signal to allow determination of the frequency of CMV- or EBV-specific CD8+ T cells in each sample, as well as the percentage of CMV- or EBV-specific CD8+ T cells expressing PD-1 within a given sample. Thus, the planned longitudinal analysis will define the effects of antiviral therapy on CD8+ T cell populations in IPF patients, as well as determine the capacity for immunologic biomarkers, such as PD-1, to identify response to therapy.
			2. While the studies described above will quantify differences in lymphocyte populations, we will also determine differences in lymphocyte activation in response to herpesvirus antigens. For these studies, PBMCs will be stimulated with pooled MHC Class I CMV- or EBV- specific peptides followed by flow cytometry with intracellular cytokine staining for IL-6, IL-2, and IFN-γ (49). Results will be reported as the percentage of CD8+ T cells that stain positive for each cytokine. As a positive control, cells will be stimulated with Staphylococcal enterotoxin B. In complementary studies, lymphocytes will be placed in 96-well plates (2x105 cells/well) and pooled MHC Class I CMV- or EBV- specific peptides will be added to cells in triplicate wells for measurement of cytokines, including Th1 cytokine (IL-2, IFN-γ and TNFα), Th2 cytokines (IL-4 and IL-10), and IL-6. Cytokine bead array (CBA) analysis will be performed on cell culture supernatants from untreated cells and MHC-I peptide-treated cells after 24 hours in culture as previously described. Together, these studies will determine functional differences in CD8+ T lymphocyte populations in response to herpesvirus antigens during the course of antiviral therapy.
			3. Similar assays will be performed on lymphocytes isolated from BAL from each patient. The typical BAL sample contains 5-10x106 cells, of which 5-10% are lymphocytes. If cells are limiting, then tetramer assays and intracellular cytokine staining will be performed on PBMCs only.
	2. Bronchoscopy:Individuals in the bronchoscopy substudy will undergo bronchoscopy at study entry and at week 12. Bronchoscopy will be performed using a standard protocol and will include airway survey, and BAL. For BAL, the bronchoscope will be wedged in the lingula or right middle lobe and 4 sequential aliquots of sterile saline (60 ml) will be instilled and gently withdrawn. Samples will be processed immediately. Bronchoscopy will be performed using standard monitoring procedures including continuous cardiac monitoring and blood pressure monitoring and pulse oximetry in the bronchoscopy suite at Vanderbilt University Hospital.
	3. Biologic specimen management:Following bronchoscopy, BAL fluid will be centrifuged for collection of both the cell pellet and the supernatant. The supernatant will be aliquoted and stored at -700C. Total and differential cell counts will be determined and remaining the remaining cell pellet will be used for flow cytometry studies. 50 ml of blood will be collected on the day of bronchoscopy, and peripheral blood mononuclear cells will be isolated using standard Ficoll-Paque™ density centrifugation. Plasma and serum will be aliquoted and stored at -700.
1. **Study drug procedures**

Valganciclovir (Valgan) tablets and placebo will be provided by Genentech, Inc. through the VUMC pharmacy. The VUMC Pharmacy Investigational Drug Service (IDS) will dispense study medication at baseline and every 4 weeks coinciding with study visits. At the final study visit, subjects will return all unused study medication. To maintain blinding, valganciclovir and placebo will be encapsulated by the IDS as follows:

 Starting Materials:

1. Valganciclovir 450 mg tablets or placebo
2. Microcrystalline cellulose
3. Size “0” orange caps
4. Capsule machine

Procedure:

1. Don appropriate attire for compounding
2. Fill capsules machine with empty capsules (tops removed)
3. Place one tablet in each empty capsule
4. Obtain verification from pharmacist
5. Cover with microcrystalline cellulose
6. Replace tops
7. Clean capsules with capsule cleaning towel
8. Package and label appropriately
9. Log in Investigational Drug Accountability Record
	1. *Contraindications, precautions, and side effects of study medications:*Valganciclovir is FDA approved for the treatment and chronic suppression of herpesviruses, and has a good safety profile post marketing. The dose of valganciclovir recommended for this study is well within the FDA approved dosing range for other indications. The major side effect of valganciclovir is bone marrow suppression causing anemia, leukopenia, or thrombocytopenia. GI upset (nausea and diarrhea) is another reported side effect. These side effects will be monitored by routine laboratory testing and monitoring of subject diaries and direct questioning. Dose adjustments of valganciclovir are required for renal impairment, and dose modifications for renal insufficiency will be made according to recommended guidelines based on estimated creatinine clearance using the Cockroft-Gault equation. For both valganciclovir and pirfenidone, there are no known major drug interactions requiring special clinical monitoring.
	2. Valganciclovir safety and handling. In animal studies ganciclovir was found to be mutagenic, teratogenic, aspermatogenic and carcinogenic. Valganciclovir is considered a potential teratogen and carcinogen in humans with the potential to cause birth defects and cancers, and may cause temporary or permanent inhibition of spermatogenesis. Women of childbearing age will be required to use a medically approved means of contraception and will have urine pregnancy tests performed with each study visit. Male patients will be advised to practice barrier contraception during and for at least 90 days following treatment with valganciclovir. Tablets will not be broken or crushed. Since valganciclovir is considered a potential teratogen and carcinogen in humans, caution will be observed in handling broken tablets. Direct contact of broken or crushed tablets with skin or mucous membranes will be avoided. If such contact occurs, thorough washing with soap and water, and washing of eyes with sterile water will be performed. Unused medication will be returned to the pharmacy for disposal.
10. **Data Management and Statistical Analysis Plan**
	1. **Data Management:** All data for this study will be stored in a password-protected, HIPAA security compliant computer database (REDCap). Vanderbilt was a founding member of the secure electronic REDCap database and houses much of this database on its servers. REDCap is a secure, web-based application designed to support data capture for research studies and registries. REDCap provides: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. Only study personnel for this project with appropriate permissions will be allowed to access the database. Authorized users can input data from anywhere in the world with secure web authentication and data logging. REDCap will be used for electronic capture of all data related to study visits with the exception of digital HRCT images. Data will be recorded directly from clinical records into REDCap by study personnel trained in this protocol. A second member of the study team will review the data for accuracy prior to the data being locked. This will avoid paper case report forms and any potential for breach of confidentiality from maintaining them or transposing data. All samples obtained for this study will be assigned a code which will not identify the subject. The only key to the code will be in the password-protected computer database. Dr. Blackwell, PI of the study, will be responsible for maintaining confidentiality of data and codes. Only Dr. Blackwell and his delegates will have access to the code and information that identifies a participant as a being in this study. The results of analyses run on those samples will not be reported to the participant or clinical providers. No one else, including relatives, doctors, employers or insurance companies will be allowed to view the test results. Informed consent documents will be maintained in a locked file cabinet in the study coordinator’s locked office. Only the study personnel will have access to this file cabinet.
	2. **Sample Size and Randomization**
		1. *Power analysis:* This pilot study is designed to determine tolerability and safety of valganciclovir as add-on therapy to pirfenidone. Pirfenidone is reported to be discontinued in 13% of subjects due to adverse events, almost all of which occur within the first 4 weeks of treatment (based on AE profiles from the ASCEND study). Therefore, we do not expect any placebo treated subjects to withdraw from this study. Using a 2:1 randomization (valganciclovir:placebo) and enrolling 30 subjects will allow us to detect an increased withdrawal rate in the valganciclovir group in the range of 25% (75% power with a one-sided =0.1). Subjects who require dose reduction, or temporary discontinuation but are able to resume treatment will be considered non-withdrawals.
		2. *Randomization:* The randomization scheme will be a ratio of 2:1 for drug versus placebo. Once a participant has signed the consent form and completed the enrollment visit (and baseline bronchoscopy in subjects who participate in the bronchoscopy substudy), the participant will be randomized. Study medication will be distributed by the VUMC investigational pharmacy according to the randomization scheme.
	3. *Statistical analysis:* Descriptive statistics of frequencies will be used to compare adverse event profiles between groups. *Secondary analysis.* A mixed-effects model will be used to test whether the change in FVC is smaller for valganciclovir therapy group (treatment group) compared to the standard of care (placebo) group. The treatment group, time, and their interaction terms will be included as fixed effects, while the time will be also assumed to be a random effect (i.e. the random intercept and slope model). In addition, the baseline FVC, age, and gender will be adjusted in the model. For repeatedly measured outcomes (such as PFTs, 6MWD, oxygenation, and biochemical/immunological endpoints), generalized linear mixed effects models will be used to adjust for correlation among repeated measurements within the same subject, while generalized linear models will be used for outcomes measured at baseline and 12 weeks only (e.g., measurement of viral load in BAL), as both continuous outcomes and binary outcomes defined for the subgroups. For both approaches, an appropriate link function and family will be chosen depending on the type of outcome, and the baseline measurement for each outcome will be adjusted in each analysis. All analyses will be performed using STATA 11.0 (StataCorp, College Station, TX) and the programming language R ([50](#_ENREF_50)).
11. **Safety and Adverse Events Monitoring:** Study personnel will meet bi-weekly to review all safety laboratory data collected on each subject and review all adverse events. The study investigators will decide the appropriate steps to be taken to include dose modifications, and further monitoring. Dr. Todd Rice will act asan on-site medical monitor who can become un-blinded to study drug if needed in order to adjudicate side effects are detected and whether they may due to valganciclovir.
	1. **Adverse Event (AE) monitoring:** An AE will be defined as any untoward new medical occurrence, or signs, symptoms, or laboratory abnormalities already existing at enrollment that worsen (i.e., increase in intensity or frequency) in a subject who signed written informed consent. The AE causal relationship with the treatment administered will be assessed as possible, probable, or unrelated to both the study drug or study procedures, and assessed as expected or unexpected. SAEs are defined as AEs that are fatal, life-threatening, emergency room visits, require hospitalization (excluding preplanned admissions or those less than twenty-four hours in duration), result in persistent or significant disability, result in a congenital anomaly, or are in the judgment of the investigator likely to result in a life threatening occurrence or hospitalization without some intervention. All AEs and SAEs will be logged into electronic case report forms. All SAEs will be followed until resolution, stabilization, or for twenty-eight days after the subject has completed the final study-related visit. Study drug will be discontinued in the event of an SAE requiring unplanned hospitalization. All SAEs that are study-drug related (valganciclovir) or study-procedure related (possible or probable), and unexpected will be reported to the institutional review board (IRB) in a timely fashion and all AEs felt to be study related (possible or probable) will be reported to the IRB at continuing review.
12. **Recruitment and Informed Consent**
	1. This project will be approved by the VUMC Institutional Review Board (IRB). Participation in this study is voluntary. Subjects will be recruited from the Vanderbilt Interstitial Lung disease (ILD) clinic. A number of recruitment strategies to enhance recruitment will be utilized. IRB approved advertisements will be posted online through an IRB-approved website post. The Vanderbilt Pulmonary Division has extensive physician referral databases that maintain referring physicians located in Kentucky, Tennessee, Alabama, Mississippi, Georgia, Arkansas, and Indiana will be mailed information and referral brochure. Recruitment and informed consent will not be coercive or unduly influenced by study staff. This trial will be registered at Clinicaltrials.gov.
	2. Written informed consent for participation will be obtained at a face-to-face interview between the individual subject and research personnel only after adequate time has been provided for consent review and discussion in a private clinic room and prior to any research-related activities. All questions will be answered by study personnel knowledgeable in the protocol. Subjects are given the opportunity and encouraged to take the written informed consent home to review with family and/or personal healthcare providers. Documentation of written informed consent will be made by the consenting study staff member placing a note into the electronic medical record. This note will include how consent was obtained, the subject’s level of comprehension, the subject’s decision-making capacity at the time of consent, the time given for the subject to consider the research and whether others were involved in the decision-making process, and who was present during the consenting process. There will be no waivers approved for any portion of the written informed consent, nor will surrogate consent be obtained. All subjects agreeing to this study must be able to understand the consent document. A copy of the signed informed consent will be given to the subject.
13. **Good Clinical Practice**
	1. The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigators abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).
	2. Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers/countries before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained. Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.
	3. Modifications to the study protocol will not be implemented by the investigator without approval of the IRB. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial patients without prior IEC/IRB/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and if appropriate, the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

**Bibliography and References Cited**

1. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810-6. Epub 2006/07/01.

2. Yonemaru M, Kasuga I, Kusumoto H, Kunisawa A, Kiyokawa H, Kuwabara S, et al. Elevation of antibodies to cytomegalovirus and other herpes viruses in pulmonary fibrosis. Eur Respir J. 1997;10(9):2040-5. Epub 1997/10/06.

3. Tang YW, Johnson JE, Browning PJ, Cruz-Gervis RA, Davis A, Graham BS, et al. Herpesvirus DNA is consistently detected in lungs of patients with idiopathic pulmonary fibrosis. J Clin Microbiol. 2003;41(6):2633-40. Epub 2003/06/07.

4. Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. Am J Physiol Lung Cell Mol Physiol. 2008;294(6):L1119-26. Epub 2008/04/09.

5. Ebrahimi B, Dutia BM, Brownstein DG, Nash AA. Murine gammaherpesvirus-68 infection causes multi-organ fibrosis and alters leukocyte trafficking in interferon-gamma receptor knockout mice. Am J Pathol. 2001;158(6):2117-25. Epub 2001/06/08.

6. Lok SS, Haider Y, Howell D, Stewart JP, Hasleton PS, Egan JJ. Murine gammaherpes virus as a cofactor in the development of pulmonary fibrosis in bleomycin resistant mice. Eur Respir J. 2002;20(5):1228-32. Epub 2002/11/27.

7. Eakin EG, Resnikoff PM, Prewitt LM, Ries AL, Kaplan RM. Validation of a new dyspnea measure: the UCSD Shortness of Breath Questionnaire. University of California, San Diego. Chest. 1998;113(3):619-24. Epub 1998/03/27.

8. Yorke J, Jones PW, Swigris JJ. Development and validity testing of an IPF-specific version of the St George's Respiratory Questionnaire. Thorax. 2010;65(10):921-6. Epub 2010/09/24.

9. Tzouvelekis A, Kouliatsis G, Anevlavis S, Bouros D. Serum biomarkers in interstitial lung diseases. Respiratory research. 2005;6:78. Epub 2005/07/27.

10. Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, et al. MMP1 and MMP7 as Potential Peripheral Blood Biomarkers in Idiopathic Pulmonary Fibrosis. PLoS Medicine. 2008;5(4):623-33.

11. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management. Am J Respir Crit Care Med. 2011;183(6):788-824. Epub 2011/04/08.

12. Flaherty KR, Toews GB, Travis WD, Colby TV, Kazerooni EA, Gross BH, et al. Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J. 2002;19(2):275-83. Epub 2002/02/28.

13. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, et al. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2004;350(2):125-33. Epub 2004/01/09.

14. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377(9779):1760-9. Epub 2011/05/17.

15. King TE, Jr., Albera C, Bradford WZ, Costabel U, Hormel P, Lancaster L, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet. 2009;374(9685):222-8. Epub 2009/07/03.

16. King Jr TE, Brown KK, Raghu G, du Bois RM, Lynch DA, Martinez F, et al. BUILD-3: A Randomized, Controlled Trial of Bosentan in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 2011. Epub 2011/04/09.

17. Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2010;363(7):620-8. Epub 2010/05/21.

18. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071-82. Epub 2014/05/20.

19. King TE, Jr., Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083-92. Epub 2014/05/20.

20. Raghu G, Anstrom KJ, King TE, Jr., Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968-77. Epub 2012/05/23.

21. Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldron JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1997;155(1):242-8. Epub 1997/01/01.

22. Baumgartner KB, Samet JM, Coultas DB, Stidley CA, Hunt WC, Colby TV, et al. Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating Centers. Am J Epidemiol. 2000;152(4):307-15. Epub 2000/09/01.

23. Hubbard R, Lewis S, Richards K, Johnston I, Britton J. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. Lancet. 1996;347(8997):284-9. Epub 1996/02/03.

24. Siegesmund KA, Funahashi A, Pintar K. Identification of metals in lung from a patient with interstitial pneumonia. Arch Environ Health. 1974;28(6):345-9. Epub 1974/06/01.

25. Egan JJ, Stewart JP, Hasleton PS, Arrand JR, Carroll KB, Woodcock AA. Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis. Thorax. 1995;50(12):1234-9. Epub 1995/12/01.

26. Tsukamoto K, Hayakawa H, Sato A, Chida K, Nakamura H, Miura K. Involvement of Epstein-Barr virus latent membrane protein 1 in disease progression in patients with idiopathic pulmonary fibrosis. Thorax. 2000;55(11):958-61. Epub 2000/10/26.

27. Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J, et al. Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). Free Radic Biol Med. 2008;45(1):50-9. Epub 2008/04/09.

28. Jorgensen E, Stinson A, Shan L, Yang J, Gietl D, Albino AP. Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. BMC cancer. 2008;8:229. Epub 2008/08/13.

29. Laing S, Wang G, Briazova T, Zhang C, Wang A, Zheng Z, et al. Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues. American journal of physiology Cell physiology. 2010;299(4):C736-49. Epub 2010/06/18.

30. Isler JA, Skalet AH, Alwine JC. Human cytomegalovirus infection activates and regulates the unfolded protein response. J Virol. 2005;79(11):6890-9. Epub 2005/05/14.

31. Cheng G, Feng Z, He B. Herpes simplex virus 1 infection activates the endoplasmic reticulum resident kinase PERK and mediates eIF-2alpha dephosphorylation by the gamma(1)34.5 protein. J Virol. 2005;79(3):1379-88. Epub 2005/01/15.

32. Pardo A, Selman M. Molecular mechanisms of pulmonary fibrosis. Front Biosci. 2002;7:d1743-61. Epub 2002/07/23.

33. Thomas AQ, Lane K, Phillips J, 3rd, Prince M, Markin C, Speer M, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med. 2002;165(9):1322-8. Epub 2002/05/07.

34. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet. 2009;84(1):52-9. Epub 2008/12/23.

35. Beers MF, Mulugeta S. Surfactant protein C biosynthesis and its emerging role in conformational lung disease. Annu Rev Physiol. 2005;67:663-96. Epub 2005/02/16.

36. Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. Am J Respir Cell Mol Biol. 2005;32(6):521-30. Epub 2005/03/22.

37. Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2008;178(8):838-46. Epub 2008/07/19.

38. Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC, et al. Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. Proc Natl Acad Sci U S A. 2011;108(26):10562-7. Epub 2011/06/15.

39. Nash AA, Dutia BM, Stewart JP, Davison AJ. Natural history of murine gamma-herpesvirus infection. Philos Trans R Soc Lond B Biol Sci. 2001;356(1408):569-79. Epub 2001/04/21.

40. Mora AL, Torres-Gonzalez E, Rojas M, Xu J, Ritzenthaler J, Speck SH, et al. Control of virus reactivation arrests pulmonary herpesvirus-induced fibrosis in IFN-gamma receptor-deficient mice. Am J Respir Crit Care Med. 2007;175(11):1139-50. Epub 2007/03/17.

41. Fell CD, Martinez FJ, Liu LX, Murray S, Han MK, Kazerooni EA, et al. Clinical Predictors of a Diagnosis of Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 2010;181(8):832-7.

42. Palmer SM, Limaye AP, Banks M, Gallup D, Chapman J, Lawrence EC, et al. Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation: a randomized, controlled trial. Ann Intern Med. 2010;152(12):761-9. Epub 2010/06/16.

43. Asberg A, Humar A, Rollag H, Jardine AG, Mouas H, Pescovitz MD, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2007;7(9):2106-13. Epub 2007/07/21.

44. Meng Q, Hagemeier SR, Fingeroth JD, Gershburg E, Pagano JS, Kenney SC. The Epstein-Barr virus (EBV)-encoded protein kinase, EBV-PK, but not the thymidine kinase (EBV-TK), is required for ganciclovir and acyclovir inhibition of lytic viral production. J Virol. 2010;84(9):4534-42. Epub 2010/02/26.

45. Kiser TH, Fish DN, Zamora MR. Evaluation of valganciclovir pharmacokinetics in lung transplant recipients. J Heart Lung Transplant. 2012;31(2):159-66. Epub 2012/02/07.

46. Standardization of spirometry--1987 update. Statement of the American Thoracic Society. Am Rev Respir Dis. 1987;136(5):1285-98. Epub 1987/11/01.

47. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J. 2005;26(4):720-35. Epub 2005/10/06.

48. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7. Epub 2002/07/02.

49. Oswald-Richter KA, Richmond BW, Braun NA, Isom J, Abraham S, Taylor TR, et al. Reversal of global CD4+ subset dysfunction is associated with spontaneous clinical resolution of pulmonary sarcoidosis. J Immunol. 2013;190(11):5446-53. Epub 2013/05/01.

50. Raghuveer TS, Cox AJ. Neonatal resuscitation: an update. Am Fam Physician. 2011;83(8):911-8. Epub 2011/04/29.