Supplement for Manuscript #14-01739: A Double-blind, Placebo-controlled, Randomized Trial of N-Acetylcysteine in Idiopathic Pulmonary Fibrosis

This supplement contains the following items:

1. PANTHER-IPF Protocols
    a. Original protocol
    b. Final protocol
    c. Summary of revisions
2. Statistical Analysis Plans
    a. Original statistical analysis plan
    b. Final statistical analysis plan
    c. Summary of revisions

PANTHER-IPF

PREDNISONE, AZATHIOPRINE, AND N-ACETYLCYSTEINE: A STUDY THAT EVALUATES RESPONSE IN IDIOPATHIC PULMONARY FIBROSIS

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Compiled by:
The PANTHER-IPF Protocol Committee

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Distributed by:
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Protocol Summary

PRODUCT	Prednisone, azathioprine, and N-acetylcysteine
CLINICALTRIALS.GOV NUMBER	NCT00650091
PROTOCOL TITLE	**P**rednisone, **A**zathioprine, and **N**-acetylcysteine: A Study **TH**at **E**valuates **R**esponse in **I**diopathic **P**ulmonary **F**ibrosis (PANTHER-IPF)
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION	Confirmed idiopathic pulmonary fibrosis, diagnosed within 48 months of enrollment; forced vital capacity ≥ 50% predicted; hemoglobin adjusted diffusing capacity of the lung ≥ 30% predicted
STUDY OBJECTIVES	To assess the safety and efficacy of N-acetylcysteine and the combination of prednisone + azathioprine + N-acetylcysteine in subjects with newly diagnosed idiopathic pulmonary fibrosis
STUDY DESIGN	Multi-center, randomized, double-blind, placebo-controlled
TREATMENT REGIMENS	1) prednisone (0.5–0.15 mg/kg/day) + azathioprine (1.0–2.0 mg/kg/day) + N-acetylcysteine (600 mg TID) **or** 2) N-acetylcysteine (600 mg TID) **or** 3) placebo
ROUTE OF ADMINISTRATION	Oral
TIME BETWEEN FIRST AND LAST DOSES OF ACTIVE STUDY AGENT	Maximum of 67 weeks
NUMBER OF SUBJECTS	390 (1:1:1)
NUMBER OF CLINICAL CENTERS	Approximately 22 US sites
PRIMARY ENDPOINT	Change in longitudinal forced vital capacity measurements over 60 weeks
INTERIM ANALYSIS	One planned interim analysis of the primary endpoint. It is expected that this evaluation will occur at the study midpoint.

Study Sponsor: **National Heart Lung and Blood Institute**

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List of Abbreviations

6MWT	6-minute walk test
A-aPO_2	alveolar-arterial PO_2 difference
ABG	arterial blood gas
AE	adverse event
AEx	acute exacerbation
A/G	albumin/globulin
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATS	American Thoracic Society
AZA	azathioprine
BAL	bronchoalveolar lavage
BUN	blood urea nitrogen
CBC	complete blood count
cGMP	Current Good Manufacturing Practice
CPI	Composite Physiologic Index
CPK	creatine phosphokinase
CT	computed tomography
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute

DLCO	diffusing capacity of the lung for carbon monoxide
DLCO%pred	diffusing capacity of the lung for carbon monoxide percent predicted
DSMB	data and safety monitoring board
eCRF	Electronic case report form
ERS	European Respiratory Society
FDA	Food and Drug Administration (U.S.)
FEV_1	forced expiratory volume in 1 second
FSH	follicle-stimulating hormone
FVC	forced vital capacity
FVC%pred	forced vital capacity percent predicted
GGT	gamma glutamyl transferase
GSH	glutathione
HAD	Hospital Anxiety and Depression
HHS	Health & Human Services (U.S. Dept . of)
HIPAA	Health Insurance Portability and Accountability Act
HRCT	high-resolution computed tomography
IBW	ideal body weight
ICE CAP	Investigating Choice Experiments for Preferences of Older People Capability Instrument
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis

IPFnet	Idiopathic Pulmonary Fibrosis Clinical Research Network
IRB	institutional review board
ITT	intent to treat
IV	intravenous
IVRS	interactive voice response system
LDH	lactate dehydrogenase
LFT	liver function test
LOCF	last observation carried forward
LSD	Least Significant Difference
MAR	missing at random
MCAR	missing completely at random
MMRM	mixed model repeated measures
MOOP	manual of operating procedures
NAC	N-acetylcysteine
NHLBI	National Heart Lung and Blood Institute (U.S.)
NIH	National Institutes of Health (U.S.)
NSIP	nonspecific interstitial pneumonia
PaO_2	partial pressure of arterial oxygen
PCP	primary care provider
PFT	pulmonary function test

PHS	Public Health Service (U.S.)
PI	principal investigator
PL	placebo
PLT	platelet
PRED	prednisone
QOL	quality of life
SAE	serious adverse event
SAP	statistical analysis plan
SGRQ	St. George's Respiratory Questionnaire
SpO_2	oxygen saturation by pulse oximetry
TPMT	thiopurine methyl transferase
UCSD SOBQ	University of California at San Diego Shortness of Breath Questionnaire
UIP	usual interstitial pneumonia
ULN	upper limit of normal
USP	United States Pharmacopoeia
VC	vital capacity
WBC	white blood cell

PREDNISONE, AZATHIOPRINE, AND N-ACETYLCYSTEINE: A STUDY THAT EVALUATES RESPONSE IN IDIOPATHIC PULMONARY FIBROSIS

1. Summary

There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) will conduct a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy and in combination with azathioprine (AZA) and prednisone (PRED) in subjects with mild or moderate IPF. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] ≥ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DL_{CO}%pred] ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Each subject will be treated up to a maximum of 60 weeks, followed by a tapering of PRED/PL and a 4-week period for safety evaluation.

The primary endpoint is the change in longitudinal measurements of FVC over the 60-week treatment period. The primary goal of this study is to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF.

2. Hypotheses and Specific Aims

2.1. Null Hypotheses

- Treatment with AZA-PRED-NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.
- Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.

2.2. Specific Aim 1

This study is designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF.

2.3. Specific Aim 2

Secondary goals of this study are to assess differences between treatment groups for the following:

1. Mortality
2. Time to death
3. Frequency of acute exacerbations (AExs)
4. Frequency of maintained FVC response
5. Time to disease-progression
6. Change in DL_{CO}
7. Change in Composite Physiologic Index (CPI)
8. Change in resting alveolar-arterial oxygen gradient
9. Change in 6-minute walk test (6MWT) distance
10. Change in 6MWT oxygen saturation area under the curve
11. Change in 6MWT distance to desaturation < 80%
12. Change in 6MWT minutes walked

13. Changes in health status as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ)
14. Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ)
15. Frequency and types of adverse events (AEs)
16. Frequency and types of respiratory complications, both infectious and noninfectious
17. Frequency of hospitalizations, both all-cause and respiratory-related

2.4. Prespecified Subgroups of Interest

Treatment effects will be estimated and compared within key subgroups:

- higher enrollment FVC (Raghu 2004; King 2005)
- typical vs. atypical HRCT reading at baseline (Flaherty, Thwaite, et al 2003)
- a recent vs. more remote diagnosis (time from initial diagnosis of IPF ≤ 1 year and > 1 year)
- lower CPI score at enrollment (Wells 2003)
- use of medical therapy for gastroesophageal reflux (Raghu, Yang, et al 2006; Raghu, Freudenberger, et al 2006)
- ethnic background
- sex
- smoking history (current/ex-smoker vs. never smoker), given potential impact on oxidant status (Kinnula 2005)
- presence of emphysema > 25% on high-resolution computed tomography (HRCT)

3. Background and Significance

3.1. Idiopathic Pulmonary Fibrosis is the Most Common Interstitial Lung Disease

IPF is the most common interstitial lung disease (ILD) of unknown etiology. The current incidence and prevalence of IPF in the United States are not known. A 1994 study of a population-based registry of subjects in Bernalillo County, New Mexico, USA, estimated an incidence of 10.7 cases per 100,000 per year for males and 7.4 cases per 100,000 per year for females; the prevalence of IPF was estimated at 20 per 100,000 for males and 13 per 100,000 for females (Coultas 1994). Extrapolating from a large healthcare claims database, a more recent review estimated the prevalence of IPF in the United States at 42.7 per 100,000 (incidence estimated at 16.3 per 100,000 per year) (Raghu, Weycker, et al 2006). Recent epidemiological studies indicate increasing mortality rates from IPF in the United States and other industrialized nations (Olson 2007; Mannino 1996; Hubbard 1996; Johnston 1990).

Approximately two-thirds of subjects with IPF are over the age of 60 at the time of presentation, and the incidence increases with age (American Thoracic Society, 2000). IPF has no distinct geographical distribution, and predilection by race or ethnicity has not been identified (American Thoracic Society, 2000). Individual subjects may remain relatively stable for prolonged periods, experience very slow declines in lung function with progression of radiographic abnormalities for a period of months to years, or experience more rapid declines and subsequent death. Only 20% to 30% of IPF patients survive for 5 years following diagnosis.

There is currently no proven, effective treatment for IPF. Anti-inflammatory and immunosuppressive agents have been the traditional approach to the management of patients with IPF. However, few controlled clinical trials have been performed to prove efficacy of this approach. In addition, multiple factors have severely limited the ability to draw conclusions from previous therapeutic trials: (a) the lack of a clear understanding of the natural history of IPF; (b) the presence of many different study designs; (c) heterogeneous subject groups; (d) disputable diagnostic certainty; (e) variable study duration; (f) differences in medication formulation, dosage, route of administration, and duration of treatment; (g) differing types and/or lack of quantitative assessment criteria; (h) variable intervals between evaluations; and most importantly, and (i) the lack of controls treated in a true PL arm. Consequently, no management approach has proven to be efficacious compared with a true PL arm, and treatment of IPF is largely based on anecdotes or small studies (Selman 2004; Thannickal 2004;

Richeldi 2003; Davies 2003). Recently, a study comparing treatment of IPF subjects with AZA-PRED-NAC vs. AZA-PRED indicated a better preservation of FVC and DLCO in subjects receiving adjunct treatment with NAC (Demedts 2005); however, a true PL group was not included in this study. Thus, it remains unknown if a combination of AZA-PRED-NAC is superior to PL; it is also not known if NAC alone or in combination with AZA-PRED will prove beneficial in IPF patients. The primary goal of this study is to establish an evidence-based standard of care and clarify the role of immunosuppressive and antioxidant pharmacotherapy for IPF.

3.2. Rationale for Placebo Control

IPF is a disorder for which there is no proven efficacious therapy. A major objective of this trial is to test, to the greatest degree possible, a proposed standard of care for patients with IPF. The current traditional therapy employs immunosuppressive and corticosteroid drugs, which have significant known side effects but have never been proven to improve outcomes in well-designed, well-powered clinical trials. In this prospective, randomized clinical trial, the inclusion of a PL arm is vital to adequately test the benefits of NAC and AZA-PRED-NAC in well-characterized subjects with IPF.

If AZA-PRED-NAC and NAC have no true efficacy, then their role as standard of care will be refuted. If a benefit compared with PL is confirmed, it will establish a benchmark against which future novel therapies for IPF will be safely compared. As there is no currently accepted therapy for IPF, there is an increasing body of published literature supporting the concept of no treatment as the "best care" option for IPF.

Posthoc analyses of PL-controlled trials suggest that subjects with milder disease may be more amenable to therapy (Raghu 2004; King 2005). It is notable that a recent international, prospective, randomized trial of interferon-gamma for IPF also included a PL arm; the study was terminated early by the data and safety monitoring board (DSMB) due to lack of treatment effect (FDA Public Health Advisory 2007). This underscores the belief that a proven effective therapy for IPF does not currently exist and that true placebo-controlled trials remain the gold standard. Similarly, recently completed trials of etanercept and bosentan in IPF have included PL-treated arms. In these trials, the treated subjects showed little, if any, objective improvement. Based on this evidence and the well-known potential for toxicity from immunosuppressive agents, we believe that clinicians and subjects should be willing to enroll in a PL-controlled study. The highly experienced investigators in the IPFnet have discussed this issue extensively and voted to include a PL arm in this trial. We strongly believe that there is clinical equipoise in this trial design in that there is no compelling reason to favor the outcome of one treatment arm over another.

3.3. Rationale for Prednisone and Azathioprine Therapy

The mechanisms by which corticosteroids affect the immune effector cells associated with lung fibrosis are not well understood. Glucocorticoids suppress neutrophil and lymphocyte migration into the lung, as well as decrease the levels of immune complexes. Glucocorticoids also alter alveolar macrophage function by inhibiting the secretion of proteolytic enzymes and by decreasing the release of chemotactic factors. Neutrophil adhesion to endothelial surfaces is also likely modified through direct effects on the surface membrane configuration.

Recent developments in understanding the fundamental mechanisms of gene transcription have led to major advances in understanding the molecular mechanisms by which corticosteroids suppress inflammation. Most inflammatory proteins are regulated by increased gene transcription, which in turn is controlled by proinflammatory transcription factors, such as nuclear factor-kappa B and activator protein-1. Glucocorticoids exert their effects on target cells by interacting with specific intracellular receptors. These receptors are members of a large family of nuclear proteins capable of binding to DNA and regulating expression of specific target genes. It is unclear why some subjects respond to corticosteroids and others do not. It has been suggested that this may be related to the altered expression of glucocorticoid surface receptors on the specific lung parenchymal cells.

Clinical data supporting the role of steroid therapy have been inconsistent (Selman 2004; Raghu 1991). Several uncontrolled studies have been reported over the last several decades with inconsistent results (Richeldi 2003; Thannickal 2005). Prospective, PL-controlled data are not available to definitively address the role of steroid therapy alone in IPF (Richeldi 2003). Flaherty and colleagues reported results of corticosteroid therapy on a multidimensional clinical, radiographic, and physiologic score in 29 IPF subjects (Flaherty 2002). A positive response was seen in 17% of subjects, while 31% remained stable and 52% were classified as nonresponders. A separate report from this group suggested that response to steroid therapy was not associated with a survival benefit; those remaining stable during short-term steroid therapy exhibited the best long-term prognosis (Flaherty 2001). In addition, lower doses have been demonstrated to favorably affect cough in IPF subjects (Hope-Gill 2003).

AZA is a purine analogue that is converted to mercaptopurine in body tissues. It appears to act by the substitution of purines in deoxyribonucleic acid synthesis and by inhibiting adenine deaminase, resulting in relatively selective lymphocyte dysfunction, given their high susceptibility to adenine deaminase deficiency. In addition to cytotoxic effects, AZA has been reported to suppress natural killer cell activity, antibody production, and antibody-dependent cellular cytotoxicity. AZA also suppresses the production of autoantibodies in animal models of autoimmune disease, although the clinical relevance of these findings to IPF remains unknown.

Numerous investigators have combined cytotoxic agents with corticosteroids in IPF subjects, although the majority of the studies have been retrospective or uncontrolled (Bouros 2005). Collard and colleagues did not identify survival differences between IPF subjects treated with combined cyclophosphamide and PRED at one institution and untreated subjects from a second institution (Collard 2004). In contrast, Pereira and colleagues suggested survival benefit to combination cyclophosphamide/steroid compared with corticosteroids alone (Pereira 2006). The lack of randomization, standardization of therapy, and open-label nature of therapy limits the interpretation. Raghu et al reported on a small, prospective, controlled trial of PRED alone compared with PRED plus AZA; subjects treated with combination therapy appeared to experience an age-adjusted survival benefit after 4 years of follow-up (Raghu 1991).

In 2000, the American Thoracic Society (ATS)/European Respiratory Society (ERS) adopted a uniform classification for IPF and also outlined a management approach for patients with IPF (American Thoracic Society 2000). The ATS/ERS consensus committee suggested that therapy was not indicated for all patients with IPF. However, if therapy was recommended to a patient, they proposed that therapy should be started at the first identification of clinical or physiological evidence of impairment or documentation of decline in lung function. Pending the availability of an efficacious therapy for IPF, combined low-dose PRED with AZA was the consensus panel recommendation for treatment of IPF. It remains unknown if there is a beneficial role with combined PRED plus AZA for IPF. Acknowledging the known side effects associated with corticosteroids and AZA, it is not clear if this immunosuppressive therapy is truly effective, or whether it is worth exposing patients to the risk of these agents.

3.4. Rationale for N-acetylcysteine

NAC is a derivative of the amino acid L-cysteine. NAC has been shown to augment levels of the naturally occurring antioxidant glutathione (GSH) (glutathione; γ-glutamyl cysteinyl glycine) both in vitro and in vivo (Borok 1991; Meyer 1994). GSH is present in all eukaryotic cells and may play an important role in protecting alveolar epithelial cells against oxidant injury. The concentration of GSH in the bronchoalveolar lavage (BAL) fluid in patients with IPF is markedly diminished compared with normal subjects. This GSH deficiency may be corrected by exogenous administration of NAC (Meyer 1994).

There is evidence of enhanced production of oxidants in an IPF lung. Both inflammatory cells and myofibroblasts derived from patients with IPF generate increased amounts of extracellular oxidants, including hydrogen peroxide (Cantin 1987; Waghray 2005). Secretion of hydrogen peroxide by activated myofibroblasts may induce the death of adjacent lung epithelial cells by paracrine mechanisms (Waghray 2005). Additionally, generation of oxidants by myofibroblasts induces oxidative crosslinking of extracellular matrix proteins (Larios 2001), a potential mechanism for aberrant matrix remodeling. Thus, an oxidant-antioxidant imbalance exists in the lungs of IPF patients (Kinnula, Fattman, et al 2005). NAC may confer protection against this imbalance by augmenting GSH levels in addition to its more direct free-radical scavenging activity.

Intravenous (IV) NAC therapy has been shown to increase total BAL GSH in 8 IPF subjects (Meyer 1995). Oral NAC (600 mg 3 times per day) has been shown to decrease markers of oxidant injury and improve both total and reduced GSH levels in the epithelial lining fluid of subjects with IPF in a small, uncontrolled study (Behr 1997); pulmonary function improved modestly with therapy. A similar study in 18 IPF subjects confirmed increased intracellular GSH concentration after 12 weeks of NAC (600 mg 3 times per day) (Behr 2002); no clinical correlates were reported.

3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy and in Combination with Azathioprine and Prednisone

Results of a double-blind, multi-center European clinical trial of 150 IPF subjects testing combinations of AZA-PRED vs. AZA-PRED-NAC have recently been reported (Demedts 2005). NAC added to AZA-PRED ("conventional therapy") had a significant positive effect on DL_{CO} ($p < 0.005$) and vital capacity (VC) (p <

0.05) at the end of 1 year (Demedts 2005). These investigators also demonstrated significant protection against bone marrow toxicity in subjects treated with AZA/PRED/NAC. With this new knowledge and awareness, it was considered by the Steering Group to be potentially inappropriate to incur the risk of bone marrow toxicity associated with AZA if NAC is not used as an adjunct therapy in this population. In addition, it was felt that little additional information would be gathered by comparing the treatment effect in subjects receiving AZA-PRED compared to those treated with AZA-PRED-NAC.

The interpretation of these data has been quite controversial. Some have suggested that the magnitude of the treatment effect, although statistically significant, is modest (Toma 2006). Others have suggested that NAC may be modulating potential toxic effects of AZA-PRED alone (Hunninghake 2005), supporting the investigation of NAC as stand-alone therapy. Still others suggest that, pending additional studies, triple therapy should be considered as standard of care in IPF (Wells 2006). However, given the lack of a PL and NAC-alone arms in this trial, whether this triple combination reflects the standard of care for IPF therapy requires a well-designed, PL-controlled trial that will contrast AZA-PRED-NAC vs. NAC alone vs. PL.

The IPFnet will complete such a trial of a 1:1:1 design including these groups. As a reflection of the clinical equipoise of the IPFnet investigators, the 1:1:1 randomization ratio was selected to balance the statistical efficiency and attractiveness to potential subjects. Potential results are illustrated in Figure 1. Panel A would suggest that neither AZA-PRED-NAC nor NAC alone alter FVC over 60 weeks in comparison with PL. These results would strongly suggest that triple-combination therapy should not be considered standard of care. Panel B would suggest that both AZA-PRED-NAC and NAC have a similar effect on FVC that is better than PL. This would suggest that NAC should be strongly considered standard therapy in IPF. Panel C suggests that the NAC alone may be superior to PRED-AZA-NAC. This would also support NAC alone and not triple-combination therapy as standard of care. Panel D suggests that NAC provides additive benefits to AZA-PRED, supporting triple-combination therapy as the standard of care.

Thus, the 1:1:1 double-blind, randomized trial as proposed (AZA-PRED-NAC vs. NAC vs. PL) provides a simple, practical, feasible, and scientifically rational design that will establish standard of care for IPF based on currently available therapeutic agents and the existing data to support their use. We anticipate that all future clinical trials of novel therapeutic agents will be tested against this to-be-established standard of care.

3.6. Rationale for the Study Design and Primary Endpoint

The optimal study design of a therapeutic trial in IPF would include survival as a primary endpoint. The published results of the IFN-γ 1b Phase 3 (GIPF-001) trial suggested a survival benefit in subjects with milder disease in retrospective analyses (Raghu 2004), although the trial was underpowered to address this question. This was likely related to the limited mortality in the PL arm of the study, which included IPF subjects with mild to moderate disease. This study documents that an IPF study powered to improve survival in a patient population with mild disease requires a larger sample size and/or duration of study. In fact, the recently aborted Phase 3 IFN-γ 1b (GIPF-007; INSPIRE) study was a survival-based study and recruited more than 800 subjects at 75 centers worldwide (FDA Public Health Advisory 2007). As such, within the context of the current IPFnet trial, survival is an impractical primary endpoint variable.

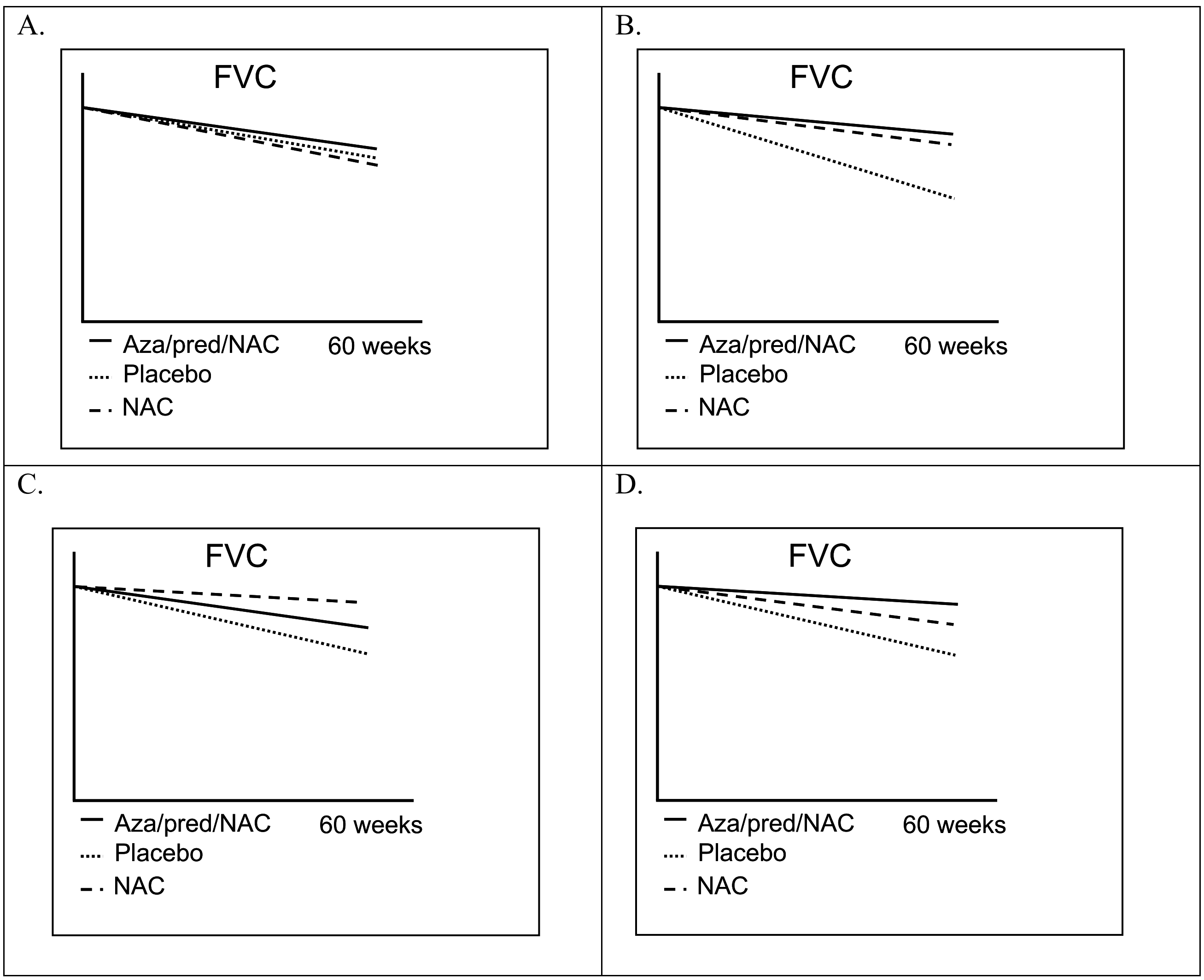


Figure 1: Potential Outcomes Based on FVC Response

Several groups have published data defining an appropriate surrogate outcome variable; a 10% decrement in FVC during 6 to 12 months is a powerful predictor of survival in IPF (Flaherty, Mumford, et al 2003; Latsi 2003; Collard 2003; Jegal 2005). Furthermore, additional evidence suggests a similar predictive ability for a 10% decrement in FVC during 3 months of follow-up (Martinez 2005). With strong supportive evidence of FVC progression being related to mortality on a per-subject basis, this study will use FVC changes in liters between treatment groups as the primary endpoint. Previously published IPF studies have shown a steady decline in FVC (and FVC%pred) among control group subjects (Demedts 2005; King 2005). The GIPF-001 study suggested a 48-week decrease in FVC of 0.16 L in the PL-treated subjects. The IFIGENIA study demonstrated a decline in FVC of approximately 0.19 L over 52 weeks in the subjects randomized to the control treatment. Figure 2 depicts the change in FVC for control groups from previously published IPF studies (Hull 2006). Based on these data, we expect that the PL group will have a decline of 0.20 L over the 60-week study period. The IPFnet Steering Group determined that a clinically meaningful improvement would be the preservation of the majority of the 0.20-L FVC decline. Therefore, a mean treatment difference of 0.15 L in mean FVC over the 60-week study period was determined to be a clinically meaningful difference.

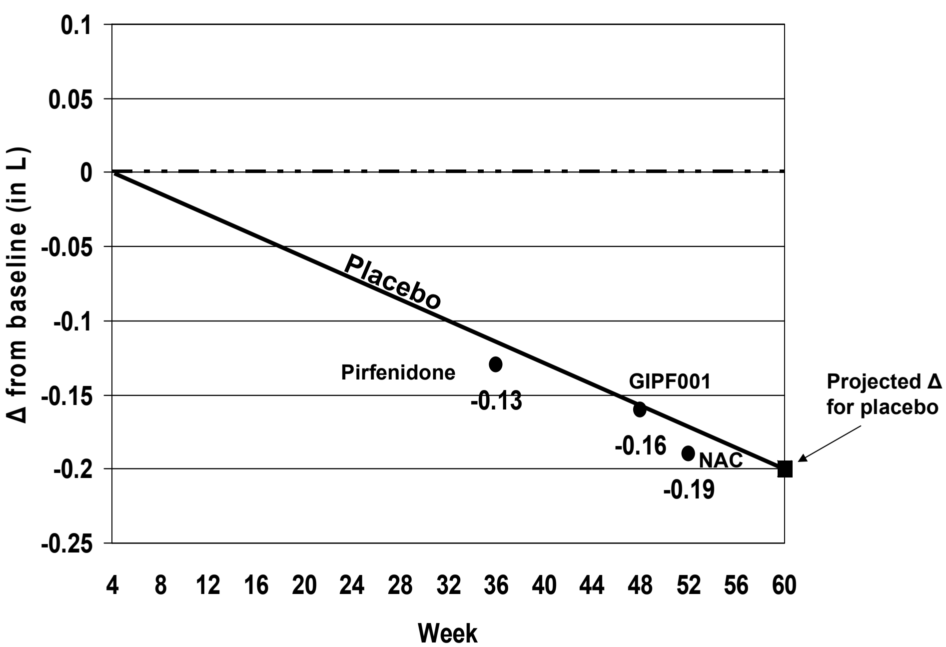


Figure 2: Changes in FVC From Baseline in Prior IPF Clinical Trials

Recent data suggest that various patient subgroups would be expected to potentially exhibit differential response to therapy. These parameters will be used to a priori separate patients by a series of baseline characteristics, including:

1. Higher enrollment FVC (Raghu 2004; King 2005)
2. Typical vs. atypical HRCT readings (Flaherty, Thwaite, et al 2003)
3. Recent vs. more remote diagnosis (time from initial diagnosis of IPF ≤ 1 year and > 1 year)
4. Lower enrollment CPI score
5. Use of medical therapy for gastroesophageal reflux (Raghu, Yang, et al 2006; Raghu, Freudenberger, et al 2006)
6. Ethnic background
7. Sex
8. Smoking history (current/ex-smoker vs. never smoker), given potential impact on oxidant status (Kinnula 2005)
9. Presence of emphysema > 25% on HRCT

3.7. Rationale for Blinding of Treatments

The issue of treatment blinding was given a great deal of consideration, with subject safety being the primary concern. After discussion among the Steering Group members, it was decided that, as long as subject safety could be ensured, blinding was necessary. Blinding allows the study to:

- Have optimal scientific validity and potential to impact the standard of care for subjects.
- Make objective assessments of treatment effects.
- Maintain clinical equipoise among investigators.
- Encourage subjects to have similar levels of contact with the medical community.
- Minimize the differential dropout rates across study arms.

4. Methods

4.1. Inclusion Criteria

1. Age 35 to 85 years, inclusive
2. FVC ≥ 50% of predicted (post-bronchodilator measurement from the screening visit)
3. DL_{CO} ≥ 30% of predicted (hemoglobin corrected and altitude corrected if >4000 ft above sea level)
4. Ability to understand and provide informed consent
5. Diagnosis of IPF according to a modified version of the ATS criteria ≤ 48 months from enrollment. The date of diagnosis is defined as the date of the first available HRCT or surgical lung biopsy characteristic of definite UIP.

4.1.1. Subjects Shown to Have Usual Interstitial Pneumonia Pattern on Surgical Lung Biopsy

Subjects who have been shown to have UIP pattern on lung biopsy must have all of the following:

1. Exclusion of other known causes of ILD, such as drug toxicity, clinically significant environmental exposures, or diagnosis of connective tissue diseases
2. Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scan

4.1.2. Subjects Who Have Not Undergone a Surgical Lung Biopsy

In addition to the criteria above, these subjects must have radiological findings considered to be definite for the diagnosis of UIP/IPF:

1. Bibasilar reticular abnormalities with minimal ground glass opacities
2. Honeycombing as the predominant feature and located in the peripheral lung bases

4.2. Diagnosis of IPF

Only subjects with definite IPF will be eligible for enrollment in this study. We will utilize a combination of clinical/physiologic features, HRCT, and review of a clinically obtained surgical lung biopsy specimen to establish the diagnosis of IPF. An algorithm for the diagnosis is provided to guide entry into the protocol as outlined in the inclusion and exclusion criteria (Figures 3 and 4). This multi-disciplinary approach uses expertise from clinicians, radiologists, and pathologists. Investigators at each site, in conjunction with central pathology, will work together to establish the diagnosis of IPF. This interactive approach to the diagnosis of IPF increases the level of agreement between observers (Flaherty 2004).

A subject with suspected ILD should be evaluated for secondary causes including, but not limited to, environmental exposures, drugs, and systemic diseases. Presence of any of these findings felt to be significant enough to cause an ILD should disqualify the subject from entry into the trial.

If secondary causes are absent, an HRCT scan may be obtained. If an HRCT of sufficiently high quality has been obtained within the last 3 months, that scan may be used for diagnosis. In the appropriate clinical setting, the diagnosis of IPF can be made by the demonstration of a typical radiographic pattern on HRCT or by demonstration of UIP pattern on a surgical lung biopsy. The following criteria for a radiographic (ie, nonsurgical) diagnosis will be used. In the absence of known exposures and/or clinical associations attributable to pulmonary fibrosis, and in the appropriate clinical setting, the presence of definite UIP pattern in HRCT images is required to meet study criteria for the diagnosis of IPF.

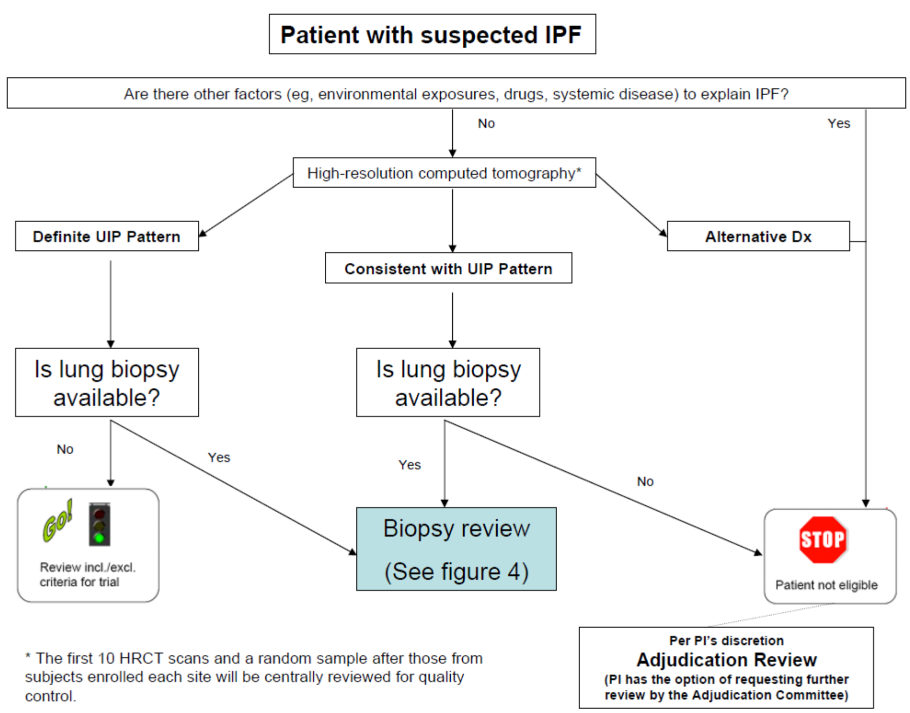


Figure 3: Diagnosis of Idiopathic Pulmonary Fibrosis in the IPFnet

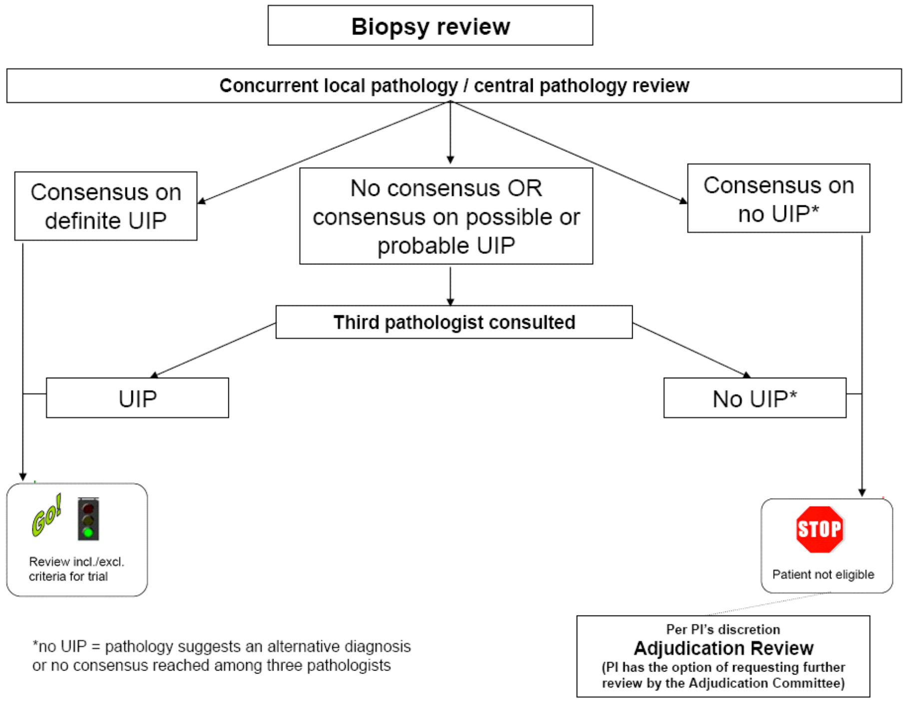


Figure 4: Pathology Flow Chart: Surgical Lung Biopsy Diagnosis

<u>Requirement for diagnosis</u>

1. **Clinical:** exclusion of other known causes (connective tissue diseases, environmental and drug exposures) of ILD
2. **Radiographic:** HRCT with bibasilar reticular abnormality and honeycomb change with minimal ground glass opacities

<u>Appropriate clinical setting</u>

1. Age > 50 years
2. Insidious onset of unexplained dyspnea
3. Duration of illness for ≥ 3 months
4. Bibasilar, inspiratory crackles

Unlike the ATS/ERS consensus criteria published in 2000, bronchoscopy will not be required for diagnosis. This decision was made based on the experience of the IPFnet Steering Group members regarding the utility of bronchoscopy in the diagnosis of IPF. The presence of an atypical HRCT finding will require documentation of a definitive diagnosis by surgical lung biopsy. As shown in Figure 4, central review of the pathology data will be required for a diagnosis of IPF.

We will not require central review of HRCT, as several studies have shown that a confident local interpretation of clinical/HRCT criteria as definite IPF/UIP is associated with a high positive predictive value for finding UIP at surgical lung biopsy (see Table 1). Differences in sensitivity in these series likely reflect subject selection, as Flaherty et al (Flaherty, Thwaite, et al 2003) evaluated only UIP and nonspecific interstitial pneumonia (NSIP), while Raghu et al (Raghu 1999) and Hunninghake et al (Hunninghake 2003) included a broader range of ILD.

Table 1: Operating Characteristics of Local HRCT Review for Diagnosis of UIP

Researcher	**# of Subjects**	**Sensitivity** (%)	**Specificity** (%)	**PPV** (%)	**NPV** (%)
Raghu et al (Raghu 1999)	59 (29 UIP by SLB)	78	90	88	82
Hunninghake et al (Hunninghake 2003)	91 (54 UIP by SLB)	74	81	85	67
Flaherty et al (Flaherty, Thwaite, et al 2003)	96 (only NSIP & UIP)	37	100	100	30

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; UIP, usual interstitial pneumonia; SLB, surgical lung biopsy; and NSIP, nonspecific interstitial pneumonia.

Furthermore, an analysis of the HRCT scans from subjects enrolled in the GIPF-001 trial confirmed that local site interpretations have a high congruity to a central radiology core. In this multi-center study, 263 HRCT scans were read as definite IPF, and a retrospective central radiology core review found 93.2% to be consistent with IPF (Lynch 2005). We will also take several additional steps to insure that the local HRCT reads are accurate, including:

1. A detailed training module has been developed and must be completed by each site radiologist before site initiation.
2. Clinical centers are to mail all HRCT scans to the HRCT core lab. The first 10 HRCT scans from subjects enrolled at each enrolling clinical center will be reviewed centrally to be certain that local reads are congruent with a central interpretation. If discrepancies are identified, additional education will be

provided, and HRCT scans will continue to be reviewed centrally until the central radiology core is confident that the local center is performing appropriately.

3. Random scans will be obtained from each center throughout the study to confirm that the local read continues to agree with central interpretation. If discrepancies are identified, they will be addressed as in #2 above.

In all cases, if a subject has a surgical lung biopsy sample, that sample will be reviewed by the local and central pathologists. Therefore, the only cases that would not be subject to a direct central review process are those where the HRCT meets the centrally defined criteria for an unequivocal diagnosis and a lung biopsy sample is not available. Table 2 below summarizes the possible combinations for making a diagnosis.

Table 2: Combining HRCT and Pathology Interpretations to Determine if IPF is Present

HRCT Diagnosis	**Pathology Diagnosis**	**Diagnosis of IPF**
Definite UIP	Definite UIP	**Yes**
Definite UIP	Probable UIP	**Yes**
Definite UIP	Possible UIP	**Yes**
Definite UIP	Not UIP	**No**
Definite UIP	Unavailable	**Yes**
Consistent with UIP	Definite UIP	**Yes**
Consistent with UIP	Probable UIP	**Yes**
Consistent with UIP	Possible UIP	**No**
Consistent with UIP	Not UIP	**No**
Consistent with UIP	Unavailable	**No**
Inconsistent with UIP	Any	**No**

Abbreviations: HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonia; Dx, diagnosis

4.3. Exclusion Criteria

1. History of clinically significant environmental exposure known to cause pulmonary fibrosis. Occupational exposures, such as asbestos, or environmental exposure to organic dust, such as occurs in pigeon breeders, may at times mimic the clinical and radiographic findings of IPF.
2. Diagnosis of connective tissue disease, felt by the principal investigator (PI) to be the etiology of the interstitial disease. Diagnosis of collagen-vascular conditions will be according to the published American College of Rheumatology criteria. As such, the presence of any documented collagen-vascular disorder or the presence of any suspicious symptom complex, whether or not associated with significantly abnormal rheumatological serologies, will exclude the subject, at the discretion of the PI.
3. Extent of emphysema greater than the extent of fibrotic change (honeycombing, reticular changes) on HRCT scan
4. Forced expiratory volume in 1 second (FEV_1)/FVC ratio < 0.65 at screening (postbronchodilator)
5. Partial pressure of arterial oxygen (PaO_2) on room air < 55 mm Hg (< 50 mm Hg at Denver site)
6. Residual volume > 120% predicted at screening (postbronchodilator)
7. Evidence of active infection
8. Significant bronchodilator response on screening spirometry, defined as a change in $FEV_1 \geq 12\%$ and absolute change > 200 mL OR change in FVC ≥ 12% and absolute change > 200 mL. The percent difference between the FVC (or FEV_1) values will be calculated by taking the absolute value of the difference and dividing it by the larger of the two FVC (or FEV_1) values
9. Screening and enrollment post-bronchodilator FVC measurements (in liters) differing by > 11%. The percent difference between the FVC values will be calculated by taking the absolute value of the difference and dividing it by the larger of the two FVC values (eg., the percent difference between FVC measurements of 1.9 and 2.0 liters would be determined by taking the difference between the two (0.1 liters) and dividing by the larger of the two (2.0 liters). So 0.1/2.0 = 5%, and these FVC measurements would not exclude the subject.
10. Listed for lung transplantation, ie., the patient has completed the evaluation process, has been accepted as a candidate for transplantation at an appropriate center, and is waiting to receive notification of an available donor organ
11. History of unstable or deteriorating cardiac disease
12. Myocardial infarction, coronary artery bypass, or angioplasty within 6 months of screening

13. Unstable angina pectoris or congestive heart failure requiring hospitalization within 6 months of screening
14. Uncontrolled arrhythmia
15. Severe uncontrolled hypertension
16. Known HIV or hepatitis C
17. Known cirrhosis and chronic active hepatitis
18. Active substance and/or alcohol abuse (as determined by site PI)
19. Pregnancy or lactation (subjects who are pregnant or breastfeeding)
20. Women of childbearing potential who are not using a medically approved means of contraception (ie, oral contraceptives, intrauterine devices, diaphragm, Norplant®, etc). 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.
21. Any clinically relevant lab abnormalities (from central lab values obtained within 30 days before enrollment), including:
    a. Creatinine > 2 x upper limit of normal (ULN)
    b. Hematology outside of specified limits:
        i. White blood cells (WBCs) < 3,500/mm^3
        ii. Hematocrit < 25% or > 59%
        iii. Platelets < 100,000/mm^3
    c. Any of the following liver function test (LFT) criteria above specified limits:
        i. Total bilirubin > 2 x ULN
        ii. Aspartate (AST) or alanine aminotransferases (ALT) (serum glutamic-oxaloacetic transaminase [SGOT], or serum glutamic pyruvic transaminase [SGPT]) > 1.5 x ULN
        iii. Alkaline phosphatase > 3 x ULN
        iv. Albumin < 3.0 mg/dL at screening
22. Homozygous for low thiopurine S-methyl transferase (TPMT)
23. Overt or persistent clinical depression as perceived by site PI and / or the patient's primary physician and supported by uncontrolled depression (depression component of the Hospital Anxiety and Depression Score [HADS-D] ≥ 15)
24. Known hypersensitivity to study medication

25. Any condition other than IPF that, in the opinion of the site PI, is likely to result in the death of the subject within the next year
26. 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
27. Any therapy directed at pulmonary fibrosis (excepting triple therapy of prednisone plus azathioprine plus NAC) requires a 30-day washout period before randomized. Triple therapy of <= 12 weeks duration in the past 4 years requires a 30-day washout period before randomization.
28. History of triple therapy of prednisone plus azathioprine plus NAC for > 12 weeks' duration in the past 4 years

4.4. Study Design and Study Visit

4.4.1. Study Design Summary

This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF.

Approximately 390 subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLCO%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Once enrolled, subjects will visit the clinical center at 4 weeks, 15 weeks, and 15-week intervals thereafter. Between visits, subjects will visit local blood-draw centers or the clinical center for monitoring of blood counts and serum chemistries on a predefined schedule. Each subject will be treated and followed for a maximum of 60 weeks.

During the 60-week visit, subjects will be taken off all study agents except PRED/PL and will be placed on a tapering dose. Approximately four weeks after the final dose of PRED/PL is taken, subjects will receive a safety phone call from the study site.

4.4.2. Study Visits

Subjects who meet entry criteria will review the informed consent, a written description of the purpose, procedures, and risks of the study, with the PI, coinvestigator, or study coordinator, and all questions will be answered. The informed consent form will be signed by the subject at screening. No protocol-specific procedures will be performed until the subject has signed and dated an informed consent form. This includes the screening procedures.

4.4.2.1. Screening

Once informed consent is obtained, subjects may immediately begin the screening process or may return within 28 days of consent. In the event a study subject has been clinically evaluated at the study site by an IPFnet study physician and has performed testing within three weeks for this clinical evaluation that meets guidelines provided in the IPFnet PANTHER-IPF Manual of Operating Procedures (MOOP), this testing may be used to satisfy the following screening criteria: medical history, physical exam, arterial blood gas (ABG) with A-a gradient, vital signs with oximetry, body height and weight, spirometry, DL_{CO}, lung volumes, and HRCT scan.

Allowing the use of previously performed test results that meet study guidelines for the screening visit is intended to permit subjects easier access to study entry, to prevent subjects from repeating testing that has been performed within the study window, and to decrease risks to subjects from repeated exposure to procedures such as arterial puncture and HRCT.

The following procedures will be performed at screening:

- Medical history and a physical examination
- Measure height and weight
- Measure vital signs including oximetry
- Blood draws performed and the following analyses conducted:
  - If not previously done, TPMT levels
  - Hematology (red cell count, white cell count, hemoglobin, hematocrit, cell indices, differential, platelet count)
  - Blood chemistries according to central laboratory protocol (see Section 4.10, Laboratory Testing)
  - FSH checked (if deemed necessary)
  - Beta human chorionic gonadotropin (serum) pregnancy test (in women of childbearing potential)
  - Urinalysis
- PFTs, including spirometry pre- and post-bronchodilator, and post-bronchodilator measurement of lung volumes, and measurement of hemoglobin adjusted diffusing capacity.
- Measure ABGs
- HRCT if a satisfactory scan has not been performed on the subject within 3 months of screening
- Surgical lung biopsies (if applicable) reviewed by local and central pathology departments
- Current medications. If required, a washout period discussed with the subject and initiated at this visit
- Depression and anxiety levels measured using the Hospital Anxiety and Depression (HAD) scale.

4.4.2.2. Enrollment

The enrollment visit is expected to take place within eight weeks of the screening visit. Enrollment visit activities include:

- Measure vital signs, including oximetry
- Measure height and weight
- Blood draw and measurement of blood cell counts and serum chemistries
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Spirometry (post-bronchodilator)
- Measure 6MWT with Borg Dyspnea Scale
- Collect Quality-of-life (QOL) data using the SF-36, EuroQol, Investigating Choice Experiments for Preferences of Older People Capability Instrument (ICE CAP), and SGRQ
- Complete HAD scale questionnaire
- Female subjects complete Gender Substudy questionnaire
- Dyspnea status collected using the UCSD SOBQ
- Evaluate Acute Exacerbation (AEx)
- Review of any Adverse Events (AEs)
- Review of concomitant medications
- Subject receives diary and instructions on its purpose and proper use
- Subject receives supply of study drug sufficient to last until his or her 15-week study visit

If the enrollment visit occurs within 21 days of the screening visit, some procedures may not need to be performed at this visit. See the Schedule of Assessments (Table 4) for more details. Subjects with screening and enrollment post-bronchodilator FVC measurements (in liters) differing by more than 11% are not eligible to be enrolled in the study.

Subjects will be asked to provide a physician of record. This physician will be considered the subject's primary care provider (PCP), and, if the subject agrees, the PCP will be informed by letter of the subject's enrollment in the trial. The subject will be informed that his or her ongoing medical care should be received from the PCP. The PCP will be informed of any safety issues identified by the study staff. The PCP will also be given information regarding communication with study personnel about pertinent health issues or clinic encounters the subject may have.

4.4.2.3. Week 1

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.4. Week 2

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.5. Week 4

The week 4 visit is expected to occur within +/- 7 days of the subject's scheduled visit time (eg., the week 4 visit should occur anytime between 3 and 5 weeks after starting study drug). Week 4 activities include:

- Targeted medical history
- Physical examination
- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries) to monitor for side effects
- Review AEs
- Evaluate for AEx
- Review concomitant medications.
- Subject will complete HAD scale questionnaire
- Review study diary

4.4.2.6. Week 6

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.7. Week 10

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.8. Week 15

The week 15 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg., the week 15 visit should occur anytime between 13 and 17 weeks after starting study drug Week 15 activities include:

- Measure vital signs with oximetry
- Measurement height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Serum pregnancy test (if applicable)
- Spirometry (post-bronchodilator) measurement
- Review of AEs
- Evaluate for AEx
- Review concomitant medications
- Subject will complete HAD scale questionnaire
- If consented, draw blood and collect urine specimen for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Review study diary and a new study diary will be given
- Provide additional supply of study drug sufficient to last until the next scheduled visit

If at anytime during the study the subject has a post-bronchodilator FVC measurement indicating a drop ≥ 10% from the baseline value, he or she must be scheduled for a follow-up visit within 6 to 8 weeks. The FVC decline is confirmed only once throughout the duration of the study. Once an FVC decline is confirmed, no further confirmation visits will be scheduled.

4.4.2.9. Week 20

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.10. Week 25

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.11. Week 30

The week 30 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg., the week 30 visit should occur anytime between 28 and 32 weeks after starting study drug). Week 30 activities include:

- Physical examination
- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Serum pregnancy test (if applicable)
- Measure spirometry (post-bronchodilator)
- 6MWT with Borg scale
- DLCO
- Review of AEs
- Evaluate for AEx
- Review concomitant medications
- Complete all QOL and dyspnea questionnaires (EuroQol, ICE CAP, SF-36, SGRQ, and UCSD SOBQ).
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Review study diary
- Subjects return used and unused study drug for the visit
- Provide additional supply of study drug sufficient to last until the next scheduled visit

4.4.2.12. Week 35

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.13. Week 40

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.14. Week 45

The week 45 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg, the week 45 visit should occur anytime between 43 and 47 weeks after starting study drug). Week 45 activities include:

- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Serum pregnancy test (if applicable)
- Measure spirometry (post-bronchodilator)
- Review of AEs
- Evaluate for AEx
- Review concomitant medications
- Subjects wll complete the HAD scale questionnaire
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Study diary reviewed
- Provide additional supply of study drug sufficient to last until the next scheduled visit

4.4.2.15. Week 50

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.16. Week 55

Subjects will visit their local blood-draw site or the clinical center to provide samples for blood counts and chemistry.

4.4.2.17. Week 60 (Early Withdrawal/Final Treatment Visit)

At week 60, or at subject withdrawal from the study (premature, by study doctor or subject's decision), a final treatment visit will occur. At this final treatment visit subjects will discontinue AZA/PL and NAC/PL abruptly. Subjects will receive a supply of PRED (or PL) sufficient to taper off of the drug. The tapering schedule will vary depending on the dose of PRED (or PL) the subject is taking at the time of withdrawal. Week 45 activities also include:

- Physical examination
- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Serum pregnancy test (if applicable);
- Spirometry (post-bronchodilator) measurement
- 6MWT with Borg scale measurement
- DLCO
- Lung volumes
- ABG
- Review of AEs
- Evaluate for AEx
- Review concomitant medications.
- Subjects will complete all QOL and dyspnea questionnaires (EuroQol, ICE CAP, SF-36, SGRQ, and UCSD SOBQ)

- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Study diary reviewed

Table 3: Tapering Dose Schedule for Prednisone

Prednisone dose at the final treatment visit :	**Subject will:**
> 10 mg/day for fewer than 15 days (and subject was <u>not</u> on any PRED before enrolling in the trial)	Stop taking PRED (or PL) abruptly, along with all other study drugs.
> 10 mg/day for more than 15 days (and/or subject had taken PRED before enrolling in trial)	Stop taking AZA and NAC abruptly. Decrease PRED (or PL) dosage by 5 mg every 4th day (ie., take dosage for 3 days, then on 4th day drop dosage by 5 mg). When subject reaches equivalent of 10 mg/day for 3 days, follow tapering schedule for 10mg/day (see below).
10 mg/day (maintenance or upon tapering to reach 10 mg/day)	Stop taking AZA and NAC abruptly. Alternate PRED (or PL) dose between 10 mg/day and 5 mg/day each day for 1 week, then move to the 5 mg/day tapering schedule (see below).
5 mg/day (maintenance or upon tapering to reach 5 mg/day)	Stop taking AZA and NAC abruptly. Take 5 mg/day of PRED (or PL) each day for 1 week, then alternate dose between 5 mg/day and 0 mg/day (ie., no tablet) each day for the next week, then decrease to twice during the next week (Monday and Thursday), and then stop completely.

Abbreviations: PRED, predisone; PL, placebo; AZA, azathioprine; NAC, N-acetylcysteine

If not tolerating this slow taper, the subject will be instructed to stop further taper and go back to the dose reached before developing new symptoms (below) and notify the clinical site for instructions on further PRED/PL withdrawal. Based on the severity of the symptoms, the subject may need to be evaluated and managed by a physician either at the site or by a physician proximal to the subject's residence.

These symptoms include the following:

- Worsening shortness of breath
- Dizziness/low blood pressure

- Abdominal pain/cramps; nausea and vomiting
- Fever
- Muscle pain
- Joint pain
- Fatigue
- Headache

4.4.2.18. Final Site Visit – FVC drop confirmation

In the event that the subject has a recorded FVC drop of >10% from baseline at the final treatment visit and the subject has not had a confirmation of such a drop at a previous study visit, the subject should return to the clinical site 6 – 8 weeks after the final treatment visit. During this visit, a post-bronchodilator spirometry test will be performed. This FVC measurement will be evaluated according to section 5.2.1 of this protocol.

4.4.2.19. Final Visit – Telephone Follow-up

Four weeks following the final dose of study medication, subjects will receive a telephone call from the study coordinator to ensure that there are no side effects related to the halting of PRED/PL and to follow up on any ongoing adverse events (AEs).

4.4.2.20. Phone Contact Between Visits

At week 2 and each month that a subject does not have a scheduled clinical center visit, his or her study coordinator will contact him or her at least once by telephone to:

- Inquire if the subject has had any hospitalizations, events that might be considered an AE, or any events significant enough to warrant an out-of-cycle visit to the clinical center
- Ensure compliance with the scheduled local blood draws and address any concerns regarding them
- Remind subjects of their current dosage levels and confirm that the subject understands them
- Address any questions or concerns the subject might have regarding other aspects of the study
- Assess adherence to the treatment regimen by reviewing diary data; verbal review of medications taken, including nutritional supplements

4.4.2.21. Long-term Follow-up

Following the above visits, subjects will have no further study visits. However, study staff will conduct a long-term follow-up 5 years after the subject completes the study visits. There are no plans to contact the subject directly during this follow-up. Study staff will be asked to collect survival information from the Social Security Death Index or other forms of public information.

Table 4: Schedule of Assessments

Procedure	Screening Visit 0	Enrollment Visit 1	Wk 4 Visit 2	Wk 15 Visit 3	Wk 30 Visit 4	Wk 45 Visit 5	Wk 60 Visit 6	Final Visit [6] (via phone)
Informed consent	X							
Medical history	X							
Targeted medical history			X					
Inclusion/exclusion criteria	X							
Serum pregnancy test (if applicable)	X			X	X	X		
Review of lung biopsy	X							
ABG	X						X	
6MWT		X			X		X	
Physical examination	X		X		X		X	
Vital signs with oximetry	X	X	X	X	X	X	X	
Body height and weight	X	X	X	X	X	X	X	
CBC[2]	X	X[1]	X	X	X	X	X	
Chemistry panel[2]	X	X[1]	X	X	X	X	X	
Monitor Lab Values[3]	X	X	X	X	X	X	X	
Urinalysis	X							
Research blood draw and urine collection (if consent granted)		X		X	X	X	X	
TPMT measurement (if not already done)	X							
FSH (if applicable)	X							
HRCT (if not completed within three months)	X							
Spirometry (pre- and post-bronchodilator)	X							
Spirometry (post-bronchodilator only)		X		X	X	X	X	
DLCO (post-bronchodilator only)	X				X		X	
Lung volumes (post-bronchodilator only)	X						X	
Evaluate for acute exacerbation		X	X	X	X	X	X	
Review AEs		X	X	X	X	X	X	X
Review concomitant meds	X	X	X	X	X	X	X	
Dispense subject diary		X		X	X	X	X	
Review subject diary		X	X	X	X	X	X	
Dispense study treatment and collect unused study agent		X		X	X	X	X[4]	
Gender Substudy questionnaire[5]		X						
HAD Scale	X	X[1]	X	X	X	X	X	
EuroQol, ICE CAP, UCSD SOBQ, SGRQ, SF-36		X			X		X	

Abbreviations: ABG, arterial blood gas; 6MWT, 6-minute walk test; CBC, complete blood count; TPMT, thiopurine methyl transferase; FSH, follicle-stimulating hormone; HRCT, high-resolution computed tomography; DLCO, diffusing capacity of the lung for carbon monoxide; AE, adverse event; HAD, Hospital Anxiety and Depression; ICE CAP, Investigating Choice Experiments for Preferences of Older People; UCSD SOBQ, University of California at San Diego Shortness of Breath Questionnaire; SGRQ, St. George's Respiratory Questionnaire

[1]If the enrollment visit occurs within 21 days of the screening visit, these procedures do not need to be repeated.
[2] There will be interim blood draws for blood cell counts and serum chemistries. These may be drawn at the clinical center or a laboratory closer to subject's home.
[3]Blood draws will also occur at local blood draw centers during the following weeks: 1, 2, 6, 10, 20, 25, 35, 40, 50, and 55.
[4]Final study kit will be provided to allow tapering of PRED/PL.
[5]Female subjects only.
[6]Follow-up visit via phone will occur four weeks after final dose of study medication

4.5. Dose Justification

The general philosophy for determining dosing levels was to apply previously examined treatment regimens. With the focus of the study being to establish a standard of care for mild/moderate IPF subjects, the goal was to develop flexible yet standardized treatment rules that allow for the temporary or permanent withholding of one or more components of treatment when necessary. Subjects developing laboratory abnormalities or symptoms that result in discontinuation of one or more components of study treatment may continue on the other components as long as there is no contraindication for this. Complete follow-up is important for the validity of any study. As a strategy to maintain protocol adherence, we are using treatment regimens that will detect potential side effects and prompt interventions proactively in the interest of patient safety. In addition, subjects who permanently stop study medications during the course of the study are encouraged to continue in the study, completing all scheduled visits and tests.

The dosing for PRED was set at relatively low doses to limit common steroid side effects. The incidence of AZA-related side effects will be reduced because the dosage is determined based on the TPMT levels that will be checked at screening. Algorithms have been developed to assist with dosage adjustments of study medication in response to specific laboratory abnormalities or symptoms. If questions arise, the IPFnet Data Coordinating Center (DCC) medical monitor and PANTHER-IPF protocol cochair Dr. Ganesh Raghu will be available for consultations about possible dose reductions and side effects management.

4.5.1. Azathioprine

Measurements of TPMT activity are required on all subjects before enrollment in the study. If previous TPMT measurements are unavailable, TPMT levels will be measured at screening. TPMT activity tests for this study will be conducted by the Estoterix CTS Laboratories in. Calabasas, CA.

The accumulation of metabolites of AZA depends on the activity of TPMT. In a review of the literature, MacDermott found the following concerning metabolites and TMPT:

> "Approximately 89% of the population has wild type TPMT, which is associated with normal or 'high' activity, while 11 percent are heterozygous and have corresponding low activity. Importantly, 0.3 percent of the population are homozygous for mutations of TPMT and thus have negligible activity. Deficiency of this enzyme causes 6-MP to be preferentially metabolized toward the excessive production of 6-TG nucleotides, which correlate with bone marrow suppression. 6-MMP correlate with liver toxicity, manifested as increased liver enzymes." (MacDermott, 2007)

Subjects who are homozygous for low TPMT levels will therefore be excluded from the protocol.

4.5.1.1. Rationale for Azathioprine Dosing

This treatment regimen is based on the original observations in a case series by Winterbauer et al (Winterbauer 1978), and the double-blind, randomized clinical trial published by Raghu et al (Raghu 1991). The described dosing schedule is a standard regimen used in clinical practice for rheumatological diseases. The ATS Consensus Statement for IPF acknowledged that there were no dose-dependent data available for AZA. However, the dose proposed for this study is in keeping with longstanding "standard of care" use of AZA. In addition, the dosing regimen corresponds to the strategy used in the IFIGENIA study, where it was generally well tolerated.

4.5.1.2. Azathioprine/Placebo Dosing

AZA/PL dosages are prescribed based on the subject's ideal body weight (IBW) in kg and adjusted based on TPMT activity and concurrent use of allopurinol (Table 5). AZA/PL capsules are 50 mg. The calculated dose for subjects should be rounded to the nearest 50 mg. For most subjects, AZA/PL dosing is initiated at a lower dose for 2 weeks and then increased to a maintenance dose (beginning of week 3 until end of AZA/PL treatment). AZA/PL capsules equivalent to the prescribed dose should be taken once or twice per day (ie, 1 capsule every other day or daily; 2 capsules—1 in the morning, 1 in the evening; 3 capsules—1 in the morning, 2 in the evening).

Table 5: Azathioprine/Placebo Dosing

	Initiation **Dosage Weeks 1 and 2**	**Maintenance** **Dosage starts Week 3**
Negligible TPMT activity (homozygous for low TPMT [< 6.3 U/mL RBC])	None (exclude from study)	None (exclude from study)
Low TPMT activity (heterozygous for low TPMT [6.3–15.0 U/mL RBC])	50 mg/day If also taking allopurinol, the starting dose is 50 mg every other day.	1 mg/kg IBW/day (maximum dose 100 mg/day) If also taking allopurinol, the maintenance dose is no greater than 50 mg every other day.
Normal TPMT activity (≥15.1 U/mL RBC)	50 mg/day If also taking allopurinol, the starting dose is 50 mg/day.	2 mg/kg IBW/day (maximum dose 150 mg/day) If also taking allopurinol, the maintenance dose is no greater than 50 mg/day.

Abbreviations: TPMT, thiopurine methyl transferase; RBC, red blood cell; IBW, ideal body weight

4.5.1.3. Azathioprine Monitoring

Screening

- Baseline CBC, including platelets
- Chemistry (including LFTs)
- TPMT level
- Amylase

Follow-up Blood Tests

Following enrollment, monitoring of blood cell counts and serum chemistries is to be conducted weekly for 2 weeks; then at week 4, week 6, and week 10; then once every 5 weeks. Additional tests may be required based on symptoms or laboratory changes as outlined in the Dosage Adjustment Algorithms (Section 4.5.4).

4.5.1.4. Dosage Adjustments for Azathioprine/Placebo (see Dosage Adjustment Algorithms)

AZA/PL dosage adjustments in response to laboratory changes or symptoms are provided in algorithm format (see Dosage Adjustment Algorithms, Section 4.5.4).

Azathioprine Dosing During Acute Infections or Suspected Acute Exacerbation

During episodes of acute infection as determined by the clinical center investigator, or if the subject is admitted to an inpatient facility, AZA/PL should be withheld. Resume the maintenance dose of AZA/PL after infection resolves or the subject has been discharged from the inpatient facility and the clinical investigator determines that it is appropriate for the subject to resume study medications.

Reasons to Discontinue Azathioprine/Placebo

The Dosage Adjustment Algorithms outline circumstances in which AZA/PL will be discontinued for the duration of the study based on laboratory abnormalities or symptoms. In addition, AZA/PL will be discontinued permanently for subjects developing:

- Pancreatitis
- Lymphoma

4.5.2. Rationale for Prednisone/Placebo Dosing

The dosage and regimen chosen for this study is a modified version of the dosage recommended by the consensus of the expert panel that led to the joint ATS/ERS Statement (American Thoracic Society 2000). Since then, this particular dosage regimen has evolved into an ongoing standard of care despite acknowledging that this is based on anecdotal experiences over decades. Nevertheless, this regimen has now been tested in subjects with IPF in a prospective manner, and subjects seem to tolerate the dosage schedule guided by the ATS (Demedts 2005). In an attempt to decrease the side effects associated with the PRED as well as increase the blinding of treatments, the dosage chosen in this study is slightly lower than the one used in the IFIGENIA study.

4.5.2.1. Prednisone Dosing

Doses of PRED/PL should be taken once each day. The doses are prescribed according to the subject's IBW expressed in kg. Doses should be rounded to the nearest 5 mg. For example, 27 mg should be rounded to 25 mg, and 28 mg should be rounded to 30 mg. PRED/PL dosing is initiated at 0.5 mg/kg IBW/day. PRED/PL doses are gradually decreased over the first 6 months of treatment as indicated in Table 6. Dosing is then sustained at 0.15 mg/kg IBW/day for the remainder of the study treatment period (until Week 60 or final treatment visit) at which point PRED/PL is tapered as described in Section 4.4.2.7.

Table 6: Prednisone/Placebo Dosing

Time Period	**Months 1 and 2**		**Months 3–6**	**Months 6–15**	
	Weeks 1–2 **Day 1–14**	**Weeks 3–8** **Day 15–56**	**Weeks 9–24** **Day 57–168**	**Weeks 25–60** **Day 169–420**	**Final treatment visit**
PRED/PL Dose	0.5 mg/kg IBW/day	0.3 mg/kg IBW/day	0.25 mg/kg IBW/day	0.15 mg/kg IBW/day	Taper per section 4.4.2.8 Week 60 /Final Treatment Visit

Abbreviations: PRED, prednisone; PL, placebo; IBW, ideal body weight

4.5.2.2. Reasons to Discontinue Prednisone

Subjects must be informed of the potential for developing avascular necrosis, acute glaucoma, increases in blood sugar requiring insulin, and profound emotional disturbances while on PRED. Subjects must also be informed of the risks of abruptly discontinuing PRED therapy and the need to taper PRED/PL. PRED/PL tapering (using the guidelines in Section 4.4.2.7) and discontinuation may be considered for:

- Diabetes mellitus not controlled by oral antihyperglycemics or insulin
- Psychoses per assessment by a mental health professional

- Development of avascular necrosis
- Glaucoma not controlled by medications

4.5.2.3. Prednisone/Placebo Dosing During Apparent Acute Exacerbation of IPF

Hold oral PRED/PL during IV corticosteroids.

4.5.2.4. Recommended Dosing of Intravenous Corticosteroid During Acute Exacerbation of IPF

IV solumedrol: 1.0 g/day for 3 days, 0.5 g/day for 3 days, and taper dosage to reach 0.5 mg/kg/day of oral PRED by the end of 2 weeks as clinically tolerated. Then follow taper guidelines in Table 3, section 4.4.2.7. When the subject is tapered off active PRED, the PRED/PL dosing should resume in accordance with the study schedule.

4.5.2.5. Prednisone Dosing During Clinical Worsening or Shortness of Breath and Cough (Not Considered Acute Exacerbation)

Temporary treatment with oral dose PRED up to 40 mg/day regardless of body weight for a short duration (7–14 days) is allowed at the discretion of the clinician involved in the care of the subject. The study treatment of PRED/PL should be continued during this time. The temporary PRED treatment should be decreased to the prescribed dose of PRED/PL by the end of a 2-week period. If the clinician judges that a slower taper is needed, the guidelines in Table 3, section 4.4.2.7, can be followed. The study treatment of PRED/PL should be continued as directed by the protocol during the temporary treatment with PRED.

4.5.3. Rationale for N-acetylcysteine Dosing

To our knowledge, there have been no IPF studies to correlate clinical outcome measures with different dosages for NAC. The dosage chosen is based on the IFIGENIA study. However, BAL lung GSH levels from subjects with IPF have been augmented with the use of oral NAC at 600 mg 3 times per day. In addition, lung GSH levels have been associated with improved PFTs (Meyer 1994; Meyer 1995; Behr 1997). The dose chosen for this study was based on previous data, including the IFIGENIA study (Demedts 2005).

4.5.3.1. Dosing of N-acetylcysteine/placebo

Dosing of NAC/PL will be 600 mg orally 3 times a day (1800 mg/day).

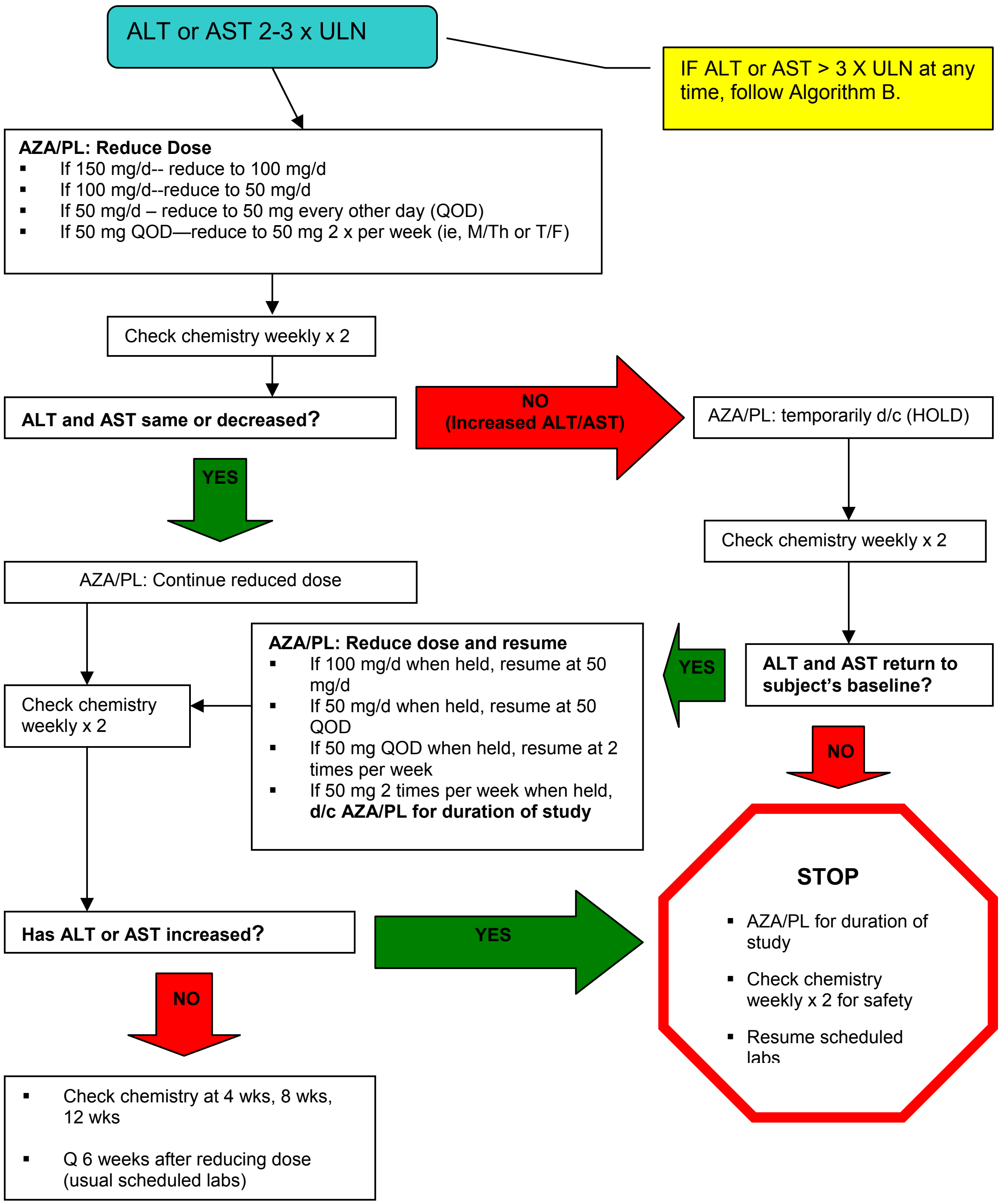
4.5.3.2. Reasons to Discontinue N-acetylcysteine/placebo

NAC/PL may be temporarily or permanently discontinued for the duration of the study for gastrointestinal symptoms or dermatologic reactions as described in the Dosage Adjustment Algorithms, Section 4.5.4.

Temporarily discontinue (hold) oral NAC/PL for subjects requiring inpatient admission for acute exacerbation (AEx) or other conditions. Resume NAC/PL after discharge.

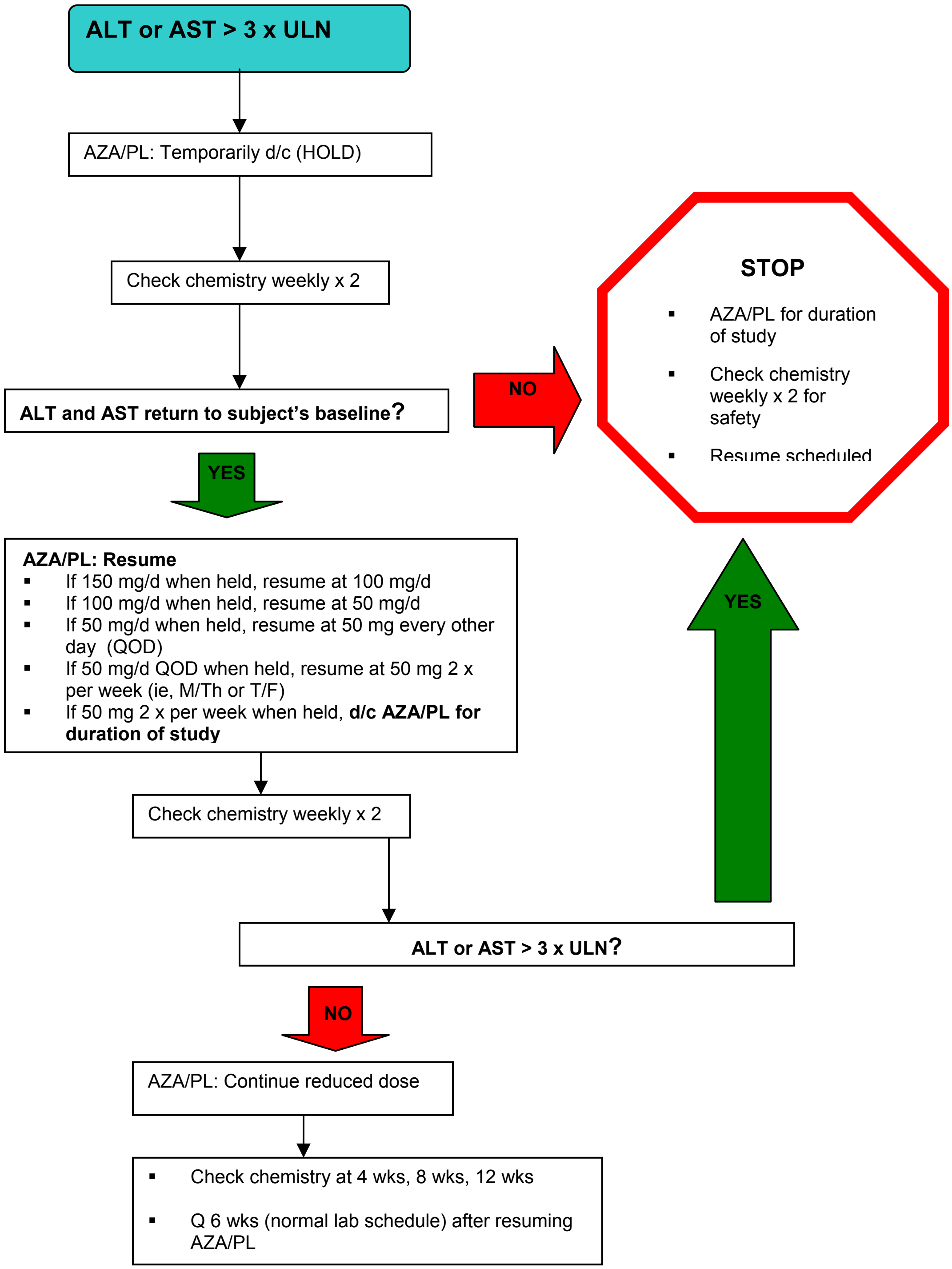
4.5.4. Dosage Algorithms (A-H)

Dosage Adjustment Algorithm A: AZA/PL* Dose Modifications for Increased Liver Enzymes: ALT or AST 2 to 3 x the ULN



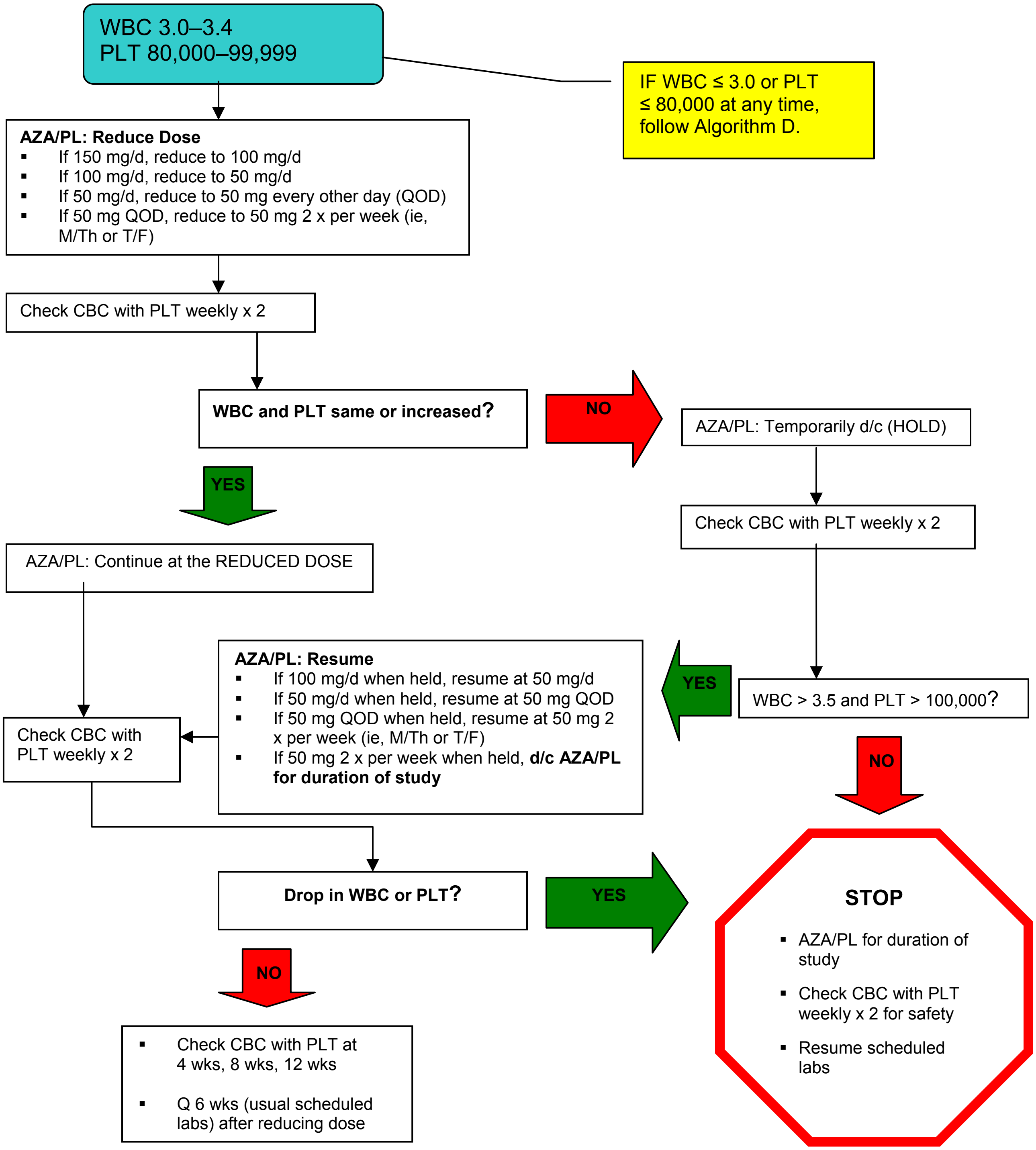
*Note: NAC/PL and PRED/PL dosing are continued without change.

Dosage Adjustment Algorithm B: AZA/PL* Dose Modifications for Increased Liver Enzymes: ALT or AST > 3 x the ULN



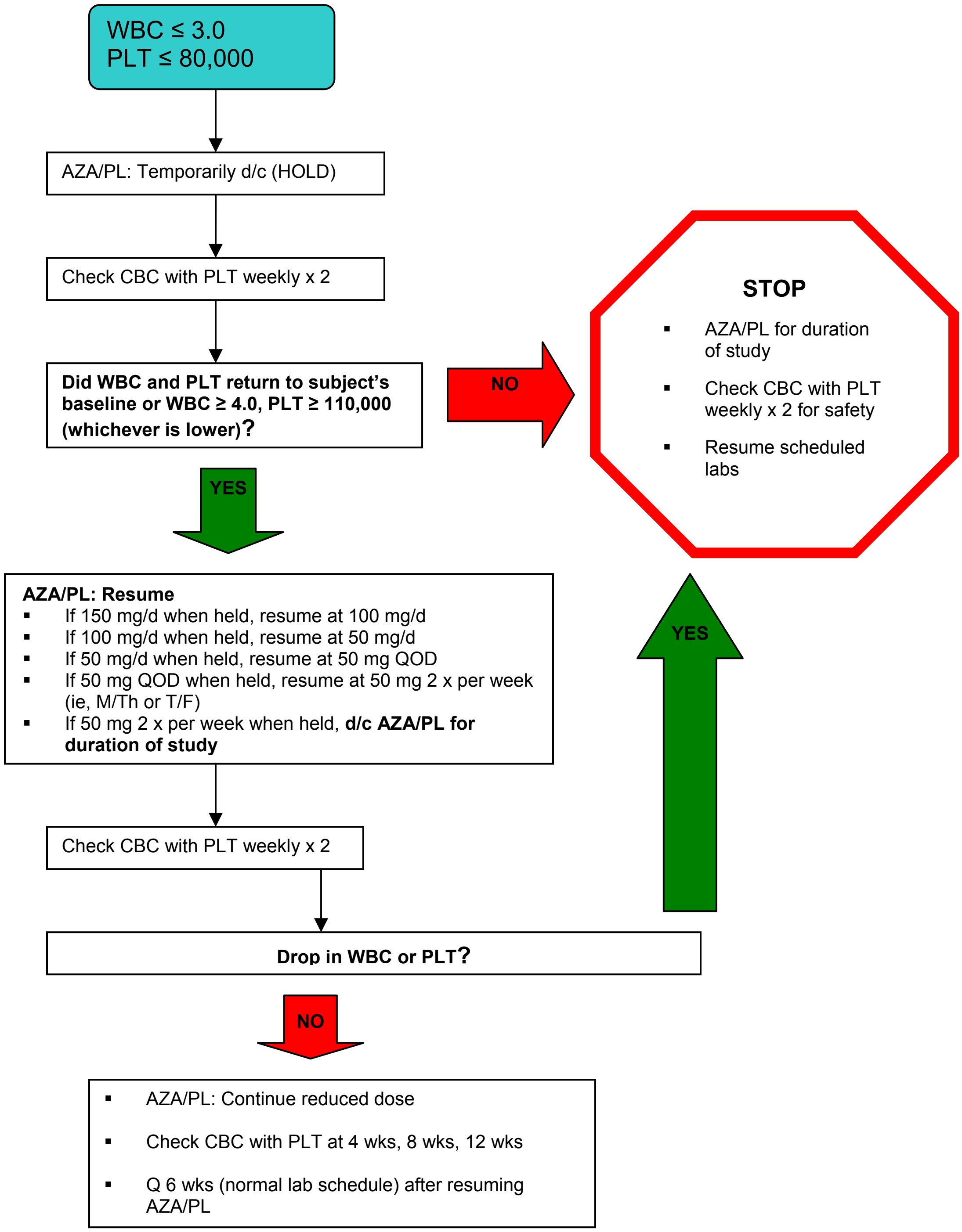
*Note: NAC/PL and PRED/PL dosing are continued without change.

Dosage Adjustment Algorithm C: AZA/PL* Dose Modifications for Decreased Blood Counts: White Blood Cell Count (WBC) 3.0–3.4 or PLTCount 80,000–99,999



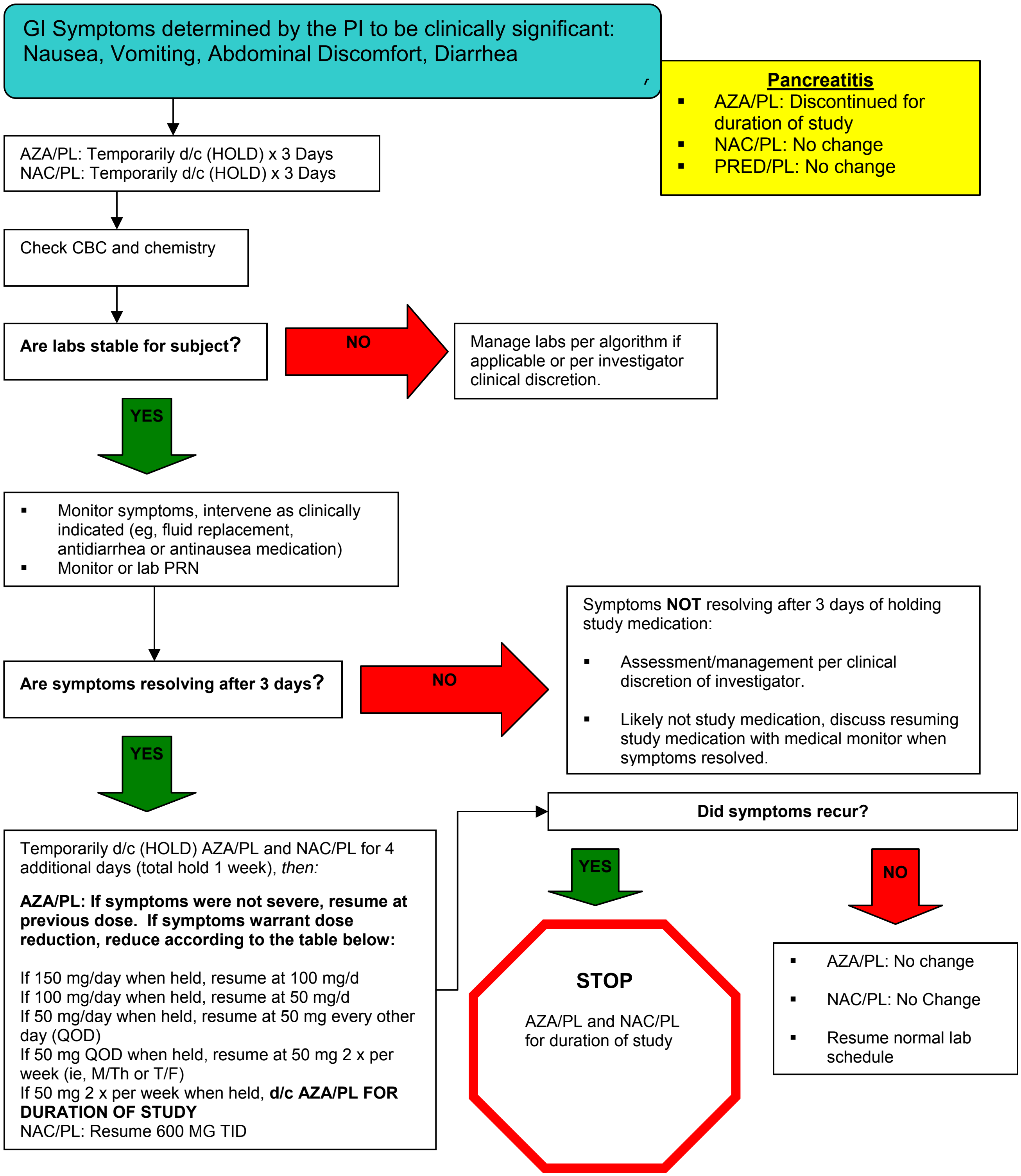
*Note: NAC/PL and PRED/PL dosing are continued without change.

Dosage Adjustment Algorithm D: AZA/PL* Dose Modifications for Decreased Blood Counts: WBC < 3.0 or PLT Count < 80,000



*Note: NAC/PL and PRED/PL dosing are continued without change.

Dosage Adjustment Algorithm E: AZA/PL and NAC/PL* Dose Modifications for Gastrointestinal Symptoms: Nausea, Vomiting, Abdominal Discomfort, Diarrhea *Not* Associated With Pancreatitis



*Note: PRED/PL dosing is continued without change

Dosage Adjustment Algorithm F: AZA/PL and NAC/PL * Dose Modifications For Dermatologic Reactions: Rash *(Not Acneiform)*, Desquamation, Generalized Itching, etc—Do Not Use This Algorithm for Hair Loss

Dermatologic Reactions (Not Acneiform) ≥ Grade 2

Grading per Common Terminology Criteria for Adverse Events V3.0
(http://ctep.cancer.gov)

For example:

- Grade 1 RASH/DESQUAMATION: Macular or papular eruption or erythema without associated symptoms.
- Grade 2 RASH/DESQUAMATION: Macular or popular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area.

AZA/PL: Temporarily d/c (HOLD) x 3 days
NAC/PL: Temporarily d/c (HOLD) x 3 days

Are symptoms resolving after 3 days?

NO →

Symptoms **not** resolving after 3 days of holding study medication:

- Assessment/management per clinical discretion of investigator.
- Likely not study medication.
- Discuss resuming study medication with medical monitor when symptoms resolved.

YES ↓

Temporarily d/c (HOLD) AZA/PL and NAC/PL for 4 additional days (total hold 1 week), *then*:

AZA/PL: Resume

- If 150 mg/day when held, resume at 100 mg/d
- If 100 mg/day when held, resume at 50 mg/d
- If 50 mg/day when held, resume at 50 mg QOD
- If 50 mg QOD when held, resume at 50 mg 2 x per week (ie, M/Th or T/F)
- If 50 mg 2 x per week when held, **d/c AZA/PL for duration of study**
- NAC/PL: resume 600 MG TID

→ **Did symptoms recur?**

YES ↓

STOP

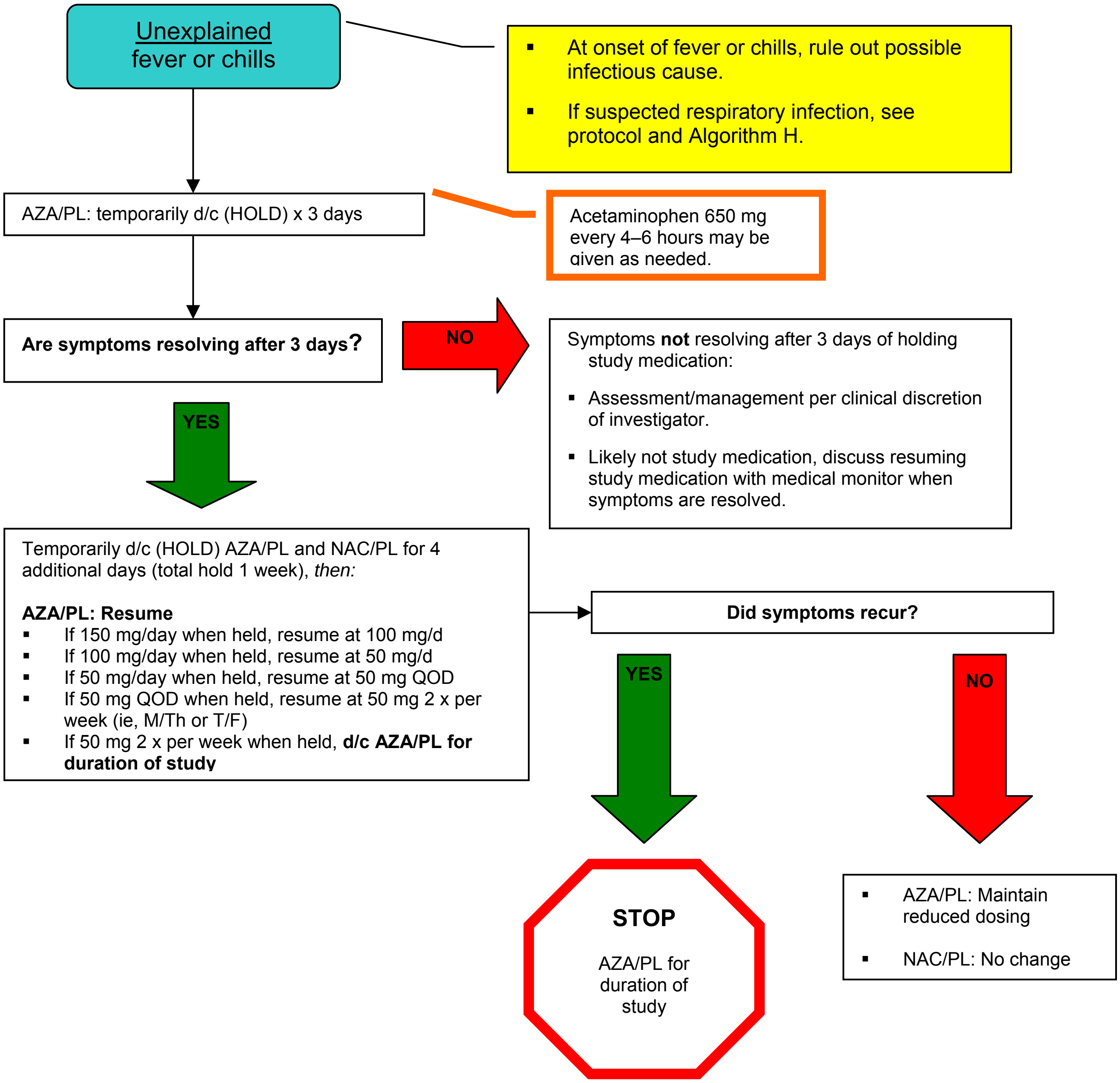
AZA/PL and NAC/PL for duration of study

NO ↓

- AZA/PL: Continue reduced dosing
- NAC/PL: No change

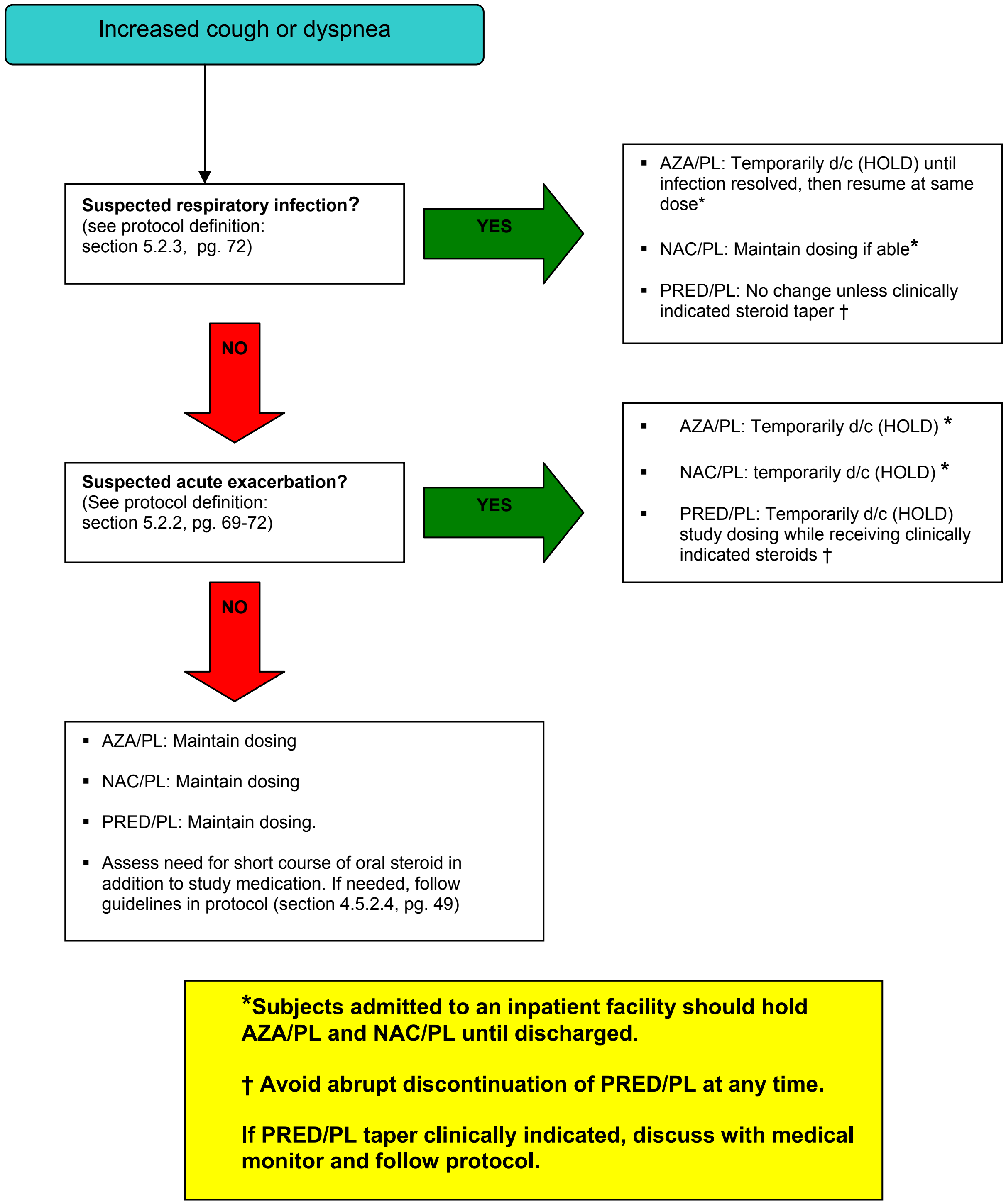
*Note: PRED/PL Dosing Is Continued Without Change.

Dosage Adjustment Algorithm G: AZA/PL* Dose Modifications for Fever or Chills Not Associated with Suspicion of an Infectious Cause in a Source Such as Tissue or Organ. If Suspected Respiratory Infection, See Protocol and Algorithm H.



*Note: NAC/PL and PRED/PL dosing are continued without change.

Dosage Adjustment Algorithm H: Cough or Dyspnea Worse Than Subject Baseline



4.6. Contraindications, Precautions, and Side Effects of Study Medications

4.6.1. Azathioprine

4.6.1.1. Contraindications

Contraindications to AZA are:

- known hypersensitivity to AZA
- breastfeeding
- pregnancy

4.6.1.2. Precautions

A gastrointestinal hypersensitivity reaction characterized by severe nausea and vomiting has been reported. These symptoms may also be accompanied by diarrhea, rash, fever, malaise, myalgias, elevations in liver enzymes, and occasionally hypotension. Symptoms of gastrointestinal toxicity most often develop within the first several weeks of therapy with AZA and are reversible upon discontinuation of the drug. This reaction can occur within hours after rechallenge with a single dose of AZA. Subjects receiving AZA with allopurinol concomitantly will receive a reduced dosage of AZA/PL per protocol. Caution will be exercised when used concomitantly with aminosalicylates, angiotensin-converting enzyme inhibitors, warfarin, and other agents affecting myelopoesis.

4.6.1.3. Side Effects

Side effects of AZA range from common to less common and serious. See Table 7.

Table 7: Side Effects of Azathioprine

Side Effect	Common— ≥ 1% and <15%	**Common— < 1%**	**Less Common— Serious**
Fever and chills	**X**		

Side Effect	Common— ≥ 1% and <15%	**Common— < 1%**	**Less Common— Serious**
Nausea	**X**		
Vomiting	**X**		
Skin rash, hives	**X**		
Stomach pain	**X**		
Arthralgias, myalgias		**X**	
Steatorrhea		**X**	
Malaise		**X**	
Negative nitrogen balance		**X**	
Alopecia		**X**	
Diarrhea		**X**	
Pancreatitis			**X**
Leukopenia			**X**
Megaloblastic anemia (HCT < 25)			**X**
Thrombocytopenia (platelet count < 80,000)			**X**
Hepatoxicity (LFT > 3 x ULN)			**X**
Increased risk of infection			**X**
Lymphoma			**Rare**

Abbreviations: HCT, hematocrit; LFT, liver function test; ULN, upper limit of normal

4.6.2. Prednisone

4.6.2.1. Contraindications

- Systemic fungal infections

- Known hypersensitivity to components

4.6.2.2. Precautions

Caution will be exercised in enrolling subjects with the pre-existing conditions listed below. These conditions do not specifically exclude subjects from participation; inclusion of subjects with the following conditions will be at the discretion of the investigator.

- Diabetes, insulin dependant
- Glaucoma, severe
- Hyperlipidemia, untreated
- Osteoporosis, untreated
- Morbid obesity
- Psychosis

4.6.2.3. Side Effects

Side effects of PRED range from mild to serious and occur more frequently with higher doses and prolonged treatment. See Table 8.

Table 8: Side Effects of Prednisone

Side Effect	Common ≥ 30%	Less Common 10–29%	Less Common and Serious—Rare
“buffalo hump”	X		
“moon face”	X		
Elevated cholesterol	X		
Fluid retention	X		
Growth of facial hair	X		
Headache	X		
Hyperglycemia (diabetes)	X		

Side Effect	Common ≥ 30%	Less Common 10–29%	Less Common and Serious—Rare
Impaired wound healing	X		
Insomnia	X		
Muscle weakness	X		
Obesity, weight gain	X		
Polydipsia*	X		
Polyphagia*	X		
Skin rash	X		
Emotional disturbances, irritability, nervousness*		X	
Hypertension		X	
Hypokalemia		X	
Stomach ulcers		X	
Thinning and easy bruising of the skin		X	
Cataracts			X
Osteoporosis—long term use			X
Worsening of diabetes*			X
Aseptic necrosis of the hip*			X
Glaucoma*			X
Psychotic behavior*			X
Seizures, involuntary muscle contractions			X

*These side effects can occur acutely within days to weeks of treatment with PRED. Other side effects listed occur with chronic dosing.

4.6.3. N-acetylcysteine

4.6.3.1. Contraindication

Contraindication to NAC is known hypersensitivity to it.

4.6.3.2. Precautions

Concomitant administration of oral NAC and antibiotics has shown a slightly reduced absorption of cephalexin and a slight increase in erythromycin serum levels. NAC contains free sulfhydryl groups. There is no evidence that individuals sensitive to sulfa drugs are sensitive to NAC.

The NAC preparation being administered in this study contains 20 mg of aspartame. Because of the phenylalanine component of aspartame, individuals with phenylketonuria should avoid or restrict aspartame intake to avoid increased blood levels of phenylalanine. Because of this risk, labeling is required on all products containing aspartame.

4.6.3.3. Side Effects

Side effects of NAC range from common to serious. See Table 9.

Table 9: Side Effects of NAC

Side Effect	Common < 1%	Rare	Rare—Serious
Stomach upset	X		
Heartburn	X		
Rash		X	
Somnolence		X	
Headache		X	
Migraine		X	
Tinnitus		X	
Bronchospasm			X

4.7. Recruitment Procedures

Subjects recruited for this study will be established patients of the investigators or physician- or self-referred to participating clinical centers in the IPFnet. Each clinical center within IPFnet has a well-developed infrastructure of local pulmonologists within the surrounding geographic area. These pulmonologists are kept informed of ongoing IPF clinical trials and regularly refer subjects to studies conducted at IPFnet clinical centers.

Additional steps will be taken to inform clinicians of the trials in progress within IPFnet, including: presentations at faculty staff meetings at local hospitals, medical grand rounds, and national conferences; direct mail notification; monthly faxes; and advertisement of network trials in pulmonary journals. Clinical center patients previously diagnosed with IPF will be notified of the trials by mail whenever possible.

Recruitment of minorities and women will be monitored by the DCC and DSMB. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate subject sample contains appropriate representation of women and minorities.

4.8. Study Procedures

The following procedures are detailed in the PANTHER-IPF MOOP accompanying this protocol:

1. PFT
2. ABG
3. HRCT scan of the chest (including imaging of pulmonary arteries)
4. CBC and serum chemistries
5. Pregnancy test
6. 6MWT/Borg Dyspnea Scale
7. TPMT
8. QOL questionnaires (EuroQol, HAD, SF-36, SGRQ, and ICE CAP)
9. UCSD SOBQ

10. Gender Substudy Questionnaire

All assessments of PFTs will be conducted by study personnel not directly involved in the treatment of the subjects.

Monitoring of Laboratory Values

Subjects will be required to visit a local blood-draw site affiliated with the central lab or the clinical center to provide samples for blood counts and chemistry.

The schedule and location for these blood draws will be at the following weeks:

Screening	(clinical center)		
Baseline	(clinical center)	25	(local blood draw center)
1	(local blood draw center)	30	(clinical center)
2	(local blood draw center)	35	(local blood draw center)
4	(clinical center)	40	(local blood draw center)
6	(local blood draw center)	45	(clinical center)
10	(local blood draw center)	50	(local blood draw center)
15	(clinical center)	55	(local blood draw center)
20	(local blood draw center)	60	(clinical center)

Additional blood draws for safety and dosage adjustment may be required.

4.8.1. Biological Specimen Management

4.8.1.1. Biological Specimen Sample Management

Subjects at clinical centers participating in the specimen repository substudy who consent to having blood drawn for research purposes and for the banking of blood, blood components, and other biologic specimens (urine and BAL fluid) will have approximately 40.5 mL of blood drawn, 17 mL blood drawn for DNA, and 20 mL of urine collected at enrollment visit. Subjects will have approximately 50 mL of blood drawn and 20 mL of urine collected at each 15-week follow-up visit. During suspected AEx, subjects will have approximately 35 mL of blood drawn for research purposes, and other clinically obtained biologic specimens (BAL) that would otherwise be discarded will be collected whenever possible. The BAL would be collectd from the subject if subject was seen at the participating clinical center. Blood specimens will be separated according to PANTHER-IPF MOOP guidelines into the following components for banking in the repository: serum, plasma, and DNA. Coding of all biologic specimens for the repository will be performed by study staff at the clinical center. The samples will be processed per PANTHER-IPF MOOP guidelines, aliquoted, labeled with barcode labels, and stored at -70°C at the clinical center. At regular intervals, samples will be batched and shipped to the central repository.

Samples shipped to the repository will be labeled with barcode labels; no demographic information or subject identifiers will be included on the label. The only identifier will be a sample ID. This sample ID will be linked in the IPFnet DCC clinical database to subject information. No subject information will be transferred to the biological-specimen database.

The subject's samples may be utilized for approved substudies relating to human disease, including, but not limited to, IPF. The studies for which an individual's samples will be made available will be determined by the subject's answers to questions on the biological-sample informed consent form. The subjects can choose to make their samples available for all options or any combination. Samples will be made available to researchers only with IPFnet Steering Group approval until such time as the samples are made public through the NHLBI repository.

4.8.1.2. Acute Exacerbation Sample Management

In the event of an AEx episode, subjects at clinical centers participating in the biospecimen repository substudy and who consent will be given an AEx kit to carry with them to the hospital or doctor's office. The kit will include blood-collection tubes for the subject's blood samples. In addition to collecting blood samples, biologic specimens (BAL) will be harvested from clinically performed procedures (specimens that would otherwise be discarded) at the IPFnet clinical centers.

4.9. Concomitant Medications

Concurrent treatment with FDA-approved therapy for IPF is allowed. Colchicine may be used for treatment of gout. Subjects receiving allopurinol will have reduced dosing of AZA/PL as delineated in section 4.5.1.2. Temporary treatment with oral or IV corticosteroids as described in section 4.5.2.3.1 for clinical worsening or suspected AEx is permitted. Nutritional supplements containing NAC are not allowed.

4.10. Laboratory Testing

Clinical laboratory parameters will be assessed throughout the study. The following tests will be performed at the time points specified in the protocol: chemistry (A/G ratio, ALT [SGPT], AST [SGOT], albumin, alkaline phosphatase, amylase, bilirubin-direct, bilirubin-indirect, bilirubin-total, BUN, BUN/creatinine ratio, calcium, carbon dioxide, cholesterol-total, chloride, CPK-total, creatinine, GGT, globulin, glucose, iron-total, LDH, lipase, magnesium, phosphorus-inorganic, potassium, protein-total, sodium, TIBC, triglycerides, uric acid) and hematology (red blood cell count, WBC count, hemoglobin, hematocrit, cell indices, differential, platelet count).

Rationale for Central Labs

Monitoring of subject blood chemistries and blood cell counts is critical in this study, as one of the agents under investigation, AZA, can generate serious bone marrow depression and liver toxicity. Particularly in the first 3 months of treatment, regular laboratory parameters must be monitored. In order to minimize the travel burden placed on subjects and to standardize laboratory testing, it was decided to utilize a central laboratory that has a large number of blood-draw locations.

4.11. Blinding of Study Drugs

Subjects and caregivers will be blinded to study treatment. Every subject will receive AZA, PRED, and NAC or matching PLs at every study visit from the baseline visit to the week 60 visit (except the week 4 safety visit). At week 60 or the final treatment visit, subjects will begin dosage adjustments as described in section 4.4.2.7.

5. Study Endpoints

5.1. Primary Study Endpoint

The primary endpoint will be the change in serial measurements of FVC over the 60-week study.

5.2. Secondary Study Endpoints

5.2.1. Time to Disease-progression

The time to death or a 10% decline in FVC will be defined as the time to disease-progression. The 10% decline in FVC from enrollment must be confirmed on 2 consecutive visits no less than 6 weeks apart. For subjects with 2 consecutive visits with a 10% decline in FVC, the time to disease-progression will be defined as the time interval between enrollment and the initial visit with a 10% FVC decline. The study doctor will discuss remaining in the study with subjects experiencing documented disease progression.

5.2.2. Acute Exacerbations

The following 3 criteria will define AEx in subjects with acute worsening of their respiratory conditions:

1. Clinical: (all of the following required)
    - A) Unexplained worsening of dyspnea or cough within 30 days, triggering unscheduled medical care (eg, emergency room, clinic, study visit, hospitalization)
    - B) No clinical suspicion or overt evidence of cardiac event, pulmonary embolism, or deep venous thrombosis to explain acute worsening of dyspnea
    - C) No pneumothorax

2. Radiologic/Physiologic: (A and B required)

A) New ground glass opacity or consolidation computed tomography (CT) scan OR new alveolar opacities on chest x-ray
B) Decline of ≥ 5% in resting room air SpO_2 from last recorded level OR decline of ≥ 8 mm Hg in resting room air PaO_2 from last recorded level

3. Microbiologic: (all of the following required)
   A) No clinical evidence for infection (ie, absence of grossly purulent sputum, fever > 39 °C orally)
   B) Lack of positive microbiological results (if done) from lower respiratory tract defined as: (1) clinically significant bacterial growth on sputum or endotracheal aspirate cultures; (2) quantitative culture by protected brush specimen ≥ 10^3 cfu/mL or BAL ≥ 10^4 cfu/mL; (3) the presence of specific pathogens on stains of any of the above
   C) Lack of positive pathogen in blood cultures (if done)

Identification of Acute Exacerbations

All subjects will be educated regarding the importance of identifying AExs. At the time of enrollment, subjects will be educated to the possibility of developing acute symptomatic worsening that might represent an AEx of IPF and instructed to contact their study clincal center coordinator within 48 to 72 hours of the apparent event.

All subjects will be contacted by phone monthly and questioned about any change in dyspnea or cough and any interim clinic visits or hospitalizations. Finally, as part of the IPFnet outreach to community referring physicians, the importance of AExs will be emphasized. When a subject is identified who meets criterion 1A, this will trigger the collection of additional clinical data to evaluate a suspected AEx. These data will be collected as part of standard clinical care (ie, this protocol does not require collection of all items). The additional items to be collected for suspected AEx include:

- IPFnet AEx case report form (CRF) (required)

- Chest x-ray, CT scan with/without pulmonary angiogram (reports should be faxed and followed by the hard copies/discs)
- Oxygen saturation (pulse oximetry)
- Arterial blood gas
- Respiratory cultures (sputum, endotracheal aspirate, lavage)
- Blood cultures
- Clinic/hospital records related to the event

All potential cases of AEx will be reviewed by the clinical center PI first, and a decision on whether the case may represent an AEx will be made. If AEx is suspected, the case will be sent to the AEx adjudication committee, which will assign a final diagnosis (see Table 10). If there is disagreement among members, the majority opinion will be recorded.

During episodes of suspected AEx, as determined by the individual clinical center investigator, treatment with study drugs will be as specified in sections 4.5.1.4 (AZA/PL), 4.5.2.3 (PRED/PL), 4.5.3.2 (NAC/PL), and 4.5.4 Algorithm H. Subjects will remain blinded and in the study.

Table 10: Final Diagnoses in Evaluation of Suspected Acute Exacerbations

Definite acute exacerbation	All criteria met; no alternative etiology
Unclassifiable acute worsening	Insufficient data to evaluate all criteria; no alternative etiology
Not acute exacerbation	Alternative etiology identified that explains acute worsening

Management of the suspected AEx will be at the discretion of the treating physician. Standard of care generally involves evaluation for respiratory infection, pulmonary embolism, cardiac events and pneumothorax, and treatment with IV corticosteroids. Because the standard of care for management of suspected AExs includes steroids, the following is recommended: IV solumedrol—1.0 g/day for 3 days, 0.5 g/day for 3 days, and taper dosage to reach 0.5 mg/kg/day of oral PRED by the end of 2 weeks as clinically tolerated. Then follow taper guidelines in Table

3, section 4.4.2.7. When the subject is tapered off active PRED, resume PRED/PL dosing in accordance with the study schedule.

Study drugs will be resumed at presuspected AEx doses after subjects clinically improve as confirmed by the local PI. All subjects should be seen at the clinical center within 2 to 4 weeks of recovery for measurement of post-bronchodilator FVC (see Figure 5). Subjects unable to return to the clinical center after suspected AEx due to medical frailty (eg, continued institutionalization, progressive disability) will be categorized as failing to maintain FVC response in secondary analyses.

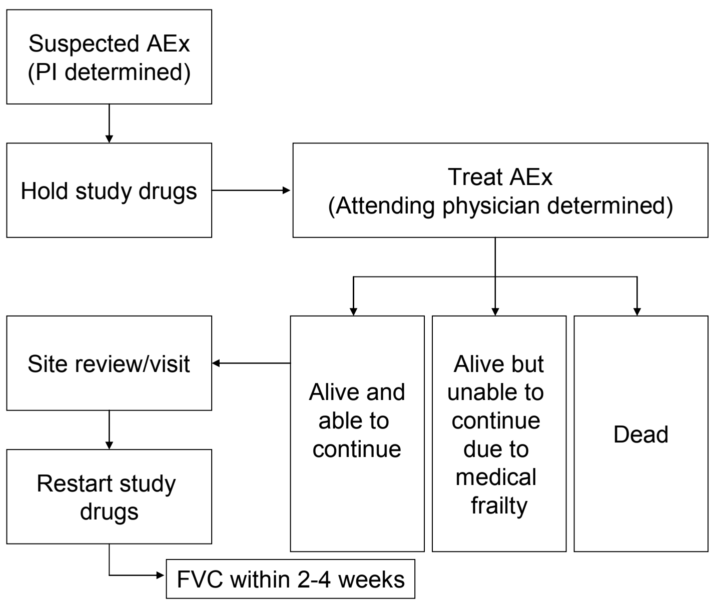


Figure 5. Acute Exacerbation Flow Chart

5.2.3. Respiratory Infections

An upper respiratory infection will be defined as:

- Change in sputum discoloration
- Increased cough of no more than 14 days' duration

A lower-respiratory infection (pneumonia) will be defined as new segmental or lobar airspace opacities visualized by image studies (chest radiograph or HRCT) in addition to any of the following:

- Positive pathogen/cultures in good sample of sputum or lower-airway secretions retrieved by fiberoptic bronchoscope
- Fever > 39°C or > 100°F
- Leukocytosis > 12,000 (unexplained; no increase in dose of corticosteroids)

5.2.4. Maintained FVC Response

Subjects with follow-up FVC%pred measurements at or above their baseline FVC%pred level will be classified as having maintained FVC response. Subjects with reduced FVC%pred levels or missing data for any reason, including death or medical frailty, will be classified as having not maintained FVC response. The FVC%pred value is used because unadjusted FVC measurements are expected to decline with age.

6. Safety Evaluations and Procedures

6.1. Adverse Events

During a clinical trial, the reporting of adverse experience information can lead to important changes in the way a new treatment is developed, as well as provide integral safety data.

6.1.1. Definitions

An **adverse event (AE)** is any untoward medical occurrence in a subject or clinical investigation subject who was administered a pharmaceutical product. The AE does not necessarily have to have a causal relationship with the drug administered. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered to be related to the medicinal product. Diseases, signs, symptoms, or laboratory abnormalities already existing at enrollment are not considered AEs unless they worsen (ie, increase in intensity or frequency). Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Surgical procedures planned before randomization and the conditions necessitating the surgery are not AEs.

A **serious adverse event** is any untoward event that:

- Is fatal
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization, with the following exceptions:
    - Preplanned (before the study) hospital admissions, unless the hospitalization is prolonged
    - Planned admissions (as part of a study, eg, routine biopsies)
    - Hospitalization lasting < 24 hours

  - Hospitalization for elective procedure
  - Emergency room visits
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Important medical events that may not result in death, be life-threatening, or require inpatient hospitalization may be considered serious adverse events (SAEs) when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening means that the subject was, in the view of the investigator, at immediate risk of death from the AE as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.

Persistent or significant disability/incapacity means that the event resulted in permanent or significant and substantial disruption of the subject's ability to carry out normal life functions.

Causality:
A reasonable possibility means the AE may have been caused by/related to the study drug. A perceived or real lack of efficacy does not satisfy the definition of relatedness.

6.1.2. Adverse Event (AE) Reporting

For the PANTHER-IPF trial, all AEs (serious and nonserious), occurring from randomization through final study visit (4 weeks after final dose of all study medication) will be recorded on the AE page of the case report form (CRF)

6.1.2.1. Serious Adverse Events (SAE) Reporting

For this trial, all deaths and all SAEs, which occur from randomization through final study visit, must be entered within the EDC system, via the SAE eCRF page within 24 hours of the investigative site's knowledge of the event. It is the responsibility of the clinical center investigator to provide a causality assessment of the event for each study medications based upon the information available at the time of the report. It is understood that complete information about the event may not be known at the time the initial report is submitted. In the event the EDC system is not accessible to the site at the time of event reporting, investigative sites will complete and forward a paper back-up SAE form to DCRI Safety Surveillance for processing:

DCRI Safety Surveillance
Telephone: 1-919-668-8624 or 1-866-668-7799 (toll free)
Fax: 1-919-668-7138 or 1-866-668-7138 (toll free)

The investigator must complete and submit a follow-up SAE information via the eCRF when important new/ follow-up information (final diagnosis, outcome, results of specific investigations, etc) becomes available. Follow-up information should be submitted according to the same process used for reporting the initial event as described above. All SAEs will be followed until resolution, stabilization, or 30 days after the subject has completed the final visit (4 weeks after the final dose of study medication), whichever occurs first. The investigator is responsible for reporting SAEs to their institutional review board (IRB) per site specific IRB reporting guidelines.

6.1.2.2. Regulatory Reporting

AEs that are serious, study drug-related, and unexpected will be reported to the regulatory authorities. The DCRI Safety Surveillance medical monitor will perform a medical review of all SAEs submitted and evaluate for "unexpectedness." DCRI Safety Surveillance will prepare MedWatch reports for those events identified as serious, study drug related and unexpected as determined by Safety Medical Monitor.

DCRI Regulatory Services will submit all unexpected, study drug-related SAEs as per 21 CFR 32. DCRI Safety Surveillance will provide a safety alert letter to the NHLBI, DSMB, and DCC clinical operation (for distribution to sites) within 15 days of initial receipt of the information.

Investigators are responsible for promptly reporting these events to their reviewing IRBs according to site specific IRB reporting guidelines.

6.2. Clinical Medical Monitoring

There will be an unblinded physician at the IPFnet DCC serving as medical monitor. The medical monitor will be available to assist with questions about dosage adjustments of study medications, including discontinuation or resumption of medications.

6.3. Unblinding Procedures

Unblinding of subjects or investigators to subject treatment is strongly discouraged. For ongoing clinical management, all subjects should be presumed to be receiving “active” study drug (PRED, AZA, or NAC). To ensure the subject’s safety, the study treatment will be dose-adjusted based on laboratory test results, clinical findings, and symptoms.

The IPFnet DCC medical monitor and PANTHER-IPF co-chairs, Drs. Ganesh Raghu and Fernando Martinez, will be available to the study physicians to discuss study drug management on a case-by-case basis. Unblinding will be considered ONLY when the knowledge of subject treatment assignment is ABSOLUTELY ESSENTIAL for subject safety and after discussion of the subject’s case with the medical monitor and either Dr. Rahgu or Dr. Martinez. Unblinding of study treatment should be minimized during the conduct of the trial. In those cases where it is felt to be medically necessary the DCC medical officer will communicate directly with the managing physician to minimize unblinding of study personnel.

7. Study Drug Procedures

At the baseline, 15-week, 30-week, and 45-week study visits, subjects will receive a supply of study drug sufficient to last until the next visit at which study drug will be dispensed. At the week 60 visit, or final study treatment visit, subjects will receive a supply of PRED/PL for tapering.

8. Data Management

8.1. Hardware and Software Configuration

8.1.1. Hardware and Database Software

Data will be stored in an Oracle database system. Oracle has advantages of processing efficiency and smooth linkage with other software systems. The application and database will be hosted on Solaris Unix servers at the IPFnet DCC.

8.1.2. Statistical Software

SAS will be used as the principal application for the management of analysis data files and statistical computations. S-Plus will be used to provide supplementary functions as needed.

8.1.3. Access Control and Confidentiality Procedures

Access to databases will be controlled centrally by the IPFnet DCC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage.

8.1.4. Security

Database and Web servers will be secured by a firewall and through controlled physical access. Oracle has many security features to ensure that any staff member accessing the database has the proper authority to perform the functions he or she requests of the system. Within the secondary SAS databases, Unix group-access control maintains similar security. The Sun workstation login is secured by extensive user-password facilities under Unix.

8.1.5. Back-up Procedures

Database back-up will be performed automatically every day, and standard IPFnet DCC policies and procedures will be applied to dictate tape rotation and retention practices.

8.1.6. Virus Protection

All disk drives that provide network services, and all user computers, will be protected using a virus-scanning software. Standard IPFnet DCC policies will be applied to update these protection systems periodically through the study.

8.2. Sources of Data

8.2.1. Design and Development

The IPFnet DCC will be responsible for development of the electronic case report forms (eCRFs), development and validation of the clinical study database, ensuring data integrity, and training clinical center staff on applicable data management procedures. A web-based distributed data entry model will be implemented. This system will be developed to ensure that guidelines and regulations surrounding the use of computerized systems used in clinical trials are upheld. The remainder of this section provides an overview of the data management plan associated with this protocol.

8.2.2. Data Collection Forms

The data collection process consists of direct data entry at the study clinical centers into the EDC system(s) provided by the DCC. A data collection worksheet will be provided to clinical centers for recording data in the event the EDC system is unavailable. Data entry of the eCRFs should be completed according to the instructions provided and project specific training. The investigator is responsible for maintaining accurate, complete and up-to-date records, and for ensuring the completion of the eCRFs for each research participant.

8.2.3. Data Acquisition and Entry

Data entry into eCRFs shall be performed by authorized individuals. Selected eCRFs may also require the investigator's written signature or electronic signature, as appropriate. Electronic CRFs will be monitored for completeness, accuracy, and attention to detail during the study.

8.2.4. Data Center Responsibilities

The IPFnet DCC will 1) develop a data management plan and will conduct data management activities, 2) provide final eCRFs for the collection of all data required by the study, 3) develop data dictionaries for each eCRF that will comprehensively define each data element, 4) conduct ongoing data monitoring activities on study data, 5) monitor any preliminary analysis data clean up activities, and 6) rigorously monitor final study data clean up.

8.2.5. Data Editing

Completed data will be entered into the IPFnet DCC automated data acquisition and management system. If incomplete or inaccurate data are found, a data clarification request will be generated and distributed to clinical centers for a response. Clinical centers will resolve data inconsistencies and errors and enter all corrections and changes into the IPFnet DCC automated data acquisition and management system.

8.2.6. Training

The training plan for clinical center staff includes provisions for training on assessments, eCRF completion guidelines, data management procedures, and the use of computerized systems.

9. Study Design and Data Analysis

9.1. Overview of the Study Design

This double-blind, PL-controlled, randomized trial will be the first study to evaluate the benefits and risks of NAC and AZA-PRED-NAC in an IPF population. We will apply a 2-step Fisher's least significant difference (LSD) procedure to control the experiment-wise error rate at 0.05. The first step of this testing procedure will be based on a 2-degree-of-freedom omnibus test. If the first test is statistically significant at the 0.05 level, then each of the 3 pairwise comparisons will be tested at the 0.05 level. The 3 pairwise comparisons are: NAC vs. PL, AZA-PRED-NAC vs. PL, and NAC vs. AZA-PRED-NAC.

9.2. General Analytic Considerations

All primary analyses will be based on intent-to-treat (ITT) principles using all randomized subjects. Baseline factors across groups will be compared using mean (standard deviation) and median (25th and 75th percentiles) summary measures. Kaplan-Meier curves will be used to display event rates. Due to clinical interest in departures from both sides of the null hypothesis, all test statistics will be 2-sided.

Reasonable caution needs to be taken when conducting multiple analyses on key clinical subgroups. For subgroup analyses, a conservative significance level of 0.001 will be used for all interaction tests. Thus, subgroup comparisons will be considered exploratory unless the p-value from the interaction test is smaller than 0.001.

9.3. Randomization, Blinding, and Reporting of Results

A permuted block-randomization scheme will be created with varying block sizes stratified by clinical center. Once a subject has completed the screening and baseline period and evaluation for inclusion/exclusion criteria, the randomization process will begin. Subjects will be randomized to receive one of the 3 treatment regimes with equal probability (1:1:1), via telephone contact with a central interactive voice response system (IVRS), using a toll-free randomization number. On the day of randomization, after the subject has successfully met all inclusion and exclusion criteria, the investigator or designee will call the central randomization number to obtain the assigned kit randomization numbers for that subject. At each subject visit, the investigator or designee will call the central randomization number to obtain the new kit randomization numbers for resupply of the subject. For resupply of the clinical center, the IVRS will monitor minimal volume of a kit type and/or expiration date and will automatically notify the pharmacy.

The trial results will be reported according to guidelines specified in the CONSORT statement. A flow diagram describing screening, recruitment, randomization, dropout, and vital status will be included in the primary manuscript. AEs and efficacy data will be presented for all 3 treatment groups. Adherence, dropout, and lost to follow-up will be carefully examined across all 3 treatment groups. Analyses of safety will be based on data from all randomized subjects who received at least 1 dose of study drug.

9.4. Stratification

Subjects will be distributed to the 3 treatment arms in a 1:1:1 allocation ratio. Stratification blocks will be based on clinical centers.

9.5. Specification of the Primary Analyses

A mixed model repeated measures (MMRM) analysis, described in section 9.6, will be used to compare differences in the slope of FVC measurements across the 3 treatment groups. Response variables are values of the FVC measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing FVC data will not be imputed for the primary analysis.

9.6. Analysis of Longitudinal Endpoints

A common goal in clinical trials is to specify models that are easily implemented and reproducible by independent data analysts. On the other hand, the models should have proper statistical behavior in terms of low bias and high precision. Many common approaches to longitudinal data analysis including last observation carried forward (LOCF) imputation rely on the missing completely at random (MCAR) assumption. However, the MCAR assumption is unlikely to hold in many clinical trials because missing data are often related to disease progression and prognosis. A more reasonable assumption, missing at random (MAR), specifies that the complete data distribution can be modeled using only the observed data. The likelihood-based MMRM approach is valid under the more general MAR assumptions. These models will be applied to analyze the longitudinal data secondary endpoints.

The advantages of MMRM analysis are that all important characteristics of the model can be prespecified, standard software can be used to implement the models, and results are based on ITT principles (Mallinckrodt 2004). In addition, the MMRM approach offers superior control of Type I and Type II errors compared with the LOCF approach.

Response variables are values of the PFTs measured at enrollment and every 15 weeks until study completion at 60 weeks and 6MWT values measured at baseline, week 30, and week 60. Covariates are treatment, time, time by treatment, and key baseline risk factors. Contrasts (along with confidence intervals) of treatment by time will be used to estimate the treatment effect.

The correlation structure involves multiple pieces, including measurement errors, random variation, and interindividual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. A careful examination of reasons for study discontinuation will be conducted to assess the validity of MCAR. Sensitivity analyses will be used to examine the untestable assumption that the observed data violate the MAR assumption. The MMRM models will be implemented using PROC MIXED in SAS.

9.7. Analysis of Binary, Time-to-Event, and Time-Lagged Endpoints

Regression modeling approaches using either the logistic regression model or Cox proportional hazards regression model will be employed when appropriate. The validity of these models will be assessed via standard modeling diagnostics and goodness-of-fit measures. Estimates of cumulative frequencies for more general time-lagged responses will be calculated using the partitioned version of the Bang-Tsiatis estimator (Bang 2000). The partitions will be set at 15-week intervals to correspond with the data-collection process. Covariate adjusted event rates will be calculated using inverse probability-weighted regression estimates (Lin 2003).

9.8. Power Analysis

9.8.1. Primary Analyses

Based on previously published IPF clinical trials, the PL group is expected to experience a drop in FVC of approximately 0.20 L over the 60-week study period (see Figure 1). The IPFnet Steering Group determined a clinically important difference would be to preserve the majority of the decline relative to PL over the 60-week study. In particular, a treatment effect of 0.15 L was determined to be a clinically meaningful difference. Potential dropout is a key factor in the proposed study. The drop-out process assumed 5% lost to follow-up after every study visit. Only 80% of subjects were assumed to be followed for the entire 60-week period. All models assumed a compound symmetry structure for the covariance matrix. Power calculations were performed using a SAS IML program for designing repeated measures studies (Rochon 1998). Based on preliminary reviews of the data from the University of Michigan, the covariance matrix parameters were estimated at approximately $\sigma^2 = 0.757$ (variance parameter) and $\rho = 0.936$ (correlation parameter). To be conservative, the power calculations for the primary analysis were performed with parameter setting of $\sigma^2 = 0.810$ (variance parameter) and $\rho = 0.925$ (correlation parameter).

We begin the power calculations by making a correction for imperfect compliance proposed by Lachin and Foulkes to allow for 2% noncompliance for each of the treatment arms (Lachin 1986). Thus, the sample size of 130 subjects per arm would be reduced to an adjusted sample size of $130*(1-0.02-0.02)^2 = 119.8$ or 120 subjects per arm. As shown in Table 11, the power for the 2-degree-of-freedom omnibus test is lowest when the middle treatment effect is halfway between the smallest and largest effect. Based on these calculations, the power for the first step of the Fisher's LSD procedure is between 87% and 95%.

Table 11: Power and Sample Size Estimates for the 2-Degree-of-Freedom Omnibus Test

Expected Drop (L) in FVC for PL	**Expected Drop (L) in FVC for NAC**	**Expected Drop (L) in FVC for PRED-AZA-NAC**	**Sample Size[1] per Group for 90% Power**	**Power with a Sample Size[1] of 120 per Group**
0.200	0.050	0.050	98	95.1%
0.200	0.100	0.050	126	88.6%
0.200	0.125	0.050	131	87.4%
0.200	0.150	0.050	126	88.6%
0.200	0.200	0.050	98	95.1%

Abbreviations: FVC, forced vital capacity; PL, placebo; NAC, N-acetylcysteine; PRED, prednisone; AZA, azathioprine

[1]Sample sizes shown are the adjusted sample sizes after accounting for possible noncompliance.

Assuming that the first step of the Fisher's LSD procedure is determined to be statistically significant, each of 3 pairwise comparisons will be conducted at the 0.05 level. Under the assumed Type I error rate of 0.05, with a correlation parameter of 0.925 and standard deviation of 0.90, the difference of 0.15 L (or 0.0025 L/week) shown in Table 12 would have power of 93%. Depending on the parameter settings, the power of the 2-step Fisher's LSD procedure to detect a particular pairwise difference would range from 81% to 88%. Therefore, the sample size of 390 subjects with a 1:1:1 randomization ratio will provide adequate power to detect clinically meaningful changes in FVC.

Table 12: Hypothetical Values of Mean FVC (L) Change from Baseline

	Week 15	Week 30	Week 45	Week 60
Active treatment	0.0125	0.0250	0.0375	0.0500
PL	0.0500	0.1000	0.1500	0.2000
Difference	0.0375	0.0750	0.1125	0.1500

Abbreviations: FVC, forced vital capacity; PL, placebo

9.8.2. Power Analysis for Maintained FVC Response

Differential dropout creates a number of problems for the analysis and interpretation of randomized clinical trials. In particular, excess dropout may be the result of toxicity or other treatment-related side effects. To account for the potential bias induced by differential dropout, we propose an analysis that treats any dropout or nonresponse as a failure to maintain FVC response. The statistical model for this power analysis assumes that 20% of subjects have incomplete data at the 60-week visit. Preliminary data suggest that approximately 10% of subjects will not survive the 60-week study period. We assumed that the change scores between baseline and 60 weeks are normally distributed with a standard deviation of 11%. The assumed mean changes in FVC%pred for the active and PL groups are -1% and -6%, respectively. Based on these assumptions, approximately 37.1% of subjects randomized to active therapy were assumed to respond (60-week FVC%pred ≥ baseline FVC%pred) compared with 23.4% for PL subjects. With a Type I error rate of 0.05 and a sample size of 130 subjects per group, the power to detect a difference would be 62%. As a sensitivity analysis, if we assume that an additional 10% of subjects drop out of the active arm due to toxicity, the power is reduced to 31%. These calculations suggest that the power to detect a statistically significant difference favoring a treatment with excess drop-out is relatively low.

9.8.3. Power Analysis for Secondary Endpoints

Power calculations for secondary endpoint measurements are shown in Table 13. Standard deviations are based on unpublished data provided by the University of Michigan. The calculations are based on a 2-sample t-test with Type I error rate set at 0.05. These calculations are likely to be conservative because the statistical approach, described in section 9.6, for analyzing these endpoints will incorporate incomplete observations as well as intermediate data points.

Table 13: Detectable Differences in Treatment Means for Selected Endpoint Measurements

Secondary Endpoints	Std Dev of the Baseline Score	Detectable Difference for 80% Power	Std Dev of the Change Score	Difference Detectable for 80% Power
DLCO%pred	16.6	5.8	9.1	3.2
6MWT Area Under the Desaturation Curve	21.9	7.6	17.5	6.1
6MWT Distance to Desaturation	22.4	7.8	31.5	11.0
6MWT Minutes Walked	2.10	0.73	2.05	0.71

Abbreviations: Std Dev, standard deviation; DLCO%pred, diffusing capacity of the lung for carbon monoxide percent predicted; 6MWT, 6-minute walk test

10. Study Administration

10.1. Cooperative Agreement Mechanism

The administrative and funding mechanism used to undertake this project is a "cooperative agreement" (U01), which is an assistance mechanism. Under the cooperative agreement, the NHLBI assists, supports, and/or stimulates the project and is substantially involved with investigators in conducting the study by facilitating performance of the effort in a "partner" role. The NHLBI project scientist serves on the IPFnet Steering Group, and he or another NHLBI scientist may serve on other project committees when appropriate. At the same time, however, NHLBI does not assume a dominant role, direction, or prime responsibility for this research program.

As described below, governance of the project is conducted through the IPFnet Steering Group. Principal investigators have lead responsibilities in all aspects of their trials and the project, including any modification of trial designs, conduct of the trials, quality control, data analysis and interpretation, preparation of publications, and collaboration with other investigators, unless otherwise provided for by the IPFnet Steering Group.

PIs retain custody of and have primary rights to their center-specific and collaborative data, subject to government rights-of-access consistent with current Health & Human Services (HHS), Public Health Service (PHS), and National Institutes of Health policies. The protocols and governance policies call for the continual submission of data centrally to the IPFnet DCC for the collaborative database, which at a minimum will contain the key variables selected by the IPFnet Steering Group for standardization across all clinical centers; the submission of copies of the collaborative datasets to each PI upon completion of the project; procedures for data analysis, reporting and publication; and procedures to protect and ensure the privacy of medical and genetic data and records of individuals. The NHLBI project scientist, on behalf of the NHLBI, will have the same access, privileges, and responsibilities regarding the collaborative data as the other members of the Steering Group.

PIs are also encouraged to publish and to publicly release and disseminate results, data, and other products of the project, concordant with the project protocols and governance and the approved plan for making data and materials available to the scientific community and to the NHLBI. However, during the 3 years after the ending date of NHLBI project support, unpublished data, unpublished results, data sets not previously released, and other study materials or products are to be made available to any third party only with the approval of the IPFnet Steering Group.

Upon completion of the project, PIs are expected to put their intervention materials and procedure manuals into the public domain and/or make them available to other investigators according to the approved plan for making data and materials available to the scientific community and the NHLBI for the conduct of research, at no charge other than the costs of reproduction and distribution.

The NHLBI reserves the right to terminate or curtail the project (or an individual award) in the event of (a) failure to develop or implement mutually agreeable collaborative measurement, subject eligibility, and data management sections of the protocols; (b) substantial shortfall in subject recruitment, follow-up, data reporting, or quality control or other major breach of protocol; (c) substantive changes in the agreed-upon protocols with which NHLBI cannot concur; (d) reaching a major project outcome, with persuasive statistical significance, substantially before schedule; or (e) human subject ethical issues that may dictate a premature termination.

Any disagreement that may arise in scientific/programmatic matters (within the scope of the award) between award recipients and the NHLBI may be brought to arbitration. An arbitration panel will be composed of 3 members—1 selected by the IPFnet Steering Group (with the NHLBI member not voting) or by the individual PI in the event of an individual disagreement, a second selected by NHLBI, and the third selected by the other 2 members. This special arbitration procedure in no way affects the PI’s right to appeal an adverse action that is otherwise appealable in accordance with the PHS regulations at 42 CFR part 50, Subpart D and HHS regulation at 45 CFR part 16 or the rights of the NHLBI under applicable statutes, regulations, and terms of the award.

10.2. IPFnet Steering Group

The IPFnet Steering Group is the main governing body of the project. It is composed of the PIs of the clinical centers, the PI of the DCC, and the NHLBI project scientist. The clinical centers, the IPFnet DCC, and the NHLBI each have 1 vote on the IPFnet Steering Group. All decisions are determined by majority vote.

All major scientific decisions are determined by the IPFnet Steering Group. It assumes overall responsibility for the design and conduct of the trial. It appoints (and disbands) committees and subcommittees as the need arises; designs, approves, and implements the study protocols; oversees the development of the MOOP; monitors subject recruitment and treatment delivery; evaluates data collection and management; oversees quality assurance procedures; and implements changes and enhancements to the study as required. It also has primary responsibility for facilitating the conduct of the trials and reporting the project's results.

10.3. Data and Safety Monitoring Board

The NHLBI will establish a DSMB in accordance with established policies (see http://www.nhlbi.nih.gov/funding/policies/dsmb_inst.htm) to ensure data quality and subject safety and to provide independent advice to the NHLBI regarding progress and the appropriateness of study continuation.

10.4. Recommendations on Interim Monitoring of Efficacy, Safety, and Futility

First and foremost the role of the DSMB will be to review subject safety and trial conduct at periodic points during the study. The DSMB may require analyses of the primary endpoint results for comparing the benefit and risks of treatment strategies. The benefit of collecting additional data on key secondary endpoints, with extended follow-up, and establishing a robust evidence base for determining a standard of care will need to be carefully considered before early termination of one or more treatment arms. After careful consideration, the IPFnet Steering Group recommends conservative thresholds for the early examinations of the safety and efficacy data.

The DSMB will be expected to meet approximately every 6 months until trial completion to review safety and toxicity data. The DSMB may recommend stopping the study based on these reviews. Because the DSMB could stop the trial for safety concerns as well as for a large efficacy benefit, there could be multiple opportunities to reject the null hypothesis (no difference in event rates between the PL and active groups). A Bonferroni approximation will be applied during the 1 planned interim analysis for efficacy. For the interim analysis, the critical value for the 2-degree-of-freedom omnibus test will be set to have $\alpha = 0.0001$. If the omnibus test is statistically significant, the 3 pairwise comparisons will be conducted. For the final efficacy analysis, the critical value of the 2-degree-of-freedom test for statistical significance will be set at $\alpha = 0.0499$.

To provide the DSMB with information on the likelihood that the null hypotheses will be rejected, the IPFnet DCC will calculate the conditional power for a positive result at the interim analysis. If the conditional power is too low, the DSMB may consider recommending that the trial be stopped. The conditional power is the probability, given the current observed data, that the test statistic at the end of the trial will reject the null hypothesis. It will be calculated using the method of Lan and Wittes (Lan 1988). Since there are 2 steps in the testing procedure, calculations of conditional power will be presented for the omnibus test and each of the 3 pairwise comparisons. The presentation of conditional power will likely occur after approximately 50% of subjects have completed their 60 weeks of treatment.

Before locking the database, a statistical analysis plan (SAP) will be developed to provide complete details on the statistical analysis. Before data analysis, the SAP will be approved by the IPFnet Steering Group and the DSMB. The SAP will include the specifics for how and when the DSMB will be notified for AEs. The IPFnet DCC will deliver to the DSMB all FDA-defined AEs at 3-month intervals. The IPFnet DCC will prepare narrative SAE reports in real time for

DSMB review including recommendations and analysis of similar events for each SAE submitted to the FDA.

11. Investigator and Sponsor Obligations

11.1. Monitoring

All monitoring activities for U.S. clinical centers will be performed in accordance with DCRI standard operating procedures. Information regarding the types of visits will be outlined in the PANTHER-IPF MOOP.

11.2. Cost and Payment

There will be no cost to subjects enrolled in this trial. Study-related procedures will be paid for by the IPFnet.

Subjects may be eligible for reimbursement for travel to the clinical center. Details of payment will be explained to each subject during the consent process.

11.3. Confidentiality and Health Insurance Portability and Accountability Act Considerations

Subject confidentiality will be protected throughout the study. All subject data will be kept strictly confidential, and no subject-identifying information will be released to anyone outside the project. Confidentiality will be assured through several mechanisms. First, each subject will be assigned an anonymous study ID, which will then be used on all study forms. Second, any study forms, blood samples, and paper records that contain subject information (eg, address lists, phone lists) will be kept at the clinical centers in secured, locked areas, coded by number. Once blood is collected, there will be no subject identifiers placed on blood samples—only the study ID number and the date of sample collection. Third, access to all subject data and information, including laboratory specimens, will be restricted to authorized personnel. In the case of computerized data, this restricted access will be assured through user logon IDs and password protection.

At the IPFnet DCC, only authorized personnel will have access to the data files containing study data. Security will be assured through user logon IDs, passwords, and appropriate access privileges. All study subjects will be identified only by their IPFnet ID numbers, and no personal identifying information, such as name, address, or Social Security number, will be entered into the IPFnet DCC database. Any subject-specific data reported to the IPFnet Steering Group will be identified only by the IPFnet ID number.

Finally, subjects will not be identified by name in any reports or publications, nor will the data be presented in such a way that the identity of individual subjects can be inferred. Analysis files created for further study by the scientific community will have no subject identifiers. These data files will be created in accordance with the Ancillary Studies and Publication Policy of the IPFnet.

11.4. Informed Consent Procedures

All IPFnet subjects will provide written informed consent using procedures reviewed and approved by each clinical center's IRB. Informed consent will be undertaken by study personnel in-person with the subject. The subject has the option of declining further participation in the study at that point. No further study procedures will be conducted until the signed documents have been provided to the IPFnet clinical center.

11.5. Institutional Review Boards

Before initiating this study, the protocol, clinical center-specific informed consent forms, Health Insurance Portability and Accountability Act (HIPAA) forms, recruitment materials, and other relevant information will be reviewed by a properly constituted IRB at each participating clinical center. A copy of the signed and dated IRB approval at each clinical center will be retrieved prior to or during the site initiation visit and archived at the IPFnet DCC. Any amendments to the protocol, other than simple administrative and typographical changes, must be approved by each IRB before they are implemented. The clinical centers will seek annual renewals of their IRB approvals in accordance with local procedures.

12. Investigator Agreement

I have read the foregoing protocol, PANTHER-IPF, and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the study.

I will fulfill all responsibilities for submitting pertinent information to the local IRB, if applicable, that is responsible for this study.

I further agree that NHLBI and/or DCRI will have access to any source documents from which eCRF information may have been generated.

____ ________________

Signature of Principal Investigator Date

____

Name of Principal Investigator (printed or typed)

Protocol version date: May 19, 2009

Protocol Amendment 1 version date: May 28, 2010

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PANTHER-IPF

PREDNISONE, AZATHIOPRINE, AND N-ACETYLCYSTEINE: A STUDY THAT EVALUATES RESPONSE IN IDIOPATHIC PULMONARY FIBROSIS

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Compiled by:

The PANTHER-IPF Protocol Committee

Version Date: May 19, 2009

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Amendment 2 Date: December 6, 2011

Distributed by:
The IPFnet Coordinating Center
Duke Clinical Research Institute
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Durham, NC 27715

Protocol Summary

PRODUCT	N-acetylcysteine
CLINICALTRIALS.GOV NUMBER	NCT00650091
PROTOCOL TITLE	**P**rednisone, **A**zathioprine, and **N**-acetylcysteine: A Study **TH**at **E**valuates **R**esponse in **I**diopathic **P**ulmonary **F**ibrosis (PANTHER-IPF)
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION	Confirmed idiopathic pulmonary fibrosis, diagnosed within 48 months of enrollment; forced vital capacity ≥ 50% predicted; hemoglobin adjusted diffusing capacity of the lung ≥ 30% predicted
STUDY OBJECTIVES	To assess the safety and efficacy of N-acetylcysteine in subjects with newly diagnosed idiopathic pulmonary fibrosis
STUDY DESIGN	Multi-center, randomized, double-blind, placebo-controlled
TREATMENT REGIMENS	1) N-acetylcysteine (600 mg TID), or 2) placebo
ROUTE OF ADMINISTRATION	Oral
TIME BETWEEN FIRST AND LAST DOSES OF ACTIVE STUDY AGENT	Maximum of 60 weeks
NUMBER OF SUBJECTS	Approximately 130 NAC, 130 placebo (1:1)
NUMBER OF CLINICAL CENTERS	Approximately 26 US sites
PRIMARY ENDPOINT	Change in longitudinal forced vital capacity measurements over 60 weeks
INTERIM ANALYSIS	Completed October 2011

Study Sponsor: **National Heart Lung and Blood Institute**

Acting Steering Group Chair: **Marvin I. Schwarz, MD**
University of Colorado - Denver

Project Officer: **Gail Weinmann, MD**
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Duke Clinical Research Institute
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List of Abbreviations

6MWT	6-minute walk test
A-aPO_2	alveolar-arterial PO_2 difference
ABG	arterial blood gas
AE	adverse event
AEx	acute exacerbation
A/G	albumin/globulin
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATS	American Thoracic Society
AZA	azathioprine
BAL	bronchoalveolar lavage
BUN	blood urea nitrogen
CBC	complete blood count
cGMP	Current Good Manufacturing Practice
CPI	Composite Physiologic Index
CPK	creatine phosphokinase
CT	computed tomography
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute

DLCO	diffusing capacity of the lung for carbon monoxide
DLCO%pred	diffusing capacity of the lung for carbon monoxide percent predicted
DSMB	data and safety monitoring board
eCRF	Electronic case report form
ERS	European Respiratory Society
FDA	Food and Drug Administration (U.S.)
FEV_1	forced expiratory volume in 1 second
FSH	follicle-stimulating hormone
FVC	forced vital capacity
FVC%pred	forced vital capacity percent predicted
GGT	gamma glutamyl transferase
GSH	glutathione
HAD	Hospital Anxiety and Depression
HHS	Health & Human Services (U.S. Dept. of)
HIPAA	Health Insurance Portability and Accountability Act
HRCT	high-resolution computed tomography
IBW	ideal body weight
ICE CAP	Investigating Choice Experiments for Preferences of Older People Capability Instrument
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis

IPFnet	Idiopathic Pulmonary Fibrosis Clinical Research Network
IRB	institutional review board
ITT	intent to treat
IV	intravenous
IVRS	interactive voice response system
LDH	lactate dehydrogenase
LFT	liver function test
LOCF	last observation carried forward
LSD	Least Significant Difference
MAR	missing at random
MCAR	missing completely at random
MMRM	mixed model repeated measures
MOOP	manual of operating procedures
NAC	N-acetylcysteine
NHLBI	National Heart Lung and Blood Institute (U.S.)
NIH	National Institutes of Health (U.S.)
NSIP	nonspecific interstitial pneumonia
PaO_2	partial pressure of arterial oxygen
PCP	primary care provider
PFT	pulmonary function test

PHS	Public Health Service (U.S.)
PI	principal investigator
PL	placebo
PLT	platelet
PRED	prednisone
QOL	quality of life
SAE	serious adverse event
SAP	statistical analysis plan
SGRQ	St. George's Respiratory Questionnaire
SpO_2	oxygen saturation by pulse oximetry
TPMT	thiopurine methyl transferase
UCSD SOBQ	University of California at San Diego Shortness of Breath Questionnaire
UIP	usual interstitial pneumonia
ULN	upper limit of normal
USP	United States Pharmacopoeia
VC	vital capacity
WBC	white blood cell

PREDNISONE, **A**ZATHIOPRINE, AND **N**-ACETYLCYSTEINE: A STUDY **TH**AT **E**VALUATES **R**ESPONSE IN **I**DIOPATHIC **P**ULMONARY **F**IBROSIS

1. Summary

There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) is conducting a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy in subjects with mild or moderate IPF.

The study initially employed a 3-arm design with 1:1:1 randomization to NAC, azathioprine (AZA)-prednisone (PRED)-NAC, and PL, with each subject to be treated up to a maximum of 60 weeks, followed by a tapering of PRED/PL and a 4-week period for safety evaluation. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] ≥ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DL_{CO}%pred] ≥ 30%) diagnosed within the past 48 months were to be enrolled.

At the pre-specified interim analysis, the DSMB recommended termination of the prednisone-azathioprine-NAC arm of the study. No additional patients will be randomized to that arm. However, the NAC and placebo arms remain open for enrollment, and we will enroll approximately 130 subjects in each arm (inclusive of the subjects enrolled at the time of the interim analysis.) Follow up for subjects enrolled into the two arms will continue for 60 weeks.

The primary endpoint is the change in longitudinal measurements of FVC over the study period. The primary goal of this study to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF has been met, in part, by demonstrating that the widely used triple therapy was harmful to patients with IPF (NHLBI press release, Oct 21, 2011). To determine the potential therapeutic benefits of NAC alone, the study will continue to enroll patients as a two-arm, double-blind, placebo-controlled study from this point on (NAC vs. placebo) as recommended by the DSMB following the pre-specified interim analysis.

2. Hypotheses and Specific Aims

2.1. Null Hypothesis

Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.

2.2. Specific Aim 1

This study is designed to assess the safety and efficacy of NAC in subjects with newly diagnosed IPF.

2.3. Specific Aim 2

Secondary goals of this study are to assess differences between treatment groups for the following:

1. Mortality
2. Time to death
3. Frequency of acute exacerbations (AExs)
4. Frequency of maintained FVC response
5. Time-to-disease progression
6. Change in DL_{CO}
7. Change in Composite Physiologic Index (CPI)
8. Change in resting alveolar-arterial oxygen gradient
9. Change in 6-minute walk test (6MWT) distance
10. Change in 6MWT oxygen saturation area under the curve
11. Change in 6MWT distance to desaturation < 80%
12. Change in 6MWT minutes walked
13. Changes in health status as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ)
14. Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ)
15. Frequency and types of adverse events (AEs)
16. Frequency and types of respiratory complications, both infectious and noninfectious
17. Frequency of hospitalizations, both all-cause and respiratory-related

2.4. Prespecified Subgroups of Interest

Treatment effects will be estimated and compared within key subgroups:

- Higher enrollment FVC[1,2]
- Typical vs. atypical high-resolution computed tomography (HRCT) reading at baseline[3]
- Recent vs. more remote diagnosis (time from initial diagnosis of IPF ≤ 1 year and > 1 year)
- Lower CPI score at enrollment[4]
- Medical therapy for gastroesophageal reflux[5]
- Ethnic background
- Sex
- Smoking history (current or ex-smoker vs. never smoker), given potential impact on oxidant status[6]
- Presence of emphysema > 25% on HRCT

3. Background and Significance

3.1. Idiopathic Pulmonary Fibrosis is the Most Common Interstitial Lung Disease

IPF is the most common interstitial lung disease (ILD) of unknown etiology. The current incidence and prevalence of IPF in the United States are not known. A 1994 study of a population-based registry of subjects in Bernalillo County, New Mexico, USA, estimated an incidence of 10.7 cases per 100,000 per year for males and 7.4 cases per 100,000 per year for females; the prevalence of IPF was estimated at 20 per 100,000 for males and 13 per 100,000 for females.[7] Extrapolating from a large healthcare claims database, a more recent review estimated the prevalence of IPF in the United States at 42.7 per 100,000 (incidence estimated at 16.3 per 100,000 per year).[8] Recent epidemiological studies indicate increasing mortality rates from IPF in the United States and other industrialized nations.[9-12]

Approximately two-thirds of subjects with IPF are over the age of 60 at the time of presentation, and the incidence increases with age.[13] IPF has no distinct geographical distribution, and predilection by race or ethnicity has not been identified.[13] Individual subjects may remain relatively stable for prolonged periods, experience very slow declines in lung function with progression of radiographic abnormalities for a period of months to years, or experience more rapid declines and subsequent death. Only 20% to 30% of IPF patients survive for 5 years following diagnosis.

There is currently no proven, effective pharmacological treatment for IPF.[13] Anti-inflammatory and immunosuppressive agents have been the traditional approach to the management of patients with IPF. Based on the results of the interim analysis of the PANTHER-IPF trial, this ‘traditional approach’ will be aborted. However, it remains unknown if NAC alone will prove beneficial in IPF patients. The primary goal of the modified study is to establish an evidence-based standard of care and clarify the role of this specific antioxidant pharmacotherapy for IPF.

3.2. Rationale for Placebo Control

IPF is a disorder for which there is no proven efficacious therapy. A major objective of this trial is to test, to the greatest degree possible, a proposed standard of care for patients with IPF. The current traditional therapy employs immunosuppressive and corticosteroid drugs. Interim review of the original PANTHER-IPF study has documented increased adverse events and lack of efficacy for AZA-PRED-NAC compared to placebo suggesting that this therapeutic approach should not be employed. Whether this applies to NAC alone, which has been advocated by international societies, has not been proven in well-designed, well-powered clinical trials. The recommendations made in the recently published evidence guidelines for NAC monotherapy was weak based on low quality.[13] Thus, this continued clinical trial randomizing patients to receive NAC or placebo is pivotal and will answer the important question of the potential therapeutic benefits of NAC monotherapy with grade A evidence. In this prospective, randomized clinical trial, the inclusion of a PL arm is therefore vital to adequately test the benefits of NAC in well-characterized subjects with IPF.

If NAC has no true efficacy, then its role as standard of care will be refuted. If a benefit compared with PL is confirmed, it will establish a benchmark against which future novel therapies for IPF will be safely compared. As there is no currently accepted therapy for IPF, there is an increasing body of published literature supporting the concept of no treatment as the "best care" option for IPF.[13]

Post hoc analyses of PL-controlled trials suggest that subjects with milder disease may be more amenable to therapy.[1,2] It is notable that a recent international, prospective, randomized trial of interferon-gamma for IPF also included a PL arm; the study was terminated early by the data and safety monitoring board (DSMB) due to lack of treatment effect.[14,15] This underscores the belief that a proven effective therapy for IPF does not currently exist and that true placebo-controlled trials remain the gold standard. Similarly, recently completed trials of etanercept, everolimus, bosentan and BIPF 1120 in IPF have included PL-treated arms.[16-19] In three of these trials, the treated subjects showed little, if any, objective improvement. Based on this evidence and the current status of IPF therapy and therapeutic trials, we believe that clinicians and subjects will continue to enroll in a PL-controlled study.

3.3. Rationale for N-acetylcysteine

NAC is a derivative of the amino acid L-cysteine. NAC has been shown to augment levels of the naturally occurring antioxidant glutathione (GSH) (glutathione; γ-glutamyl cysteinyl glycine) both in vitro and in vivo.[20,21] GSH is present in all eukaryotic cells and may play an important role in protecting alveolar epithelial cells against oxidant injury. The concentration of GSH in the bronchoalveolar lavage (BAL) fluid in patients with IPF is markedly diminished compared with normal subjects. This GSH deficiency may be corrected by exogenous administration of NAC.[21,22]

There is evidence of enhanced production of oxidants in an IPF lung. Both inflammatory cells and myofibroblasts derived from patients with IPF generate increased amounts of extracellular oxidants, including hydrogen peroxide.[23,24] Secretion of hydrogen peroxide by activated myofibroblasts may induce the death of adjacent lung epithelial cells by paracrine mechanisms.[24] Additionally, generation of oxidants by myofibroblasts induces oxidative crosslinking of extracellular matrix proteins,[25] a potential mechanism for aberrant matrix remodeling. Thus, an oxidant-antioxidant imbalance exists in the lungs of IPF patients.[26] NAC may confer protection against this imbalance by augmenting GSH levels in addition to its more direct free-radical scavenging activity.

Intravenous (IV) NAC therapy has been shown to increase total BAL GSH in 8 IPF subjects.[27] Oral NAC (600 mg 3 times per day) has been shown to decrease markers of oxidant injury and improve both total and reduced GSH levels in the epithelial lining fluid of subjects with IPF in a small, uncontrolled study;[22] pulmonary function improved modestly with therapy. A similar study in 18 IPF subjects confirmed increased intracellular GSH concentration after 12 weeks of NAC (600 mg 3 times per day);[22,28] no clinical correlates were reported. Inhaled NAC was suggested to improve pulmonary function in an open label study.[29]

3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy

Results of a double-blind, multi-center European clinical trial of 150 IPF subjects testing combinations of AZA-PRED vs. AZA-PRED-NAC have been reported.[30]. NAC added to AZA-PRED ("conventional therapy") had a significant positive effect on DLCO ($p < 0.005$) and vital capacity (VC) ($p < 0.05$) at the end of 1 year.[30] The recent ATS/ERS position statement, after much discussion, concluded that NAC alone should not be considered in the majority of patients with IPF without additional data from well-designed studies.

The interpretation of these data has been quite controversial. Some have suggested that the magnitude of the treatment effect, although statistically significant, is modest.[31] Others have suggested that NAC may be modulating potential toxic effects of AZA-PRED alone,[32] supporting the investigation of NAC as stand-alone therapy.

The IPFnet is now completing the PANTHER-IPF trial with a 1:1 randomization (NAC vs. placebo) and a simple, practical, feasible, and scientifically rational design that will establish standard of care with NAC for IPF based on a currently available therapeutic agent and the existing data to support its use. We anticipate that all future clinical trials of novel therapeutic agents will be tested against this to-be-established standard of care.

3.6. Rationale for the Study Design and Primary Endpoint

The optimal study design of a therapeutic trial in IPF would include survival as a primary endpoint. The published results of the IFN-γ 1b Phase 3 (GIPF-001) trial suggested a survival benefit in subjects with milder disease in retrospective analyses,[1] although the trial was underpowered to address this question. This was likely related to the limited mortality in the PL arm of the study, which included IPF subjects with mild to moderate disease. This study documents that an IPF study powered to improve survival in a patient population with mild disease requires a larger sample size and/or duration of study. In fact, the recently aborted Phase 3 IFN-γ 1b (GIPF-007; INSPIRE) study was a survival-based study and recruited more than 800 subjects at 75 centers worldwide.[14,15] As such, within the context of the current IPFnet trial, survival is an impractical primary endpoint variable.

Several groups have published data defining an appropriate surrogate outcome variable; a 10% decrement in FVC during 6 to 12 months is a powerful predictor of survival in IPF.[33-36] Furthermore, additional evidence suggests a similar predictive ability for a 10% decrement in FVC during 3 months of follow-up.[37] With strong supportive evidence of FVC progression being related to mortality on a per-subject basis, this study will use FVC changes in liters between treatment groups as the primary endpoint. Previously published IPF studies have shown a steady decline in FVC (and FVC%pred) among control group subjects.[2,30] The GIPF-001 study suggested a 48-week decrease in FVC of 0.16 L in the PL-treated subjects. The IFIGENIA study demonstrated a decline in FVC of approximately 0.19 L over 52 weeks in the subjects randomized to the control treatment. Figure 1 depicts the change in FVC for control groups from previously published IPF studies.[38] Based on these data, we expect that the PL group will have a decline of 0.20 L over the 60-week study period. The IPFnet Steering Group determined that a clinically meaningful improvement would be the preservation of the majority of the 0.20-L FVC decline. Therefore, a mean treatment difference of 0.15 L in mean FVC over the 60-week study period was determined to be a clinically meaningful difference.

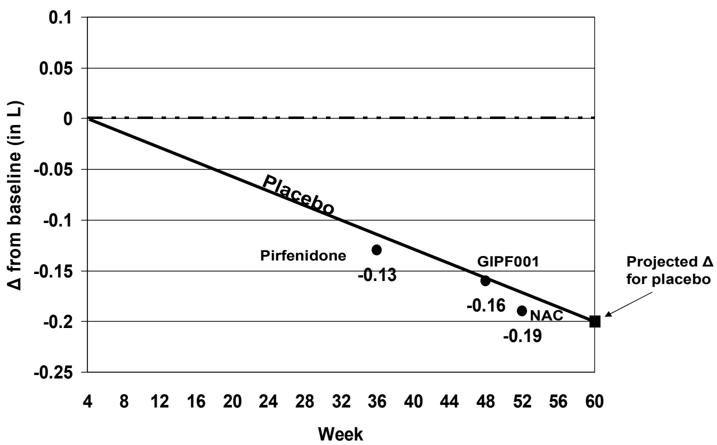


Figure 1: Changes in FVC From Baseline in Prior IPF Clinical Trials

Recent data suggest that various patient subgroups would be expected to potentially exhibit differential response to therapy. These parameters will be used to a priori separate patients by a series of baseline characteristics, including:

1. Higher enrollment FVC[1,2]
2. Typical vs. atypical high-resolution computed tomography (HRCT) reading at baseline[3]
3. Recent vs. more remote diagnosis (time from initial diagnosis of IPF ≤ 1 year and > 1 year)
4. Lower CPI score at enrollment[4]
5. Medical therapy for gastroesophageal reflux[5]
6. Ethnic background
7. Sex
8. Smoking history (current or ex-smoker vs. never smoker), given potential impact on oxidant status[6]
9. Presence of emphysema > 25% on HRCT

3.7. Rationale for Blinding of Treatments

The issue of treatment blinding was given a great deal of consideration, with subject safety being the primary concern. After discussion among the Steering Group members, it was decided that, as long as subject safety could be ensured, blinding was necessary. Blinding allows the study to:

- Have optimal scientific validity and potential to impact the standard of care for subjects.

- Make objective assessments of treatment effects.
- Maintain clinical equipoise among investigators.
- Encourage subjects to have similar levels of contact with the medical community.
- Minimize the differential dropout rates across study arms.

4. Methods

4.1. Inclusion Criteria

1. Age 35 to 85 years, inclusive
2. FVC ≥ 50% of predicted (post-bronchodilator measurement from the screening visit)
3. DL_{CO} ≥ 30% of predicted (hemoglobin corrected and altitude corrected if >4000 ft above sea level)
4. Ability to understand and provide informed consent
5. Diagnosis of IPF according to a modified version of the ATS criteria ≤ 48 months from enrollment. The date of diagnosis is defined as the date of the first available HRCT or surgical lung biopsy characteristic of definite UIP.

4.1.1. Subjects Shown to Have Usual Interstitial Pneumonia Pattern on Surgical Lung Biopsy

Subjects who have been shown to have UIP pattern on lung biopsy must have all of the following:

1. Exclusion of other known causes of ILD, such as drug toxicity, clinically significant environmental exposures, or diagnosis of connective tissue diseases
2. Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scan

4.1.2. Subjects Who Have Not Undergone a Surgical Lung Biopsy

In addition to the criteria above, these subjects must have radiological findings considered to be definite for the diagnosis of UIP/IPF:

1. Bibasilar reticular abnormalities with minimal ground glass opacities
2. Honeycombing as the predominant feature and located in the peripheral lung bases

4.2. Diagnosis of IPF

Only subjects with definite IPF will be eligible for enrollment in this study. We will utilize a combination of clinical/physiologic features, HRCT, and review of a clinically obtained surgical lung biopsy specimen to establish the diagnosis of IPF. An algorithm for the diagnosis is provided to guide entry into the protocol as outlined in the inclusion and exclusion criteria (Figures 2 and 3). This multi-disciplinary approach uses expertise from clinicians, radiologists, and pathologists. Investigators at each site, in conjunction with central pathology, will work together to establish the diagnosis of IPF. This interactive approach to the diagnosis of IPF increases the level of agreement between observers.[39]

A subject with suspected ILD should be evaluated for secondary causes including, but not limited to, environmental exposures, drugs, and systemic diseases. Presence of any of these findings felt to be significant enough to cause an ILD should disqualify the subject from entry into the trial.

If secondary causes are absent, an HRCT scan may be obtained. If an HRCT of sufficiently high quality has been obtained within the last 3 months, that scan may be used for diagnosis. In the appropriate clinical setting, the diagnosis of IPF can be made by the demonstration of a typical radiographic pattern on HRCT or by demonstration of UIP pattern on a surgical lung biopsy. The following criteria for a radiographic (ie, nonsurgical) diagnosis will be used. In the absence of known exposures and/or clinical associations attributable to pulmonary fibrosis, and in the appropriate clinical setting, the presence of definite UIP pattern in HRCT images is required to meet study criteria for the diagnosis of IPF.

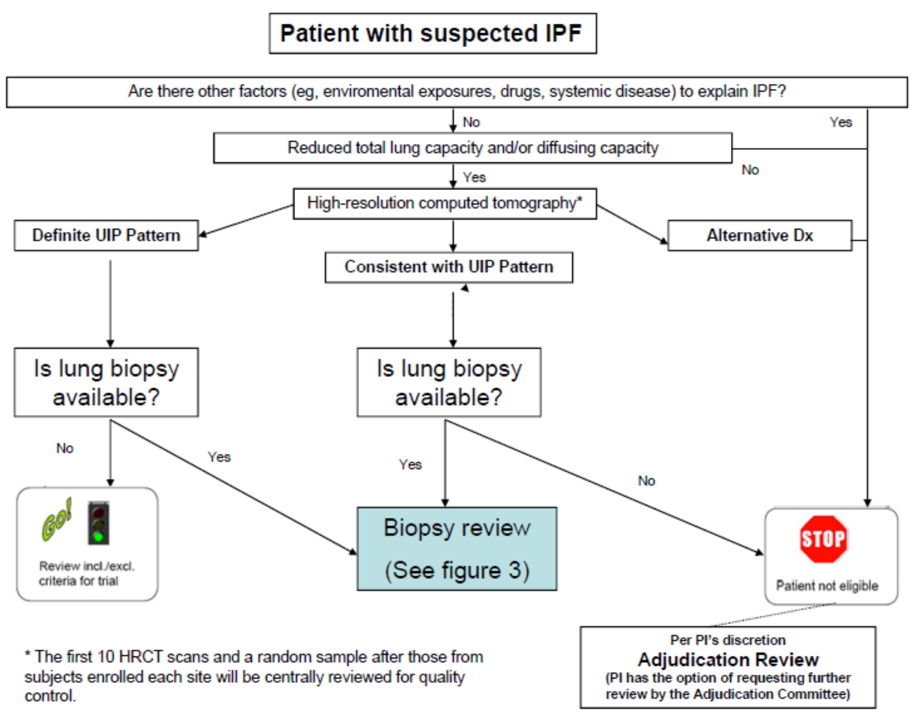


Figure 2: Diagnosis of Idiopathic Pulmonary Fibrosis in the IPFnet

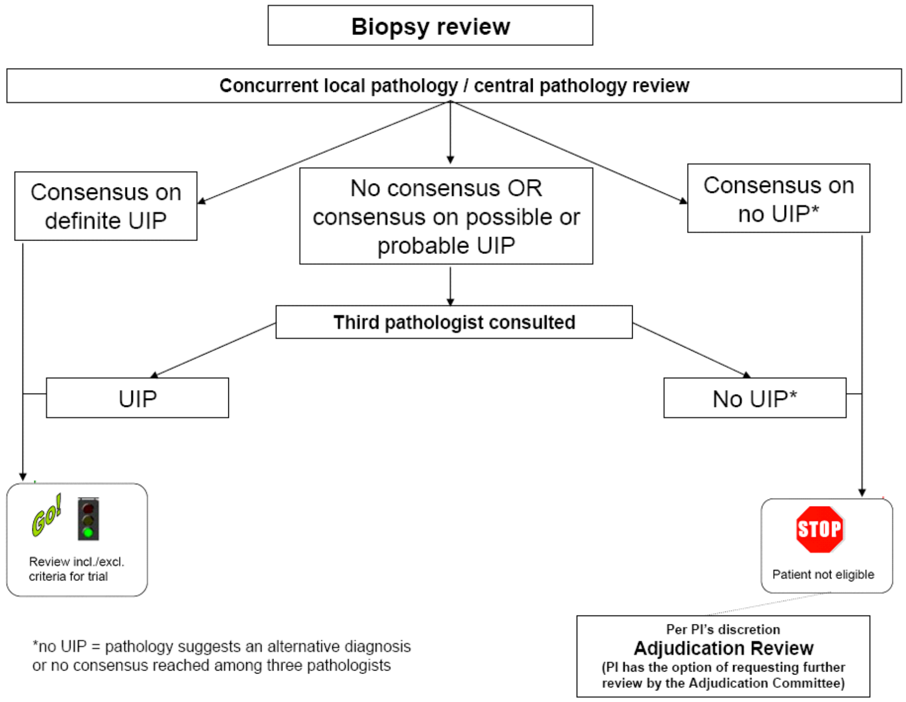


Figure 3: Pathology Flow Chart: Surgical Lung Biopsy Diagnosis

<u>Requirement for diagnosis</u>

1. **Clinical:** exclusion of other known causes (connective tissue diseases, environmental and drug exposures) of ILD
2. **Radiographic:** HRCT with bibasilar reticular abnormality and honeycomb change with minimal ground glass opacities

<u>Appropriate clinical setting</u>

1. Age > 50 years
2. Insidious onset of unexplained dyspnea
3. Duration of illness for ≥ 3 months
4. Bibasilar, inspiratory crackles

Unlike the ATS/ERS consensus criteria published in 2000, bronchoscopy will not be required for diagnosis. This decision was made based on the experience of the IPFnet Steering Group members regarding the utility of bronchoscopy in the diagnosis of IPF. The presence of an atypical HRCT finding will require documentation of a definitive diagnosis by surgical lung biopsy. In fact, this is in keeping with the recently published evidence based guidelines for diagnosis and management of IPF.[13] As shown in Figure 3, central review of the pathology data will be required for a diagnosis of IPF.

We will not require central review of HRCT, as several studies have shown that a confident local interpretation of clinical/HRCT criteria as definite IPF/UIP is associated with a high positive predictive value for finding UIP at surgical lung biopsy (see Table 1). Differences in sensitivity in these series likely reflect subject selection, as Flaherty et al[3] evaluated only UIP and nonspecific interstitial pneumonia (NSIP), while Raghu et al[40] and Hunninghake et al[41] included a broader range of ILD.

Table 1: Operating Characteristics of Local HRCT Review for Diagnosis of UIP

Researcher	**# of Subjects**	**Sensitivity** (%)	**Specificity** (%)	**PPV** (%)	**NPV** (%)
Raghu et al [40]	59 (29 UIP by SLB)	78	90	88	82
Hunninghake et al [41]	91 (54 UIP by SLB)	74	81	85	67
Flaherty et al [3]	96 (only NSIP & UIP)	37	100	100	30

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; UIP, usual interstitial pneumonia; SLB, surgical lung biopsy; and NSIP, nonspecific interstitial pneumonia.

Furthermore, an analysis of the HRCT scans from subjects enrolled in the GIPF-001 trial confirmed that local site interpretations have a high congruity to a central radiology core. In this multi-center study, 263 HRCT scans were read as definite IPF, and a retrospective central radiology core review found 93.2% to be consistent with IPF [42]. We will also take several additional steps to insure that the local HRCT reads are accurate, including:

1. A detailed training module has been developed and must be completed by each site radiologist before site initiation.
2. Clinical centers are to mail all HRCT scans to the HRCT core lab. The first 10 HRCT scans from subjects enrolled at each enrolling clinical center will be reviewed centrally to be certain that local reads

are congruent with a central interpretation. If discrepancies are identified, additional education will be provided, and HRCT scans will continue to be reviewed centrally until the central radiology core is confident that the local center is performing appropriately.

3. Random scans will be obtained from each center throughout the study to confirm that the local read continues to agree with central interpretation. If discrepancies are identified, they will be addressed as in #2 above.

In all cases, if a subject has a surgical lung biopsy sample, that sample will be reviewed by the local and central pathologists. Therefore, the only cases that would not be subject to a direct central review process are those where the HRCT meets the centrally defined criteria for an unequivocal diagnosis and a lung biopsy sample is not available. Table 2 below summarizes the possible combinations for making a diagnosis.

Table 2: Combining HRCT and Pathology Interpretations to Determine if IPF is Present

HRCT Diagnosis	**Pathology Diagnosis**	**Diagnosis of IPF**
Definite UIP	Definite UIP	**Yes**
Definite UIP	Probable UIP	**Yes**
Definite UIP	Possible UIP	**Yes**
Definite UIP	Not UIP	**No**
Definite UIP	Unavailable	**Yes**
Consistent with UIP	Definite UIP	**Yes**
Consistent with UIP	Probable UIP	**Yes**
Consistent with UIP	Possible UIP	**No**
Consistent with UIP	Not UIP	**No**
Consistent with UIP	Unavailable	**No**
Inconsistent with UIP	Any	**No**

Abbreviations: HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis;

UIP, usual interstitial pneumonia; Dx, diagnosis

4.3. Exclusion Criteria

1. History of clinically significant environmental exposure known to cause pulmonary fibrosis. Occupational exposures, such as asbestos, or environmental exposure to organic dust, such as occurs in pigeon breeders, may at times mimic the clinical and radiographic findings of IPF.
2. Diagnosis of connective tissue disease, felt by the principal investigator (PI) to be the etiology of the interstitial disease. Diagnosis of collagen-vascular conditions will be according to the published American College of Rheumatology criteria. As such, the presence of any documented collagen-vascular disorder or the presence of any suspicious symptom complex, whether or not associated with significantly abnormal rheumatological serologies, will exclude the subject, at the discretion of the PI.
3. Extent of emphysema greater than the extent of fibrotic change (honeycombing, reticular changes) on HRCT scan.
4. Forced expiratory volume in 1 second (FEV_1)/FVC ratio < 0.65 at screening (postbronchodilator).
5. Partial pressure of arterial oxygen (PaO_2) on room air < 55 mm Hg (< 50 mm Hg at Denver site).
6. Residual volume > 120% predicted at screening (postbronchodilator).
7. Evidence of active infection.
8. Significant bronchodilator response on screening spirometry, defined as a change in $FEV_1 \geq 12\%$ and absolute change > 200 mL OR change in FVC ≥ 12% and absolute change > 200 mL. The percent difference between the FVC (or FEV_1) values will be calculated by taking the absolute value of the difference and dividing it by the larger of the two FVC (or FEV_1) values.
9. Screening and enrollment post-bronchodilator FVC measurements (in liters) differing by > 11%. The percent difference between the FVC values will be calculated by taking the absolute value of the difference and dividing it by the larger of the two FVC values (eg., the percent difference between FVC measurements of 1.9 and 2.0 liters would be determined by taking the difference between the two (0.1 liters) and dividing by the larger of the two (2.0 liters). So 0.1/2.0 = 5%, and these FVC measurements would not exclude the subject.
10. Listed for lung transplantation, i.e., the patient has completed the evaluation process, has been accepted as a candidate for transplantation at an appropriate center, and is waiting to receive notification of an available donor organ.
11. History of unstable or deteriorating cardiac disease.
12. Myocardial infarction, coronary artery bypass, or angioplasty within 6 months of screening.

13. Unstable angina pectoris or congestive heart failure requiring hospitalization within 6 months of screening.
14. Uncontrolled arrhythmia.
15. Severe uncontrolled hypertension.
16. Known HIV or hepatitis C.
17. Known cirrhosis and chronic active hepatitis.
18. Active substance and/or alcohol abuse (as determined by site PI).
19. Pregnancy or lactation (subjects who are pregnant or breastfeeding).
20. Known hypersensitivity to study medication.
21. Any condition other than IPF that, in the opinion of the site PI, is likely to result in the death of the subject within the next year.
22. 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.
23. Any therapy directed at pulmonary fibrosis (excepting triple therapy of prednisone plus azathioprine plus NAC) requires a 30-day washout period before randomized. Triple therapy of <= 12 weeks duration in the past 4 years requires a 30-day washout period before randomization.
24. History of triple therapy of prednisone plus azathioprine plus NAC for > 12 weeks' duration in the past 4 years or previous enrollment in the triple-therapy arm of the PANTHER-IPF study.

4.4. Study Design and Study Visit

4.4.1. Study Design Summary

This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC in subjects with newly diagnosed IPF.

Subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLCO%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. Screening will continue until April 30, 2012.

The study will employ a 2-arm design with 1:1 randomization to NAC or PL. Once enrolled, subjects will visit the clinical center at 15 weeks and 15-week intervals thereafter. Each subject will be treated and followed for a maximum of 60 weeks.

During the 60-week visit, subjects will be taken off all study agents. Approximately four weeks after the final dose of study agent is taken, subjects will receive a safety phone call from the study site.

4.4.2. Study Visits

Subjects who meet entry criteria will review the informed consent, a written description of the purpose, procedures, and risks of the study, with the PI, co-investigator, or study coordinator, and all questions will be answered. The informed consent form will be signed by the subject at screening. No protocol-specific procedures will be performed until the subject has signed and dated an informed consent form. This includes the screening procedures.

4.4.2.1. Screening

Once informed consent is obtained, subjects may immediately begin the screening process or may return within 28 days of consent. In the event a study subject has been clinically evaluated at the study site by an IPFnet study physician and has performed testing within three weeks for this clinical evaluation that meets guidelines provided in the IPFnet PANTHER-IPF Manual of Operating Procedures (MOOP), this testing may be used to satisfy the following screening criteria: medical history, physical exam, arterial blood gas (ABG) with A-a gradient, vital signs with oximetry, body height and weight, spirometry, DL_{CO}, lung volumes, and HRCT scan.

Allowing the use of previously performed test results that meet study guidelines for the screening visit is intended to permit subjects easier access to study entry, to prevent subjects from repeating testing that has been performed within the study window, and to decrease risks to subjects from repeated exposure to procedures such as arterial puncture and HRCT.

The following procedures will be performed at screening:

- Medical history and a physical examination

- Measure height and weight
- Serum pregnancy test (if applicable)
- Measure vital signs including oximetry
- Blood draws performed and the following analyses conducted:
    - Hematology (red cell count, white cell count, hemoglobin, hematocrit, cell indices, differential, platelet count)
    - Blood chemistries according to central laboratory protocol (see Section 4.10, Laboratory Testing)
    - Beta human chorionic gonadotropin (serum) pregnancy test (in women of childbearing potential)
    - Urinalysis
- Pulmonary Function Tests (PFTs), including spirometry, pre- and post- bronchodilator, and post-bronchodilator measurement of lung volumes, and measurement of hemoglobin adjusted diffusing capacity.
- Measure ABGs
- HRCT if a satisfactory scan has not been performed on the subject within 3 months of screening (see PANTHER-IPF MOOP for more details)
- Surgical lung biopsies (if applicable) reviewed by local and central pathology departments
- Current medications. If required, a washout period discussed with the subject and initiated at this visit

4.4.2.2. Enrollment

The enrollment visit is expected to take place within eight weeks of the screening visit. Enrollment visit activities include:

- Measure vital signs, including oximetry
- Measure height and weight
- Serum pregnancy test (if applicable)
- If consent has been given, blood will be drawn and a urine specimen collected for the bio-specimen repository
- Spirometry (post-bronchodilator)
- Measure 6MWT with Borg Dyspnea Scale

- Collect Quality-of-life (QOL) data using the SF-36, EuroQol, Investigating Choice Experiments for Preferences of Older People Capability Instrument (ICE CAP), and SGRQ
- Female subjects complete Gender Substudy questionnaire
- Dyspnea status collected using the UCSD SOBQ
- Evaluate Acute Exacerbation (AEx)
- Review of any Adverse Events (AEs)
- Review of concomitant medications
- Subject receives diary and instructions on its purpose and proper use
- Subject receives supply of study drug sufficient to last until his or her 15-week study visit

See the Schedule of Assessments (Table 3) for more details. Subjects with screening and enrollment post-bronchodilator FVC measurements (in liters) differing by more than 11% are not eligible to be enrolled in the study.

Subjects will be asked to provide a physician of record. This physician will be considered the subject's primary care provider (PCP), and, if the subject agrees, the PCP will be informed by letter of the subject's enrollment in the trial. The subject will be informed that his or her ongoing medical care should be received from the PCP. The PCP will be informed of any safety issues identified by the study staff. The PCP will also be given information regarding communication with study personnel about pertinent health issues or clinic encounters the subject may have.

4.4.2.3. Week 15

The week 15 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg., the week 15 visit should occur anytime between 13 and 17 weeks after starting study drug Week 15 activities include:

- Measure vital signs with oximetry
- Measurement height and weight
- Serum pregnancy test (if applicable)
- Spirometry (post-bronchodilator) measurement
- Review of AEs
- Evaluate for AEx

- Review concomitant medications
- If consented, draw blood and collect urine specimen for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Review study diary and a new study diary will be given
- Provide additional supply of study drug sufficient to last until the next scheduled visit

4.4.2.4. Week 30

The week 30 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg., the week 30 visit should occur anytime between 28 and 32 weeks after starting study drug). Week 30 activities include:

- Physical examination
- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Serum pregnancy test (if applicable)
- Measure spirometry (post-bronchodilator)
- 6MWT with Borg scale
- DLCO
- Review of AEs
- Evaluate for AEx
- Review concomitant medications
- Complete all QOL and dyspnea questionnaires (EuroQol, ICE CAP, SF-36, SGRQ, and UCSD SOBQ).
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Review study diary
- Subjects return used and unused study drug for the visit
- Provide additional supply of study drug sufficient to last until the next scheduled visit

4.4.2.5. Week 45

The week 45 visit is expected to occur within +/- 14 days of the subject's scheduled visit time (eg, the week 45 visit should occur anytime between 43 and 47 weeks after starting study drug). Week 45 activities include:

- Measure vital signs with oximetry
- Measure height and weight
- Serum pregnancy test (if applicable)
- Measure spirometry (post-bronchodilator)
- Review of AEs
- Evaluate for AEx
- Review concomitant medications
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Study diary reviewed
- Provide additional supply of study drug sufficient to last until the next scheduled visit

4.4.2.6. Week 60 (Early Withdrawal or Final Treatment Visit)

At week 60, or at subject withdrawal from the study (premature, by study doctor or subject's decision), a final treatment visit will occur. At this final treatment visit subjects will discontinue NAC/PL abruptly. Week 60 activities also include:

- Physical examination
- Measure vital signs with oximetry
- Measure height and weight
- Laboratory values (complete blood count [CBC] and serum chemistries)
- Spirometry (post-bronchodilator) measurement
- 6MWT with Borg scale measurement
- DLCO
- Lung volumes
- ABG
- Review of AEs

- Evaluate for AEx
- Review concomitant medications.
- Subjects will complete all QOL and dyspnea questionnaires (EuroQol, ICE CAP, SF-36, SGRQ, and UCSD SOBQ)
- If consent has been given, blood will be drawn and a urine specimen collected for the biospecimen repository
- Subjects return used and unused study drug for the visit
- Study diary reviewed

4.4.2.7. Final Site Visit – FVC drop confirmation

In the event that the subject has a recorded FVC drop of >10% from baseline at the final treatment visit and the subject has not had a confirmation of such a drop at a previous study visit, the subject should return to the clinical site 6 to 8 weeks after the final treatment visit. During this visit, a post-bronchodilator spirometry test will be performed. This FVC measurement will be evaluated according to section 5.2.1 of this protocol.

4.4.2.8. Final Visit – Telephone Follow-up

Four weeks following the final dose of study medication, subjects will receive a telephone call from the study coordinator to ensure that there are no side effects and to follow up on any ongoing adverse events (AEs).

4.4.2.9. Phone Contact Between Visits

At week 2 and each month that a subject does not have a scheduled clinical center visit, his or her study coordinator will contact him or her at least once by telephone to:

- Inquire if the subject has had any hospitalizations, events that might be considered an AE, or any events significant enough to warrant an out-of-cycle visit to the clinical center.
- Remind subjects of their current dosage levels and confirm that the subject understands them.
- Address any questions or concerns the subject might have regarding other aspects of the study.
- Assess adherence to the treatment regimen by reviewing diary data; verbal review of medications taken, including nutritional supplements.

Table 3: Schedule of Assessments

Procedure	Screening Visit 0	Enrollment Visit 1	Week 15 Visit 2	Week 30Visit 3	Week 45 Visit 4	Week 60 / Early Withdrawal Visit 5	Final[1] Visit (via phone)
Informed consent	X						
Medical history	X						
Inclusion/exclusion criteria	X						
Serum pregnancy test (if applicable)	X	X	X	X	X		
Review of lung biopsy	X						
Arterial Blood Gas	X					X	
6-Minute Walk Test		X		X		X	
Physical examination	X			X		X	
Vital signs with oximetry	X	X	X	X	X	X	
Body height and weight	X	X	X	X	X	X	
Complete Blood Count	X			X		X	
Chemistry panel	X			X		X	
Monitor Lab Values	X			X		X	
Urinalysis	X						
Specimen repository substudy blood draw and urine collection (if consented)		X	X	X	X	X	
HRCT (if not completed within three months)	X						
Spirometry (pre- and post-bronchodilator)	X						
Spirometry (post-bronchodilator only)		X	X	X	X	X	
DLCO (post-bronchodilator only)	X			X		X	
Lung volumes (post-bronchodilator only)	X					X	
Evaluate for acute exacerbation		X	X	X	X	X	
Review Adverse Events		X	X	X	X	X	X
Review concomitant meds	X	X	X	X	X	X	
Dispense subject diary		X	X	X	X		
Review subject diary		X	X	X	X	X	
Dispense study agent		X	X	X	X		
Gender Substudy questionnaire (female subjects only)		X					
EuroQol, ICECAP, UCSD SOBQ, SGRQ, SF-36		X		X		X	

Abbreviations: DLCO, diffusing capacity of the lung for carbon monoxide; QOL, Quality of Life; ICECAP, Investigating Choice Experiments for Preferences of Older People; UCSD SOBQ, University of California Shortness of Breath Questionnaire; SGRQ, St. George's Respiratory Questionnaire; SF-36, Short Form 36 Health Survey

[1]Follow-up visit via phone will occur four weeks after final dose of study medication

4.5. Dose Justification

The general philosophy for determining dosing levels was to apply previously examined treatment regimens. With the focus of the study being to establish a standard of care for mild/moderate IPF subjects, the goal was to develop flexible yet standardized treatment rules that allow for the temporary or permanent withholding of one or more components of treatment when necessary. Subjects developing laboratory abnormalities or symptoms that result in discontinuation of one or more components of study treatment may continue on the other components as long as there is no contraindication for this. Complete follow-up is important for the validity of any study. As a strategy to maintain protocol adherence, we are using a treatment regimen that will detect potential side effects and prompt interventions proactively in the interest of patient safety. In addition, subjects who permanently stop study medications during the course of the study are encouraged to continue in the study, completing all scheduled visits and tests.

4.5.1. Rationale for N-acetylcysteine Dosing

To our knowledge, there have been no IPF studies to correlate clinical outcome measures with different dosages for NAC. The dosage chosen is based on the IFIGENIA study. However, BAL lung GSH levels from subjects with IPF have been augmented with the use of oral NAC at 600 mg 3 times per day. In addition, lung GSH levels have been associated with improved PFTs.[21,22,27] The dose chosen for this study was based on previous data, including the IFIGENIA study.[30]

4.5.1.1. Dosing of N-acetylcysteine/placebo

Dosing of NAC/PL will be 600 mg orally 3 times a day (1800 mg/day).

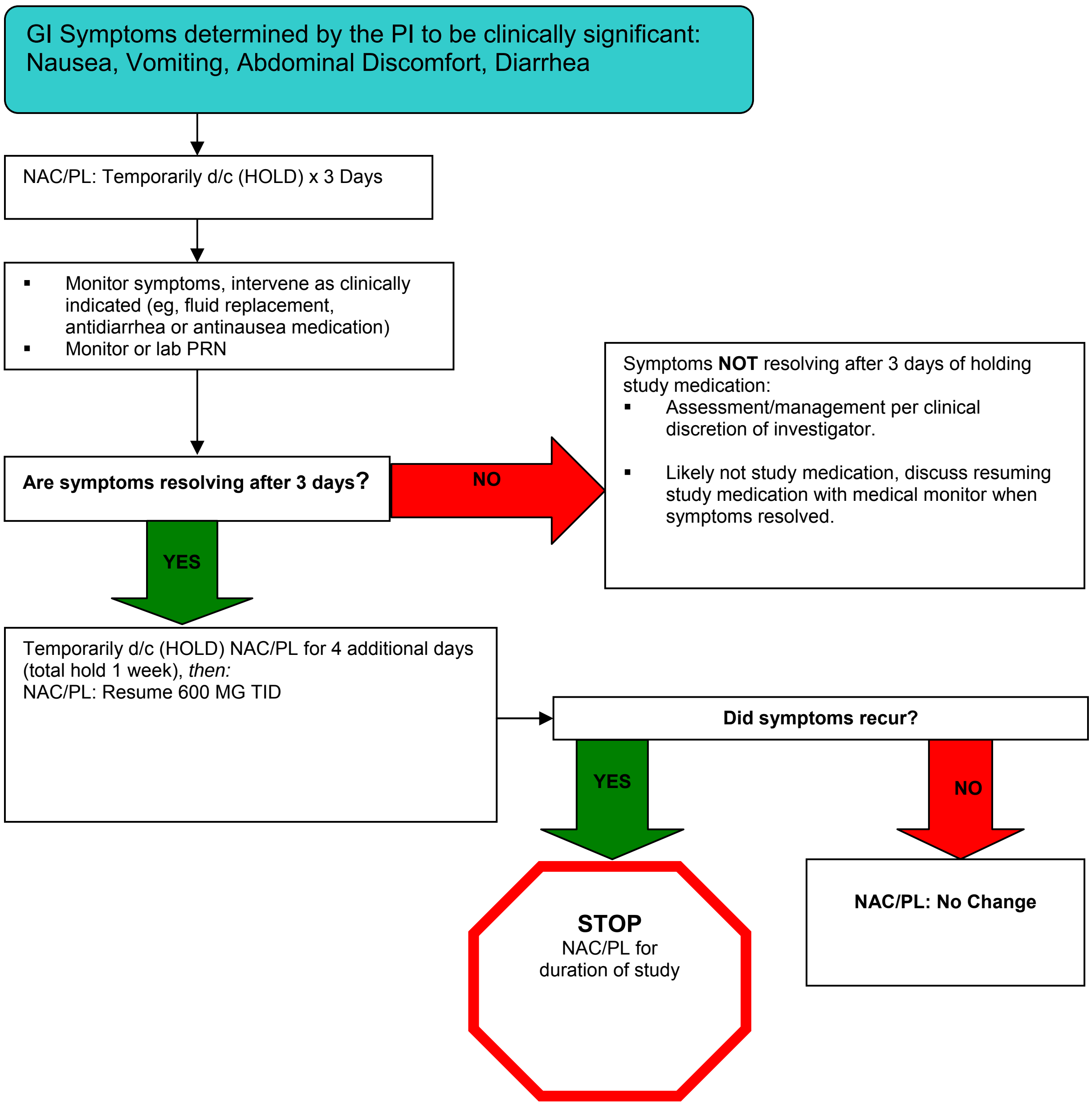
4.5.1.2. Reasons to Discontinue N-acetylcysteine/placebo

NAC/PL may be temporarily or permanently discontinued for the duration of the study for gastrointestinal symptoms or dermatologic reactions.

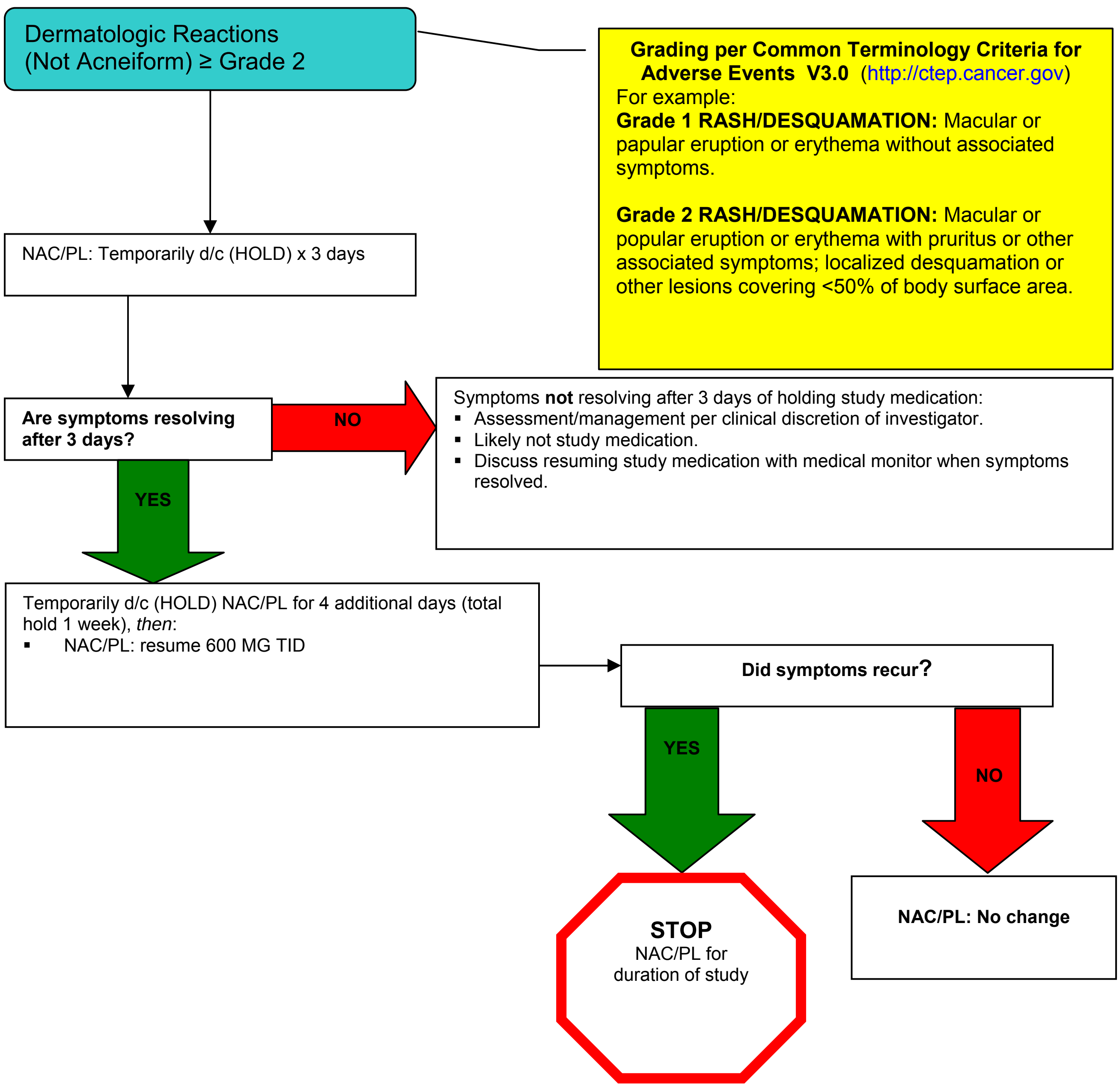
Temporarily discontinue (hold) oral NAC/PL for subjects requiring inpatient admission for acute exacerbation (AEx) or other conditions. Resume NAC/PL after discharge.

4.5.2. Dosage Algorithms

Dosage Adjustment Algorithm #1: NAC/PL Dose Modifications for Gastrointestinal Symptoms: Nausea, Vomiting, Abdominal Discomfort, Diarrhea



Dosage Adjustment Algorithm #2: NAC/PL Dose Modifications For Dermatologic Reactions



4.6. Contraindications, Precautions, and Side Effects of Study Medications

4.6.1. Contraindication

Contraindication to NAC is known hypersensitivity to it.

4.6.2. Precautions

Concomitant administration of oral NAC and antibiotics has shown a slightly reduced absorption of cephalexin and a slight increase in erythromycin serum levels. NAC contains free sulfhydryl groups. There is no evidence that individuals sensitive to sulfa drugs are sensitive to NAC.

The NAC preparation being administered in this study contains 20 mg of aspartame. Because of the phenylalanine component of aspartame, individuals with phenylketonuria should avoid or restrict aspartame intake to avoid increased blood levels of phenylalanine. Because of this risk, labeling is required on all products containing aspartame.

4.6.3. Side Effects

Side effects of NAC range from common to serious. See Table 4.

Table 4: Side Effects of NAC

System-organ class	Undesirable Effects Uncommon (≥1/1,000; <1/100)	Rare (≥1/10,000; <1/1,000)	Very rare (<1/10,000)	Not known
Immune system disorders	Hypersensitivity		Anaphylactic shock / reaction	
Nervous system disorders	Headache			
Ear and labyrinth disorders	Tinnitus			
Cardiac disorders	Tachycardia			
Vascular disorders			Hemorrhage	
Respiratory, thoracic, and mediastinal disorders		Bronchospasm, Dyspnea		
Gastrointestinal disorders*	Vomiting, diarrhea, stomatitis, abdominal pain, nausea	Dyspepsia		
Skin and subcutaneous tissue disorders	Urticaria, rash, angioedema, itching			
General disorders and administration site conditions	Pyrexia			
Investigations	Reduced arterial pressure			Face edema

*In very rare cases the onset of severe skin reactions, such as Stevens-Johnson syndrome and Lyell syndrome, was reported to have a temporal relationship with N-acetylcysteine administration. Although in most cases at least another suspect drug probably most involved in the genesis of the above mentioned mucocutaneous syndromes has been identified, in case of mucocutaneous alterations it is appropriate to contact one's doctor, and the administration of N-acetylcysteine should be immediately discontinued.

Some studies confirmed a reduction of platelet aggregation during N-acetylcysteine administration. The clinical significance of these findings has not been defined yet. [Source: Fluimucil Investigator Brochure]

4.7. Recruitment Procedures

Subjects recruited for this study will be established patients of the investigators or physician- or self-referred to participating clinical centers in the IPFnet. Each clinical center within IPFnet has a well-developed infrastructure of local pulmonologists within the surrounding geographic area. These pulmonologists are kept informed of ongoing IPF clinical trials and regularly refer subjects to studies conducted at IPFnet clinical centers.

Additional steps will be taken to inform clinicians of the trials in progress within IPFnet, including: presentations at faculty staff meetings at local hospitals, medical grand rounds, and national conferences; direct mail notification; monthly faxes; and advertisement of network trials in pulmonary journals. Clinical center patients previously diagnosed with IPF will be notified of the trials by mail whenever possible.

Recruitment of minorities and women will be monitored by the DCC and DSMB. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate subject sample contains appropriate representation of women and minorities.

4.8. Study Procedures

The following procedures are detailed in the PANTHER-IPF MOOP accompanying this protocol:

1. PFT
2. ABG
3. HRCT scan of the chest (including imaging of pulmonary arteries)
4. CBC and serum chemistries
5. Pregnancy test
6. 6MWT/Borg Dyspnea Scale
7. QOL questionnaires (EuroQol, HAD, SF-36, SGRQ, and ICE CAP)
8. UCSD SOBQ
9. Gender Substudy Questionnaire

All assessments of PFTs will be conducted by study personnel not directly involved in the treatment of the subjects.

4.8.1. Biological Specimen Management

4.8.1.1. Biological Specimen Sample Management

Subjects at clinical centers participating in the specimen repository substudy who consent to having blood drawn for research purposes and for the banking of blood, blood components, and other biologic specimens (urine and BAL fluid) will have approximately 40.5 mL of blood drawn, 17 mL blood drawn for DNA, and 20 mL of urine collected at enrollment visit. Subjects will have approximately 50 mL of blood drawn and 20 mL of urine collected at each 15-week follow-up visit. Blood specimens will be separated according to PANTHER-IPF MOOP guidelines into the following components for banking in the repository: serum, plasma, and DNA. Coding of all biologic specimens for the repository will be performed by study staff at the clinical center. The samples will be processed per PANTHER-IPF MOOP guidelines, aliquoted, labeled with barcode labels, and stored at -70°C at the clinical center, and shipped to the central repository.

The only subject identifiers will be a sample ID number and subject initials. This sample ID will be linked in the IPFnet DCC clinical database to subject information. No subject information will be transferred to the biological-specimen database.

The subject's samples may be used for approved sub-studies relating to human disease, including, but not limited to IPF. The studies for which an individual's samples will be made available will be determined by the subject's answers to questions on the biological-sample informed consent form. The subjects can choose to make their samples available for all options or any combination. Samples will be made available to researchers only with IPFnet Steering Group approval until such time as the samples are made public through the NHLBI repository.

4.9. Concomitant Medications

Concurrent treatment with FDA-approved therapy for IPF is allowed. Colchicine may be used for treatment of gout. Temporary treatment with oral or IV corticosteroids for clinical worsening or suspected AEx is permitted. Nutritional supplements containing NAC are not allowed.

4.10. Laboratory Testing

Clinical laboratory parameters will be assessed at screening and at the end of the study. The following tests will be performed at the two time points specified in the protocol: chemistry (A/G ratio, ALT [SGPT], AST [SGOT], albumin, alkaline phosphatase, amylase, bilirubin-direct, bilirubin-indirect, bilirubin-total, BUN, BUN/creatinine ratio, calcium, carbon dioxide, cholesterol-total, chloride, CPK-total, creatinine, GGT, globulin, glucose, iron-total, LDH, lipase, magnesium, phosphorus-inorganic, potassium, protein-total, sodium, TIBC, triglycerides, uric acid) and hematology (red blood cell count, WBC count, hemoglobin, hematocrit, cell indices, differential, platelet count).

4.11. Blinding of Study Drugs

Subjects and caregivers will be blinded to study treatment. Every subject will receive NAC or matching PLs at every study visit from the baseline visit to the week-45 visit. No study agent will be dispensed at the week-60 visit.

5. Study Endpoints

5.1. Primary Study Endpoint

The primary endpoint will be the change in serial measurements of FVC over the study period.

5.2. Secondary Study Endpoints

5.2.1. Time to Disease-progression

The time-to-death or a 10% decline in FVC will be defined as the time-to-disease progression. The 10% decline in FVC from enrollment must be confirmed on 2 consecutive visits no less than 6 weeks apart. For subjects with 2 consecutive visits with a 10% decline in FVC, the time-to-disease progression will be defined as the time interval between enrollment and the initial visit with a 10% FVC decline. For subjects who experience disease progression, the study doctor will determine whether or not the subject will remain in the study.

5.2.2. Acute Exacerbations

The following 3 criteria will define AEx in subjects with acute worsening of their respiratory conditions:

1. Clinical (all of the following required):
   - A) Unexplained worsening of dyspnea or cough within 30 days, triggering unscheduled medical care (e.g., emergency room, clinic, study visit, hospitalization).
   - B) No clinical suspicion or overt evidence of cardiac event, pulmonary embolism, or deep venous thrombosis to explain acute worsening of dyspnea.
   - C) No pneumothorax.

2. Radiologic/Physiologic (A and B required):
   A) New ground glass opacity or consolidation computed tomography (CT) scan, OR new alveolar opacities on chest x-ray.
   B) Decline of ≥ 5% in resting room air SpO_2 from last recorded level OR decline of ≥ 8 mm Hg in resting room air PaO_2 from last recorded level.

3. Microbiologic (all of the following required):
   A) No clinical evidence for infection (i.e., absence of grossly purulent sputum, fever > 39 °C orally).
   B) Lack of positive microbiological results (if done) from lower respiratory tract defined as:
      (1) Clinically significant bacterial growth on sputum or endotracheal aspirate cultures;
      (2) Quantitative culture by protected brush specimen ≥ 10^3 cfu/mL or BAL ≥ 10^4 cfu/mL;
      (3) The presence of specific pathogens on stains of any of the above.
   C) Lack of positive pathogen in blood cultures (if done).

Identification of Acute Exacerbations

All subjects will be educated about the importance of identifying AExs. At the time of enrollment, subjects will be told about the possibility of developing acute symptomatic worsening that might represent an AEx of IPF, and instructed to contact their study clinical center coordinator within 48 to 72 hours of the apparent event.

All subjects will be contacted by phone monthly, and questioned about any change in dyspnea or cough and any interim clinic visits or hospitalizations. Finally, as part of the IPFnet outreach to community referring physicians, the importance of AExs will be emphasized. When a subject is identified who meets criterion 1A, this will trigger the collection of additional clinical data to evaluate a suspected AEx. These data will be collected as part of standard clinical care (i.e., this protocol does not require collection of all items). The additional items to be collected for suspected AEx include:

- IPFnet AEx case report form (eCRF) (required)

- Chest x-ray, CT scan with/without pulmonary angiogram (reports should be faxed and followed by the hard copies/discs)
- Oxygen saturation (pulse oximetry)
- ABG
- Respiratory cultures (sputum, endotracheal aspirate, lavage)
- Blood cultures
- Clinic/hospital records related to the event

All potential cases of AEx will be reviewed by the clinical center PI first, and a decision on whether the case may represent an AEx will be made. If AEx is suspected, the case will be sent to the IPFnet adjudication committee, which will assign a final diagnosis (see Table 5). If there is disagreement among members, the majority opinion will be recorded.

During episodes of suspected AEx, as determined by the individual clinical center investigator, subjects will remain blinded and in the study.

Table 5: Final Diagnoses in Evaluation of Suspected Acute Exacerbations

Definite acute exacerbation	All criteria met; no alternative etiology
Unclassifiable acute worsening	Insufficient data to evaluate all criteria; no alternative etiology
Not acute exacerbation	Alternative etiology identified that explains acute worsening

Management of the suspected AEx will be at the discretion of the treating physician. Standard of care generally involves evaluation for respiratory infection, pulmonary embolism, cardiac events and pneumothorax, and treatment with IV corticosteroids.

Study drugs will be resumed at pre-suspected AEx doses after subjects clinically improve as confirmed by the local PI. All subjects should be seen at the clinical center within 2 to 4 weeks of recovery for measurement of post-bronchodilator FVC (see Figure 4). Subjects unable to return to the clinical center after suspected AEx due to medical frailty (e.g., continued institutionalization, progressive disability) will be categorized as failing to maintain FVC response in secondary analyses.

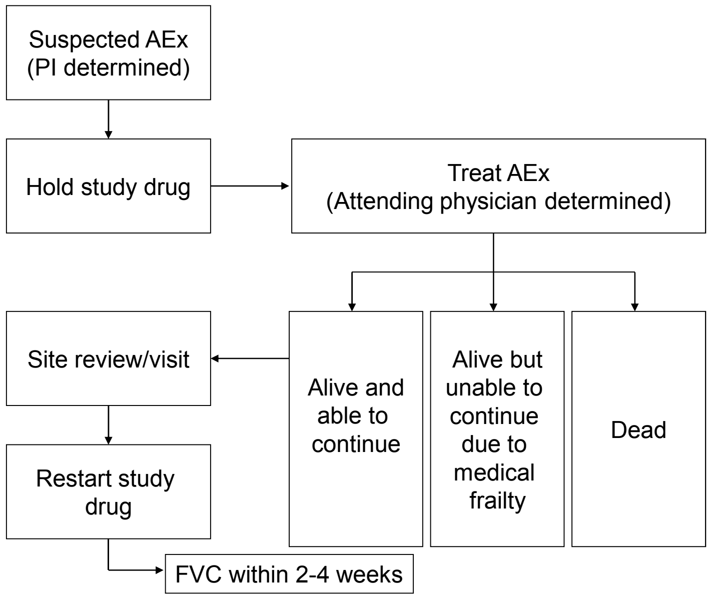


Figure 4. Acute Exacerbation Flow Chart

5.2.3. Respiratory Infections

An upper respiratory infection will be defined as:

- Change in sputum discoloration
- Increased cough of no more than 14 days' duration

A lower-respiratory infection (pneumonia) will be defined as new segmental or lobar airspace opacities visualized by image studies (chest radiograph or HRCT) in addition to any of the following:

- Positive pathogen/cultures in good sample of sputum or lower-airway secretions retrieved by fiberoptic bronchoscope
- Fever > 39°C or > 100°F
- Leukocytosis > 12,000 (unexplained; no increase in dose of corticosteroids)

5.2.4. Maintained FVC Response

Subjects with follow-up FVC%pred measurements at or above their baseline FVC%pred level will be classified as having maintained FVC response. Subjects with reduced FVC%pred levels or missing data for any reason, including death or medical frailty, will be classified as having not maintained FVC response. The FVC%pred value is used because unadjusted FVC measurements are expected to decline with age.

6. Safety Evaluations and Procedures

6.1. Adverse Events

During a clinical trial, the reporting of adverse experience information can lead to important changes in the way a new treatment is developed, as well as provide integral safety data.

6.1.1. Definitions

An **adverse event (AE)** is any untoward medical occurrence in a subject or clinical investigation subject who was administered a pharmaceutical product. The AE does not necessarily have to have a causal relationship with the drug administered. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered to be related to the medicinal product. Diseases, signs, symptoms, or laboratory abnormalities already existing at enrollment are not considered AEs unless they worsen (ie, increase in intensity or frequency). Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Surgical procedures planned before randomization and the conditions necessitating the surgery are not AEs.

A **serious adverse event** is any untoward event that:

- Is fatal
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization, with the following exceptions:
  - Preplanned (before the study) hospital admissions, unless the hospitalization is prolonged
  - Planned admissions (as part of a study, eg, routine biopsies)
  - Hospitalization lasting < 24 hours

  - Hospitalization for elective procedure
  - Emergency room visits
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Important medical events that may not result in death, be life-threatening, or require inpatient hospitalization may be considered serious adverse events (SAEs) when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening means that the subject was, in the view of the investigator, at immediate risk of death from the AE as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.

Persistent or significant disability/incapacity means that the event resulted in permanent or significant and substantial disruption of the subject's ability to carry out normal life functions.

Causality:
A reasonable possibility means the AE may have been caused by/related to the study drug. A perceived or real lack of efficacy does not satisfy the definition of relatedness.

6.1.2. Adverse Event (AE) Reporting

For the PANTHER-IPF trial, all AEs (serious and nonserious), occurring from randomization through final study visit (4 weeks after final dose of all study medication) will be recorded on the AE page of the case report form (CRF)

6.1.2.1. Serious Adverse Events (SAE) Reporting

For this trial, all deaths and all SAEs, which occur from randomization through final study visit, must be entered within the EDC system, via the SAE eCRF page within 24 hours of the investigative site's knowledge of the event. It is the responsibility of the clinical center investigator to provide a causality assessment of the event for each study medications based upon the information available at the time of the report. It is understood that complete information about the event may not be known at the time the initial report is submitted. In the event the EDC system is not accessible to the site at the time of event reporting, investigative sites will complete and forward a paper back-up SAE form to DCRI Safety Surveillance for processing:

DCRI Safety Surveillance

Telephone: 1-919-668-8624 or 1-866-668-7799 (toll free)

Fax: 1-919-668-7138 or 1-866-668-7138 (toll free)

The investigator must complete and submit follow-up SAE information via the eCRF when important new or follow-up information (e.g., final diagnosis, outcome, results of specific investigations) becomes available. Follow-up information should be submitted according to the same process used for reporting the initial event as described above. All SAEs will be followed until resolution, stabilization, or 30 days after the subject has completed the final visit (4 weeks after the final dose of study medication), whichever occurs first. The investigator is responsible for reporting SAEs to their institutional review board (IRB) per site specific IRB reporting guidelines.

6.1.2.2. Regulatory Reporting

AEs that are serious, study drug related, and unexpected will be reported to the regulatory authorities. The DCRI Safety Surveillance medical monitor will perform a medical review of all SAEs submitted and evaluate for “unexpectedness.” DCRI Safety Surveillance will prepare MedWatch reports for those events identified as serious, study drug related and unexpected as determined by Safety Medical Monitor.

DCRI Regulatory Services will submit all unexpected, study drug-related SAEs as per 21 CFR 32. DCRI Safety Surveillance will provide a safety alert letter to the NHLBI, DSMB, and DCC clinical operation (for distribution to sites) within 15 days of initial receipt of the information.

Investigators are responsible for promptly reporting these events to their reviewing IRBs according to site specific IRB reporting guidelines.

6.2. Clinical Medical Monitoring

There will be an unblinded physician at the IPFnet DCC serving as medical monitor. The medical monitor will be available to assist with questions about dosage adjustments of study medications, including discontinuation or resumption of medications.

6.3. Unblinding Procedures

Unblinding of subjects or investigators to subject treatment is strongly discouraged. For ongoing clinical management, all subjects should be presumed to be receiving “active” study drug. To ensure the subject’s safety, the study treatment will be dose-adjusted based on laboratory test results, clinical findings, and symptoms.

The IPFnet DCC medical monitor and PANTHER-IPF co-chairs, Drs. Ganesh Raghu and Fernando Martinez, will be available to the study physicians to discuss study drug management on a case-by-case basis. Un-blinding will be considered ONLY when the knowledge of subject treatment assignment is ABSOLUTELY ESSENTIAL for subject safety and after discussion of the subject’s case with the medical monitor and either Dr. Raghu or Dr. Martinez. Unblinding of study treatment should be minimized during the conduct of the trial. In those cases where it is felt to be medically necessary the DCC medical officer will communicate directly with the managing physician to minimize unblinding of study personnel.

7. Study Drug Procedures

At the baseline, 15-week, 30-week, and 45-week study visits, subjects will receive a supply of study drug sufficient to last until the next visit at which study drug will be dispensed.

8. Data Management

8.1. Hardware and Software Configuration

8.1.1. Hardware and Database Software

Data will be stored in an Oracle database system. Oracle has advantages of processing efficiency and smooth linkage with other software systems. The application and database will be hosted on Solaris Unix servers at the IPFnet DCC.

8.1.2. Statistical Software

SAS will be used as the principal application for the management of analysis data files and statistical computations. S-Plus will be used to provide supplementary functions as needed.

8.1.3. Access Control and Confidentiality Procedures

Access to databases will be controlled centrally by the IPFnet DCC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage.

8.1.4. Security

Database and Web servers will be secured by a firewall and through controlled physical access. Oracle has many security features to ensure that any staff member accessing the database has the proper authority to perform the functions he or she requests of the system. Within the secondary SAS databases, Unix group-access control maintains similar security. The Sun workstation login is secured by extensive user-password facilities under Unix.

8.1.5. Back-up Procedures

Database back-up will be performed automatically every day, and standard IPFnet DCC policies and procedures will be applied to dictate tape rotation and retention practices.

8.1.6. Virus Protection

All disk drives that provide network services, and all user computers, will be protected using virus-scanning software. Standard IPFnet DCC policies will be applied to update these protection systems periodically through the study.

8.2. Sources of Data

8.2.1. Design and Development

The IPFnet DCC will be responsible for development of the electronic case report forms (eCRFs), development and validation of the clinical study database, ensuring data integrity, and training clinical center staff on applicable data management procedures. A web-based distributed data entry model will be implemented. This system will be developed to ensure that guidelines and regulations surrounding the use of computerized systems used in clinical trials are upheld. The remainder of this section provides an overview of the data management plan associated with this protocol.

8.2.2. Data Collection Forms

The data collection process consists of direct data entry at the study clinical centers into the EDC system(s) provided by the DCC. A data collection worksheet will be provided to clinical centers for recording data in the event the EDC system is unavailable. Data entry of the eCRFs should be completed according to the instructions provided and project specific training. The investigator is responsible for maintaining accurate, complete and up-to-date records, and for ensuring the completion of the eCRFs for each research participant.

8.2.3. Data Acquisition and Entry

Data entry into eCRFs shall be performed by authorized individuals. Selected eCRFs may also require the investigator's written signature or electronic signature, as appropriate. Electronic CRFs will be monitored for completeness, accuracy, and attention to detail during the study.

8.2.4. Data Center Responsibilities

The IPFnet DCC will 1) develop a data management plan and will conduct data management activities, 2) provide final eCRFs for the collection of all data required by the study, 3) develop data dictionaries for each eCRF that will comprehensively define each data element, 4) conduct ongoing data monitoring activities on study data, 5) monitor any preliminary analysis data cleanup activities, and 6) rigorously monitor final study data clean up.

8.2.5. Data Editing

Completed data will be entered into the IPFnet DCC automated data acquisition and management system. If incomplete or inaccurate data are found, a data clarification request will be generated and distributed to clinical centers for a response. Clinical centers will resolve data inconsistencies and errors and enter all corrections and changes into the IPFnet DCC automated data acquisition and management system.

8.2.6. Training

The training plan for clinical center staff includes provisions for training on assessments, eCRF completion guidelines, data management procedures, and the use of computerized systems.

9. Study Design and Data Analysis

9.1. General Analytic Considerations

All primary analyses will be based on intent-to-treat (ITT) principles using all randomized subjects. Baseline factors across groups will be compared using mean (standard deviation) and median (25th and 75th percentiles) summary measures. Kaplan-Meier curves will be used to display event rates. Due to clinical interest in departures from both sides of the null hypothesis, all test statistics will be 2-sided.

Reasonable caution needs to be taken when conducting multiple analyses on key clinical subgroups. For subgroup analyses, a conservative significance level of 0.001 will be used for all interaction tests. Thus, subgroup comparisons will be considered exploratory unless the p-value from the interaction test is smaller than 0.001.

9.2. Randomization, Blinding, and Reporting of Results

A permuted block-randomization scheme will be created with varying block sizes stratified by clinical center. Once a subject has completed the screening and baseline period and evaluation for inclusion/exclusion criteria, the randomization process will begin. Subjects will be randomized to receive NAC or matching placebo with equal probability (1:1), via telephone contact with a central interactive voice response system (IVRS), using a toll-free randomization number. On the day of randomization, after the subject has successfully met all inclusion and exclusion criteria, the investigator or designee will call the central randomization number to obtain the assigned kit randomization numbers for that subject. At each subject visit, the investigator or designee will call the central randomization number to obtain the new kit randomization numbers for resupply of the subject. For resupply of the clinical center, the IVRS will monitor minimal volume of a kit type and/or expiration date and will automatically notify the pharmacy.

The trial results will be reported according to guidelines specified in the CONSORT statement. A flow diagram describing screening, recruitment, randomization, dropout, and vital status will be included in the primary manuscript. AEs and efficacy data will be presented for all treatment groups. Adherence, dropout, and lost to follow-up will be carefully examined across all treatment groups. Analyses of safety will be based on data from all randomized subjects who received at least one dose of study drug.

9.3. Stratification

Subjects will be distributed to the two treatment arms in a 1:1 allocation ratio. Stratification blocks will be based on clinical centers.

9.4. Specification of the Primary Analyses

A mixed model repeated measures (MMRM) analysis, described in section 9.5, will be used to compare differences in the slope of FVC measurements across the treatment groups. Response variables are values of the FVC measured at baseline and every 15 weeks until study completion. Variables in the model will include: treatment, time, and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment over time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing FVC data will not be imputed for the primary analysis.

9.5. Analysis of Longitudinal Endpoints

A common goal in clinical trials is to specify models that are easily implemented and reproducible by independent data analysts. On the other hand, the models should have proper statistical behavior in terms of low bias and high precision. Many common approaches to longitudinal data analysis including last observation carried forward (LOCF) imputation rely on the missing completely at random (MCAR) assumption. However, the MCAR assumption is unlikely to hold in many clinical trials because missing data are often related to disease progression and prognosis. A more reasonable assumption, missing at random (MAR), specifies that the complete data distribution can be modeled using only the observed data. The likelihood-based MMRM approach is valid under the more general MAR assumptions. These models will be applied to analyze the longitudinal data secondary endpoints.

The advantages of MMRM analysis are that all important characteristics of the model can be pre-specified, standard software can be used to implement the models, and results are based on ITT principles.[43] In addition, the MMRM approach offers superior control of Type I and Type II errors compared with the LOCF approach.

Response variables are values of the PFTs measured at enrollment and every 15 weeks until study completion and 6MWT values measured at baseline, week 30, and week 60. Covariates are treatment, time, time by treatment, and key baseline risk factors. Contrasts (along with confidence intervals) of treatment by time will be used to estimate the treatment effect.

The correlation structure involves multiple pieces, including measurement errors, random variation, and interindividual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. A careful examination of reasons for study discontinuation will be conducted to assess the validity of MCAR. Sensitivity analyses will be used to examine the untestable assumption that the observed data violate the MAR assumption. The MMRM models will be implemented using PROC MIXED in SAS.

9.6. Analysis of Binary, Time-to-Event, and Time-Lagged Endpoints

Regression modeling approaches using either the logistic regression model or Cox proportional hazards regression model will be employed when appropriate. The validity of these models will be assessed via standard modeling diagnostics and goodness-of-fit measures. Estimates of cumulative frequencies for more general time-lagged responses will be calculated using the partitioned version of the Bang-Tsiatis estimator.[44] The partitions will be set at 15-week intervals to correspond with the data-collection process. Covariate adjusted event rates will be calculated using inverse probability-weighted regression estimates.[45]

9.7. Power Analysis

9.7.1. Primary Analyses

Based on previously published IPF clinical trials, the PL group is expected to experience a drop in FVC of approximately 0.20 L over the study period (see Figure 1). The IPFnet Steering Group determined a clinically important difference would be to preserve the majority of the decline relative to PL over the study period. In particular, a treatment effect of 0.15 L was determined to be a clinically meaningful difference. Potential dropout is a key factor in the proposed study. The drop-out process assumed 5% lost to follow-up after every study visit. Only 80% of subjects were assumed to be followed for the entire study period. All models assumed a compound symmetry structure for the covariance matrix. Power calculations were performed using a SAS IML program for designing repeated measures studies.[46] Based on preliminary reviews of the data from the University of Michigan, the covariance matrix parameters were estimated at approximately $\sigma^2 = 0.757$ (variance parameter) and $\rho = 0.936$ (correlation parameter). To be conservative, the power calculations for the primary analysis were performed with parameter setting of $\sigma^2 = 0.810$ (variance parameter) and $\rho = 0.925$ (correlation parameter).

The power calculations assume a correction for imperfect compliance proposed by Lachin and Foulkes to allow for 2% noncompliance for each of the treatment arms.[47] Thus, the sample size of 130 subjects per arm would be reduced to an adjusted sample size of $130*(1-0.02-0.02)^2 = 119.8$ or 120 subjects per arm.

Under the assumed Type I error rate of 0.05, with a correlation parameter of 0.925 and standard deviation of 0.90, the difference of 0.15 L (or 0.0025 L/week) shown in Table 6 would have power of 93%.

Table 6: Hypothetical Values of Mean FVC (L) Change from Baseline

	Week 15	Week 30	Week 45	Week 60
NAC	0.0125	0.0250	0.0375	0.0500
Placebo	0.0500	0.1000	0.1500	0.2000
Difference	0.0375	0.0750	0.1125	0.1500

Abbreviations: FVC, forced vital capacity; PL, placebo

9.7.2. Power Analysis for Secondary Endpoints

Power calculations for secondary endpoint measurements are shown in Table 7. Standard deviations are based on unpublished data provided by the University of Michigan. The calculations are based on a 2-sample t-test with Type I error rate set at 0.05. These calculations are likely to be conservative because the statistical approach, described in section 9.5, for analyzing these endpoints will incorporate incomplete observations as well as intermediate data points.

Table 7: Detectable Differences in Treatment Means for Selected Endpoint Measurements

Secondary Endpoints	Std Dev of the Baseline Score	Detectable Difference for 80% Power	Std Dev of the Change Score	Difference Detectable for 80% Power
DLCO%pred	16.6	5.8	9.1	3.2
6MWT Area Under the Desaturation Curve	21.9	7.6	17.5	6.1
6MWT Distance to Desaturation	22.4	7.8	31.5	11.0
6MWT Minutes Walked	2.10	0.73	2.05	0.71

Abbreviations: Std Dev, standard deviation; DLCO%pred, diffusing capacity of the lung for carbon monoxide percent predicted; 6MWT, 6-minute walk test

10. Study Administration

10.1. Cooperative Agreement Mechanism

The administrative and funding mechanism used to undertake this project is a "cooperative agreement" (U01), which is an assistance mechanism. Under the cooperative agreement, the NHLBI assists, supports, and/or stimulates the project and is substantially involved with investigators in conducting the study by facilitating performance of the effort in a "partner" role. The NHLBI project scientist serves on the IPFnet Steering Group, and he or another NHLBI scientist may serve on other project committees when appropriate. At the same time, however, NHLBI does not assume a dominant role, direction, or prime responsibility for this research program.

As described below, governance of the project is conducted through the IPFnet Steering Group. Principal investigators have lead responsibilities in all aspects of their trials and the project, including any modification of trial designs, conduct of the trials, quality control, data analysis and interpretation, preparation of publications, and collaboration with other investigators, unless otherwise provided for by the IPFnet Steering Group.

PIs retain custody of and have primary rights to their center-specific and collaborative data, subject to government rights-of-access consistent with current Health & Human Services (HHS), Public Health Service (PHS), and National Institutes of Health policies. The protocols and governance policies call for the continual submission of data centrally to the IPFnet DCC for the collaborative database, which at a minimum will contain the key variables selected by the IPFnet Steering Group for standardization across all clinical centers; the submission of copies of the collaborative datasets to each PI upon completion of the project; procedures for data analysis, reporting and publication; and procedures to protect and ensure the privacy of medical and genetic data and records of individuals. The NHLBI project scientist, on behalf of the NHLBI, will have the same access, privileges, and responsibilities regarding the collaborative data as the other members of the Steering Group.

PIs are also encouraged to publish and to publicly release and disseminate results, data, and other products of the project, concordant with the project protocols and governance and the approved plan for making data and materials available to the scientific community and to the NHLBI. However, during the 3 years after the ending date of NHLBI project support, unpublished data, unpublished results, data sets not previously released, and other study materials or products are to be made available to any third party only with the approval of the IPFnet Steering Group.

Upon completion of the project, PIs are expected to put their intervention materials and procedure manuals into the public domain and/or make them available to other investigators according to the approved plan for making data and materials available to the scientific community and the NHLBI for the conduct of research, at no charge other than the costs of reproduction and distribution.

The NHLBI reserves the right to terminate or curtail the project (or an individual award) in the event of (a) failure to develop or implement mutually agreeable collaborative measurement, subject eligibility, and data management sections of the protocols; (b) substantial shortfall in subject recruitment, follow-up, data reporting, or quality control or other major breach of protocol; (c) substantive changes in the agreed-upon protocols with which NHLBI cannot concur; (d) reaching a major project outcome, with persuasive statistical significance, substantially before schedule; or (e) human subject ethical issues that may dictate a premature termination.

Any disagreement that may arise in scientific/programmatic matters (within the scope of the award) between award recipients and the NHLBI may be brought to arbitration. An arbitration panel will be composed of 3 members—1 selected by the IPFnet Steering Group (with the NHLBI member not voting) or by the individual PI in the event of an individual disagreement, a second selected by NHLBI, and the third selected by the other 2 members. This special arbitration procedure in no way affects the PI's right to appeal an adverse action that is otherwise appealable in accordance with the PHS regulations at 42 CFR part 50, Subpart D and HHS regulation at 45 CFR part 16 or the rights of the NHLBI under applicable statutes, regulations, and terms of the award.

10.2. IPFnet Steering Group

The IPFnet Steering Group is the main governing body of the project. It is composed of the PIs of the clinical centers, the PI of the DCC, and the NHLBI project scientist. The clinical centers, the IPFnet DCC, and the NHLBI each have 1 vote on the IPFnet Steering Group. All decisions are determined by majority vote.

All major scientific decisions are determined by the IPFnet Steering Group. It assumes overall responsibility for the design and conduct of the trial. It appoints (and disbands) committees and subcommittees as the need arises; designs, approves, and implements the study protocols; oversees the development of the MOOP; monitors subject recruitment and treatment delivery; evaluates data collection and management; oversees quality assurance procedures; and implements changes and enhancements to the study as required. It also has primary responsibility for facilitating the conduct of the trials and reporting the project's results.

10.3. Data and Safety Monitoring Board

The NHLBI will establish a DSMB in accordance with established policies (see http://www.nhlbi.nih.gov/funding/policies/dsmb_inst.htm) to ensure data quality and subject safety and to provide independent advice to the NHLBI regarding progress and the appropriateness of study continuation.

10.4. Recommendations on Interim Monitoring of Efficacy, Safety, and Futility

First and foremost the role of the DSMB will be to review subject safety and trial conduct at periodic points during the study. The DSMB may require analyses of the primary endpoint results for comparing the benefit and risks of treatment strategies. The benefit of collecting additional data on key secondary endpoints, with extended follow-up, and establishing a robust evidence base for determining a standard of care will need to be carefully considered before early termination of one or more treatment arms. After careful consideration, the IPFnet Steering Group recommends conservative thresholds for the early examinations of the safety and efficacy data.

The DSMB will be expected to meet approximately every 6 months until trial completion to review safety and toxicity data. The DSMB may recommend stopping the study based on these reviews. Because the DSMB could stop the trial for safety concerns as well as for a large efficacy benefit, there could be multiple opportunities to reject the null hypothesis (no difference in event rates between the PL and NAC groups). A Bonferroni approximation will be applied during the one planned interim analysis for efficacy.

Before locking the database, a statistical analysis plan (SAP) will be developed to provide complete details on the statistical analysis. The SAP will include the specifics for how and when the DSMB will be notified for AEs. The IPFnet DCC will deliver to the DSMB all FDA-defined AEs at 3-month intervals. The IPFnet DCC will prepare narrative SAE reports in real time for DSMB review including recommendations and analysis of similar events for each SAE submitted to the FDA.

11. Investigator and Sponsor Obligations

11.1. Monitoring

All monitoring activities for U.S. clinical centers will be performed in accordance with DCRI standard operating procedures. Information regarding the types of visits will be outlined in the PANTHER-IPF MOOP.

11.2. Cost and Payment

There will be no cost to subjects enrolled in this trial. Study-related procedures will be paid for by the IPFnet.

Subjects may be eligible for reimbursement for travel to the clinical center. Details of payment will be explained to each subject during the consent process.

11.3. Confidentiality and Health Insurance Portability and Accountability Act Considerations

Subject confidentiality will be protected throughout the study. All subject data will be kept strictly confidential, and no subject-identifying information will be released to anyone outside the project. Confidentiality will be assured through several mechanisms. First, each subject will be assigned an anonymous study ID, which will then be used on all study forms. Second, any study forms, blood samples, and paper records that contain subject information (eg, address lists, phone lists) will be kept at the clinical centers in secured, locked areas, coded by number. Once blood is collected, there will be no subject identifiers placed on blood samples—only the study ID number and the date of sample collection. Third, access to all subject data and information, including laboratory specimens, will be restricted to authorized personnel. In the case of computerized data, this restricted access will be assured through user logon IDs and password protection.

At the IPFnet DCC, only authorized personnel will have access to the data files containing study data. Security will be assured through user logon IDs, passwords, and appropriate access privileges. All study subjects will be identified only by their IPFnet ID numbers, and no personal identifying information, such as name, address, or Social Security number, will be entered into the IPFnet DCC database. Any subject-specific data reported to the IPFnet Steering Group will be identified only by the IPFnet ID number.

Finally, subjects will not be identified by name in any reports or publications, nor will the data be presented in such a way that the identity of individual subjects can be inferred. Analysis files created for further study by the scientific community will have no subject identifiers. These data files will be created in accordance with the Ancillary Studies and Publication Policy of the IPFnet.

11.4. Informed Consent Procedures

All IPFnet subjects will provide written informed consent using procedures reviewed and approved by each clinical center's IRB. Informed consent will be undertaken by study personnel in-person with the subject. The subject has the option of declining further participation in the study at that point. No further study procedures will be conducted until the signed documents have been provided to the IPFnet clinical center.

11.5. Institutional Review Boards

Before initiating this study, the protocol, clinical center-specific informed consent forms, Health Insurance Portability and Accountability Act (HIPAA) forms, recruitment materials, and other relevant information will be reviewed by a properly constituted IRB at each participating clinical center. A copy of the signed and dated IRB approval at each clinical center will be retrieved prior to or during the site initiation visit and archived at the IPFnet DCC. Any amendments to the protocol, other than simple administrative and typographical changes, must be approved by each IRB before they are implemented. The clinical centers will seek annual renewals of their IRB approvals in accordance with local procedures.

12. Investigator Agreement

I have read the foregoing protocol, PANTHER-IPF, and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the study.

I will fulfill all responsibilities for submitting pertinent information to the local IRB, if applicable, that is responsible for this study.

I further agree that NHLBI and/or DCRI will have access to any source documents from which eCRF information may have been generated.

____ ________________

Signature of Principal Investigator Date

____

Name of Principal Investigator (printed or typed)

Protocol version date: May 19, 2009
Protocol Amendment 1 version date: May 28, 2010
Protocol Amendment 2 version date: December 6, 2011

13. References

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IPFnet

PANTHER-IPF Protocol Table of Revisions Amendment 2

Revision	Applicable Section(s)
Added Amendment 2 date December 6, 2011	Title Page
Revised wording for PRODUCT Previously read: Prednisone, azathioprine, and N-acetylcysteine Now reads: N-acetylcysteine	Protocol Summary
Revised wording for STUDY OBJECTIVES Previously read: To assess the safety and efficacy of N-acetylcysteine and the combination of prednisone + azathioprine + N-acetylcysteine in subjects with newly diagnosed idiopathic pulmonary fibrosis Now reads: To assess the safety and efficacy of N-acetylcysteine in subjects with newly diagnosed idiopathic pulmonary fibrosis	Protocol Summary
Revised wording for TREATMENT REGIMENS Previously read: 1) prednisone (0.5–0.15 mg/kg/day) **+** azathioprine (1.0–2.0 mg/kg/day) **+** N-acetylcysteine (600 mg TID) **or** 2) N-acetylcysteine (600 mg TID) **or** 3) placebo Now reads: 1) N-acetylcysteine (600 mg TID), or 2) placebo	Protocol Summary
Revised wording for **TIME BETWEEN FIRST AND LAST DOSES OF ACTIVE STUDY AGENT** Previously read: Maximum of 67 weeks Now reads: Maximum of 60 weeks	Protocol Summary
Revised wording for **NUMBER OF SUBJECTS** Previously read: 390 (1:1:1) Now reads: Approximately 130 NAC, 130 placebo (1:1)	Protocol Summary

PANTHER-IPF Protocol Table of Revisions Amendment 2

Change Number of Clinical Centers Previously read: At least 22 US sites Now reads: 26 US sites	Protocol Summary
Revised wording for **INTERIM ANALYSIS** Previously read: One planned interim analysis of the primary endpoint. It is expected that this evaluation will occur at the study midpoint. Now reads: Completed October 2012	Protocol Summary
Change in Steering Group Chair Previously read: Galen B. Toews, MD University of Michigan Now reads: Marvin I. Schwarz, MD University of Colorado - Denver	Protocol Summary
Change in Project Officer Previously read: Herbert Y. Reynolds, MD Now reads: Gail Weinmann, MD	Protocol Summary
Revised the Table of Contents	Table of Contents
Revised the List of Tables	List of Tables
Revised the List of Figures	List of Figures

PANTHER-IPF Protocol Table of Revisions Amendment 2

Revised wording in 1st Paragraph Previously read: There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) will conduct a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy and in combination with azathioprine (AZA) and prednisone (PRED) in subjects with mild or moderate IPF. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] ≥ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DLCO%pred] ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Each subject will be treated up to a maximum of 60 weeks, followed by a tapering of PRED/PL and a 4-week period for safety evaluation. Now reads: There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) is conducting a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy in subjects with mild or moderate IPF.	1. Summary

PANTHER-IPF Protocol Table of Revisions Amendment 2

Revised wording in 2nd paragraph Previously read: The primary endpoint is the change in longitudinal measurements of FVC over the 60-week treatment period. The primary goal of this study is to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF. Now reads: The study initially employed a 3-arm design with 1:1:1 randomization to NAC, azathioprine (AZA)-prednisone (PRED)-NAC, and PL, with each subject to be treated up to a maximum of 60 weeks, followed by a tapering of PRED/PL and a 4-week period for safety evaluation. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] $\geq$ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DLCO%pred] $\geq$ 30%) diagnosed within the past 48 months were to be enrolled.	1. Summary
Added 3rd & 4th paragraph At the pre-specified interim analysis, the DSMB recommended termination of the prednisone-azathioprine-NAC arm of the study. No additional patients will be randomized to that arm. However, the NAC and placebo arms remain open for enrollment, and we will enroll approximately 130 subjects in each arm (inclusive of the subjects enrolled at the time of the interim analysis.) Follow up for subjects enrolled into the two arms will continue for 60 weeks. The primary endpoint is the change in longitudinal measurements of FVC over the study period. The primary goal of this study to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF has been met, in part, by demonstrating that the widely used triple therapy was harmful to patients with IPF (NHLBI press release, Oct 21, 2011). To determine the potential therapeutic benefits of NAC alone, the study will continue to enroll patients as a two-arm, double-blind, placebo-controlled study from this point on (NAC vs. placebo) as recommended by the DSMB following the pre-specified interim analysis.	1. Summary

Revised wording Previously read: • Treatment with AZA-PRED-NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC. • Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC. Now reads: Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.	2.1 Null Hypotheses
Revised wording Previously read: This study is designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF. Now reads: This study is designed to assess the safety and efficacy of NAC in subjects with newly diagnosed IPF.	2.2 Specific Aim 1

Revised 3rd paragraph Previously read: There is currently no proven, effective treatment for IPF. Anti-inflammatory and immunosuppressive agents have been the traditional approach to the management of patients with IPF. However, few controlled clinical trials have been performed to prove efficacy of this approach. In addition, multiple factors have severely limited the ability to draw conclusions from previous therapeutic trials: (a) the lack of a clear understanding of the natural history of IPF; (b) the presence of many different study designs; (c) heterogeneous subject groups; (d) disputable diagnostic certainty; (e) variable study duration; (f) differences in medication formulation, dosage, route of administration, and duration of treatment; (g) differing types and/or lack of quantitative assessment criteria; (h) variable intervals between evaluations; and most importantly, and (i) the lack of controls treated in a true PL arm. Consequently, no management approach has proven to be efficacious compared with a true PL arm, and treatment of IPF is largely based on anecdotes or small studies (Selman 2004; Thannickal 2004; Richeldi 2003; Davies 2003). Recently, a study comparing treatment of IPF subjects with AZA-PRED-NAC vs. AZA-PRED indicated a better preservation of FVC and DLCO in subjects receiving adjunct treatment with NAC (Demedts 2005); however, a true PL group was not included in this study. Thus, it remains unknown if a combination of AZA-PRED-NAC is superior to PL; it is also not known if NAC alone or in combination with AZA-PRED will prove beneficial in IPF patients. The primary goal of this study is to establish an evidence-based standard of care and clarify the role of immunosuppressive and antioxidant pharmacotherapy for IPF. Now reads: There is currently no proven, effective pharmacological treatment for IPF.[13] Anti-inflammatory and immunosuppressive agents have been the traditional approach to the management of patients with IPF. Based on the results of the interim analysis of the PANTHER-IPF trial, this 'traditional approach' will be aborted. . However, it remains unknown if NAC alone will prove beneficial in IPF patients. The primary goal of the modified study is to establish an evidence-based standard of care and clarify the role of this specific antioxidant pharmacotherapy for IPF.	3.1. Idiopathic Pulmonary Fibrosis is the Most Common Interstitial Lung Disease

Revised 1st paragraph Previously read: IPF is a disorder for which there is no proven efficacious therapy. A major objective of this trial is to test, to the greatest degree possible, a proposed standard of care for patients with IPF. The current traditional therapy employs immunosuppressive and corticosteroid drugs, which have significant known side effects but have never been proven to improve outcomes in well-designed, well-powered clinical trials. In this prospective, randomized clinical trial, the inclusion of a PL arm is vital to adequately test the benefits of NAC and AZA-PRED-NAC in well-characterized subjects with IPF Now reads: IPF is a disorder for which there is no proven efficacious therapy. A major objective of this trial is to test, to the greatest degree possible, a proposed standard of care for patients with IPF. The current traditional therapy employs immunosuppressive and corticosteroid drugs. Interim review of the original PANTHER-IPF study has documented increased adverse events and lack of efficacy for AZA-PRED-NAC compared to placebo suggesting that this therapeutic approach should not be employed. Whether this applies to NAC alone, which has been advocated by international societies, has not been proven in well-designed, well-powered clinical trials. The recommendations made in the recently published evidence guidelines for NAC monotherapy was weak based on low quality.[13] Thus, this continued clinical trial randomizing patients to receive NAC or placebo is pivotal and will answer the important question of the potential therapeutic benefits of NAC monotherapy with grade A evidence. In this prospective, randomized clinical trial, the inclusion of a PL arm is therefore vital to adequately test the benefits of NAC in well-characterized subjects with IPF.	3.2. Rationale for Placebo Control
Revised sentence one in 2nd paragraph Previously read: If AZA-PRED-NAC and NAC have no true efficacy, then their role as standard of care will be refuted. Now reads: If NAC has no true efficacy, then its role as standard of care will be refuted.	3.2. Rationale for Placebo Control

Revised sentence four and five 2nd paragraph Previously read: Similarly, recently completed trials of etanercept and bosentan in IPF have included PL-treated arms. In these trials, the treated subjects showed little, if any, objective improvement. Based on this evidence and the well-known potential for toxicity from immunosuppressive agents, we believe that clinicians and subjects should be willing to enroll in a PL-controlled study. The highly experienced investigators in the IPFnet have discussed this issue extensively and voted to include a PL arm in this trial. Now reads: Similarly, recently completed trials of etanercept, everolimus, bosentan and BIPF 1120 in IPF have included PL-treated arms.[16-19] In three of these trials, the treated subjects showed little, if any, objective improvement. Based on this evidence and the current status of IPF therapy and therapeutic trials, we believe that clinicians and subjects will continue to enroll in a PL-controlled study.	3.2. Rationale for Placebo Control
Deleted sentence six and seven from 2nd paragraph The highly experienced investigators in the IPFnet have discussed this issue extensively and voted to include a PL arm in this trial. We strongly believe that there is clinical equipoise in this trial design in that there is no compelling reason to favor the outcome of one treatment arm over another.	3.2. Rationale for Placebo Control
Deleted section 3.3	3.3. Rationale for Prednisone and Azathioprine Therapy
Revised the section title 3.5 Previously read: 3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy and in Combination with Azathioprine and Prednisone Now reads: 3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy	3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy and in Combination with Azathioprine and Prednisone
Revised section 3.5 to remove all mention of the AZA-PRED-NAC and AZA-PRED combinations	3.5. Rationale for N-acetylcysteine as a Stand-alone Therapy
Revised Figure 1	3.6. Rationale for the Study Design and Primary Endpoint
Revised reference in Figures 2 and 3	4.2. Diagnosis of IPF
Added sentence four in 4th paragraph In fact, this is in keeping with the recently published evidence based guidelines for diagnosis and management of IPF.[13]	4.2. Diagnosis of IPF

Deleted exclusion criteria # 20, 21, 22, and 23 Previously read: 20. Women of childbearing potential who are not using a medically approved means of contraception (ie, oral contraceptives, intrauterine devices, diaphragm, Norplant®). 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. 21. Any clinically relevant lab abnormalities (from central lab values obtained within 30 days before enrollment), including: a. Creatinine > 2 x upper limit of normal (ULN) b. Hematology outside of specified limits: iii. White blood cells (WBCs) < 3,500/mm^3 iv. Hematocrit < 25% or > 59% v. Platelets < 100,000/mm^3 22. Any of the following liver function test (LFT) criteria above specified limits: a. Total bilirubin > 2 x ULN b. Aspartate (AST) or alanine aminotransferases (ALT) (serum glutamic-oxaloacetic transaminase [SGOT], or serum glutamic pyruvic transaminase [SGPT]) > 1.5 x ULN c. Alkaline phosphatase > 3 x ULN d. Albumin < 3.0 mg/dL at screening e. Homozygous for low thiopurine S-methyl transferase (TPMT) 23. Overt or persistent clinical depression as perceived by site PI and / or the patient's primary physician and supported by uncontrolled depression (depression component of the Hospital Anxiety and Depression Score [HADS-D] ≥ 15)	4.3 Exclusion Criteria

Revised exclusion criteria # 28 Previously read: History of triple therapy of prednisone plus azathioprine plus NAC for > 12 weeks' duration in the past 4 years Now reads: History of triple therapy of prednisone plus azathioprine plus NAC for > 12 weeks' duration in the past 4 years or previous enrollment in the triple-therapy arm of the PANTHER-IPF study.	4.3 Exclusion Criteria

Revised section 4.4.1 Previously read: This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF. Approximately 390 subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLCO%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Once enrolled, subjects will visit the clinical center at 4 weeks, 15 weeks, and 15-week intervals thereafter. Between visits, subjects will visit local blood-draw centers or the clinical center for monitoring of blood counts and serum chemistries on a predefined schedule. Each subject will be treated and followed for a maximum of 60 weeks. During the 60-week visit, subjects will be taken off all study agents except PRED/PL and will be placed on a tapering dose. Approximately four weeks after the final dose of PRED/PL is taken, subjects will receive a safety phone call from the study site. Now reads: This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC in subjects with newly diagnosed IPF. Subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLCO%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. Enrollment will continue until April 30, 2012. The study will employ a 2-arm design with 1:1 randomization to NAC or PL. Once enrolled, subjects will visit the clinical center at 15 weeks and 15-week intervals thereafter. Each subject will be treated and followed for a maximum of 60 weeks. During the 60-week visit, subjects will be taken off all study agents. Approximately four weeks after the final dose of study agent is taken, subjects will receive a safety phone call from the study site.	4.4.1. Study Design Summary
Deleted procedures from screening: TPMT FSH Hospital Anxiety and Depression (HAD) questionnaire	4.4.2.1. Screening

Deleted procedures from enrollment: Blood draw and measurement of blood cell counts and serum chemistries Complete HAD scale questionnaire	4.4.2.2. Enrollment
Deleted sentence once in 2nd paragraph If the enrollment visit occurs within 21 days of the screening visit, some procedures may not need to be performed at this visit.	4.4.2.2. Enrollment
Deleted sections related to Week 1, Week 2, Week 4, Week 6, Week 10, Week 20, Week 25, Week 35, Week 40, Week 50, Week 55	4.4.2.3 …
Deleted the following procedures from week 15 Laboratory values (complete blood count [CBC] and serum chemistries) Subject will complete HAD scale questionnaire	4.4.2.8. Week 15
Deleted paragraph under section 4.4.2.8	4.4.2.8. Week 15
Deleted the following procedures from week 45 Laboratory values (complete blood count [CBC] and serum chemistries) Subject will complete HAD scale questionnaire	4.4.2.14. Week 45
Revised paragraph for week 60 Previously read: At week 60, or at subject withdrawal from the study (premature, by study doctor or subject's decision), a final treatment visit will occur. At this final treatment visit subjects will discontinue AZA/PL and NAC/PL abruptly. Subjects will receive a supply of PRED (or PL) sufficient to taper off of the drug. The tapering schedule will vary depending on the dose of PRED (or PL) the subject is taking at the time of withdrawal. Week 45 activities also include: Now reads: At week 60, or at subject withdrawal from the study (premature, by study doctor or subject's decision), a final treatment visit will occur. At this final treatment visit subjects will discontinue NAC/PL abruptly. Week 60 activities also include:	4.4.2.17. Week 60 (Early Withdrawal/Final Treatment Visit)
Delete the following procedure for week 60 Serum pregnancy test (if applicable)	4.4.2.17. Week 60 (Early Withdrawal/Final Treatment Visit)
Delete Table 3 Tapering Dose Schedule for Prednisone and paragraph and bulleted items below it	4.4.2.17. Week 60 (Early Withdrawal/Final Treatment Visit)
Revised section Previously read: Four weeks following the final dose of study medication, subjects will receive a telephone call from the study	4.4.2.19. Final Visit – Telephone Follow-up

Revision	Applicable Section(s)
coordinator to ensure that there are no side effects related to the halting of PRED/PL and to follow up on any ongoing adverse events (AEs). Now reads: Four weeks following the final dose of study medication, subjects will receive a telephone call from the study coordinator to ensure that there are no side effects and to follow up on any ongoing adverse events (AEs).	
Deleted second bulleted item: Ensure compliance with the scheduled local blood draws and address any concerns regarding them	4.4.2.20. Phone Contact Between Visits
Deleted section.	4.4.2.21. Long-term Follow-up
Revised Schedule of Assessments	Table 4 Schedule of Assessments
Deleted 2nd paragraph The dosing for PRED was set at relatively low doses to limit common steroid side effects. The incidence of AZA-related side effects will be reduced because the dosage is determined based on the TPMT levels that will be checked at screening. Algorithms have been developed to assist with dosage adjustments of study medication in response to specific laboratory abnormalities or symptoms. If questions arise, the IPFnet Data Coordinating Center (DCC) medical monitor and PANTHER-IPF protocol cochair Dr. Ganesh Raghu will be available for consultations about possible dose reductions and side effects management.	4.5. Dose Justification
Deleted the following sections and tables under 4.5 4.5.1. Azathioprine 4.5.1.1. Rationale for Azathioprine Dosing 4.5.1.2. Azathioprine/Placebo Dosing Table 5: Azathioprine/Placebo Dosing 4.5.1.3. Azathioprine Monitoring 4.5.1.4. Dosage Adjustments for Azathioprine/Placebo (see Dosage Adjustment Algorithms) 4.5.2. Rationale for Prednisone/Placebo Dosing 4.5.2.1. Prednisone Dosing Table 6: Prednisone/Placebo Dosing 4.5.2.2. Reasons to Discontinue Prednisone 4.5.2.3. Prednisone/Placebo Dosing During Apparent Acute Exacerbation of IPF 4.5.2.4. Recommended Dosing of Intravenous Corticosteroid During Acute Exacerbation of IPF 4.5.2.5. Prednisone Dosing During Clinical Worsening or Shortness of Breath and Cough (Not Considered Acute Exacerbation)	4.5. Dose Justification
Deleted references	4.5.3. Rationale for N-acetylcysteine Dosing

PANTHER-IPF Protocol Table of Revisions Amendment 2

Revision	Applicable Section(s)
Deleted reference to Dosing Algorithms 4.5.4	4.5.3.2. Reasons to Discontinue N-acetylcysteine/placebo
Deleted dosing algorithms A-H Replaced with algorithms 1-2	4.5.4. Dosage Algorithms (A-H)
Deleted the following sections and tables under 4.6 4.6.1 Azathioprine 4.6.1.1 Contraindications 4.6.1.2. Precautions 4.6.1.3. Side Effects Table 7: Side Effects of Azathioprine 4.6.2. Prednisone 4.6.2.1 Contraindications 4.6.2.2. Precautions 4.6.2.3. Side Effects Table 8: Side Effects of Prednisone	4.6. Contraindications, Precautions, and Side Effects of Study Medications
Revised Table 9: Side Effects of NAC	4.6.3.3. Side Effects
Deleted bullet # 7 - TPMT Deleted Monitoring of Laboratory Values	4.8. Study Procedures
Deleted sentences three and four in 1st paragraph: During suspected AEx, subjects will have approximately 35 mL of blood drawn for research purposes, and other clinically obtained biologic specimens (BAL) that would otherwise be discarded will be collected whenever possible. The BAL would be collected from the subject if subject was seen at the participating clinical center.	4.8.1.1. Biological Specimen Sample Management
Revised last sentence in 1st paragraph Previously read: At regular intervals, samples will be batched and shipped to the central repository. Now reads: The samples will be processed per PANTHER-IPF MOOP guidelines, aliquoted, labeled with barcode labels, and stored at -70°C at the clinical center, and shipped to the central repository	4.8.1.1. Biological Specimen Sample Management
Deleted first sentence in 2nd paragraph Samples shipped to the repository will be labeled with barcode labels; no demographic information or subject identifiers will be included on the label.	4.8.1.1. Biological Specimen Sample Management
Revised second sentence in 2nd paragraph Previously read: The only identifier will be a sample ID Now reads: The only subject identifiers will be a sample ID number and subject initials.	4.8.1.1. Biological Specimen Sample Management

PANTHER-IPF Protocol Table of Revisions Amendment 2

Revision	Applicable Section(s)
Deleted the following section 4.8.1.2. Acute Exacerbation Sample Management	4.8.1. Biological Specimen Management
Deleted second sentence: Subjects receiving allopurinol will have reduced dosing of AZA/PL as delineated in section 4.5.1.2.	4.9. Concomitant Medications
Revised third sentence: Previously read: Temporary treatment with oral or IV corticosteroids as described in section 4.5.2.3.1 for clinical worsening or suspected AEx is permitted. Now reads: Temporary treatment with oral or IV corticosteroids for clinical worsening or suspected AEx is permitted. Nutritional supplements containing NAC are not allowed	4.9. Concomitant Medications
Revised first sentence in 1st paragraph Previously read: Clinical laboratory parameters will be assessed throughout the study. Now reads: Clinical laboratory parameters will be assessed at screening and at the end of the study.	4.10. Laboratory Testing
Revised the beginning of the second sentence Previously read: The following tests will be performed at the time points… Now reads: The following tests will be performed at the *two* time points…	4.10. Laboratory Testing
Deleted the following section: Rational for Central Labs	4.10. Laboratory Testing
Revised section Previously read: Subjects and caregivers will be blinded to study treatment. Every subject will receive AZA, PRED, and NAC or matching PLs at every study visit from the baseline visit to the week 60 visit (except the week 4 safety visit). At week 60 or the final treatment visit, subjects will begin dosage adjustments as described in section 4.4.2.7. Now reads: Subjects and caregivers will be blinded to study treatment. Every subject will receive NAC or matching PLs at every study visit from the baseline visit to the week-45 visit.	4.11. Blinding of Study Drugs

Revision	Applicable Section(s)
No study agent will be dispensed at the week-60 visit.	
Revised section: Previously read: The primary endpoint will be the change in serial measurements of FVC over the 60-week study. Now reads: The primary endpoint will be the change in serial measurements of FVC over the study period	5.1. Primary Study Endpoint
Revised the last sentence Previously read: The study doctor will discuss remaining in the study with subjects experiencing documented disease progression. Now reads: For subjects who experience disease progression, the study doctor will determine whether or not the subject will remain in the study.	5.2.1. Time to Disease-progression
Revised the first sentence under <u>Identification of Acute Exacerbations</u> Previously read: All subjects will be educated regarding the importance of identifying AExs. At the time of enrollment, subjects will be educated … Now reads: All subjects will be educated about the importance of identifying AExs. At the time of enrollment, subjects will be told about…	5.2.2. Acute Exacerbations
Revised the paragraph 4 under <u>Identification of Acute Exacerbations</u> Previously read: During episodes of suspected AEx, as determined by the individual clinical center investigator, treatment with study drugs will be as specified in sections 4.5.1.4 (AZA/PL), 4.5.2.3 (PRED/PL), 4.5.3.2 (NAC/PL), and 4.5.4 Algorithm H. Subjects will remain blinded and in the study. Now reads: During episodes of suspected AEx, as determined by the individual clinical center investigator, subjects will remain blinded and in the study.	5.2.2. Acute Exacerbations
Deleted sentences in paragraph 5 under Identification of Acute Exacerbations Because the standard of care for management of suspected AExs includes steroids, the following is recommended: IV solumedrol—1.0 g/day for 3 days, 0.5 g/day for 3 days, and	5.2.2. Acute Exacerbations

Revision	Applicable Section(s)
taper dosage to reach 0.5 mg/kg/day of oral PRED by the end of 2 weeks as clinically tolerated. Then follow taper guidelines in Table 3, section 4.4.2.7. When the subject is tapered off active PRED, resume PRED/PL dosing in accordance with the study schedule.	
Deleted reference to AZA-PRED-NAC	6.3. Unblinding Procedures
Revised section Previously read: At the baseline, 15-week, 30-week, and 45-week study visits, subjects will receive a supply of study drug sufficient to last until the next visit at which study drug will be dispensed. At the week 60 visit, or final study treatment visit, subjects will receive a supply of PRED/PL for tapering. Now reads: At the baseline, 15-week, 30-week, and 45-week study visits, subjects will receive a supply of study drug sufficient to last until the next visit at which study drug will be dispensed.	7. Study Procedures
Deleted section	9.1 Overview of the Study Design
Revised references	13. References

PANTHER-IPF Trial
Statistical Analysis Plan

Version 20JUL2011

Prepared by

Duke Clinical Research Institute

Table of Contents

I. Study Summary

PREDNISONE, **A**ZATHIOPRINE, AND **N**-ACETYLCYSTEINE: A STUDY **TH**AT **E**VALUATES **R**ESPONSE IN **I**DIOPATHIC **P**ULMONARY **F**IBROSIS

There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) will conduct a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy and in combination with azathioprine (AZA) and prednisone (PRED) in subjects with mild or moderate IPF. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] ≥ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DLco%pred] ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Each subject will be treated up to a maximum of 60 weeks, followed by a tapering of PRED/ NAC and a 4-week period for safety evaluation.

The primary endpoint is the change in longitudinal measurements of FVC over the 60-week treatment period. The primary goal of this study is to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF.

Further explanation can be found in the PANTHER-IPF protocol, MOOP, and DSMB charter.

II. Study Design

i. Overview

This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF. The 3 treatment regimens are:

1) AZA-PRED-NAC

2) NAC

3) PL

Approximately 390 subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLco%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Once enrolled, subjects will visit the clinical center at 15-week intervals thereafter. Between visits, subjects will visit local blood-draw centers or the clinical center for monitoring of blood counts and serum chemistries on a predefined schedule. Each subject will be treated and followed for a maximum of 60 weeks.

After the 60-week visit, subjects will be taken off all study agents except for PRED/PL and will be placed on a tapering dose for up to 3 weeks. Four weeks after the final dose of PRED/PL is taken, subjects will return for a final safety checkup.

ii. Randomization

Patients are randomized in a 1:1:1 ratio to one of 3 treatment regimes. The study is double blinded with regard to the treatment regime. The randomization scheme consists of a permuted block design with stratification by clinical site.

iii. Data Sources

A database of case report form and lab data will be created in the Inform, a web based data entry system. The database will be converted SAS for statistical analysis. The randomization treatment assignment and kit information will be provided though ALMAC nightly transfer to the DCRI server and converted to SAS.

iv. Analysis Population and Missing Data

All randomized patients will be included in the analysis population for assessing the primary and secondary endpoints.

v. General Methodology

Definition of Statistical Significance: The statistical plan will test non-directional hypotheses, i.e., all tests will be 2-sided. The level of significance for all efficacy and safety analyses will be 0.05.

Statistical Tests: For situations were one observation per patient is observed, like safety comparisons at individual time points, a general analysis convention will be used unless otherwise specified. For continuous and pseudo-continuous variables, treatment group differences will be tested using the Wilcoxon rank-sum test for two groups and Kruskal-Wallis one-way analysis of variance for comparisons of more than two groups. For censored data, like time to event, treatment group differences will be tested using the log rank test. For discrete variables, treatment group differences will be tested using the chi-square test. In the situation of low cell counts the treatment group differences will be tested using Fisher's exact method.

Descriptive Statistics: For continuous and pseudo-continuous variables the number of observations, number of missing values, mean, standard deviation, median, twenty-fifth percentile, and seventy-fifth percentile will be given. For yes/no, categorical, and/or ordinal variables a simple count and percent or tally will be given. Other statistics may be considered if necessary.

Descriptive Plots: Descriptive plots may replace or produced in addition to descriptive statistics if deemed appropriate. If deemed necessary plots of descriptive statistics such as spaghetti, mosaic, box, cumulative distribution, loess, and etc… will be provided.

Study Listings: Study data will be listed by treatment group, visit if applicable, and patient where appropriate.

Software and Validation Procedures: All data presented in interim and final analyses will be generated and validated under the guidance of the DCRI Statistical SOPs.

vi. Common Variable Definitions

Common pulmonary studies predicted values will be calculated with the following equations:

- **FVC Prediction Equations**
  Males:
  $FVC(L) = 0.060(height(cm)) - 0.0214(age) - 4.650$

Females:
$FVC(L) = 0.0491(height(cm)) - 0.0261(age) - 3.590$

African-Americans: use scaling factor of 0.88 in addition to gender equations

- **FEV_1 Prediction Equations**
  Males:
  $FEV_1 = 0.0414(height(cm)) - 0.0244(age) - 2.190$
  Females:
  $FEV_1 = 0.0342(height(cm)) - 0.255(age) - 1.578$

  African Americans: use scaling factor of 0.88 in addition to gender equations

- **DLCO Prediction Equations**
  Males:
  $DLCO(\text{mL/min/mmHg}) = 0.416(height(cm)) - 0.219(age) - 26.34$
  Females:
  $DLCO(\text{mL/min/mmHg}) = 0.256(height(cm)) - 0.144(age) - 8.36$

- **DLCO Correction Equations**
  Altitude Correction:
  National Jewish Center, Denver Colorado
  Salt Lake City, Utah

  $DLCO_c = DLCO_{measured}[1.0 + 0.0035(PAO_2 - 120)$

  Hemoglobin (Hgb) Correction:
  a=0.7
  Hgb=g/dL

  $DLCO_c = (DLCO_{measured}(14.6a + Hgb))/((1 + a)Hgb)$

III. Schedule of Assessments

Procedure	Screening Visit 0	Enrollment Visit 1	Wk 4 Visit 2	Wk 15 Visit 3	Wk 30 Visit 4	Wk 45 Visit 5	Wk 60 Visit 6	Final Safety Visit 7
Informed consent	X							
Medical history	X							
Inclusion/exclusion criteria	X							
Pregnancy test (if applicable)	X			X	X	X		
Review of lung biopsy	X							
ABG	X						X	
6MWT		X			X		X	
Physical examination	X		X		X		X	X
Vital signs with oximetry	X	X	X	X	X	X	X	X
Body height and weight	X	X	X	X	X	X	X	X
CBC[2]	X	X[1]	X	X	X	X	X	
Chemistry panel[2]	X	X[1]	X	X	X	X	X	
Urinalysis	X							
Research blood draw and urine collection (if consent granted)		X		X	X	X	X	
TPMT measurement (if not already done)	X							
FSH (if applicable)	X							
HRCT (if necessary)	X							
Spirometry	X	X		X	X	X	X	
DLco	X				X		X	
Lung volumes	X						X	
Evaluate for acute exacerbation		X	X	X	X	X	X	X
Review AEs		X	X	X	X	X	X	X
Review concomitant meds		X	X	X	X	X	X	
Dispense subject diary		X		X	X	X	X	
Review subject diary		X	X	X	X	X	X	
Dispense study treatment		X		X	X	X	X[3]	
Gender Substudy questionnaire[4]		X						
HAD Scale	X	X[1]	X	X	X	X	X	
EuroQol		X			X		X	
ICE CAP		X			X		X	
UCSD SOBQ		X			X		X	
SGRQ		X			X		X	
SF-36		X			X		X	

Abbreviations: ABG, arterial blood gas; 6MWT, 6-minute walk test; CBC, complete blood count; TPMT, thiopurine methyl transferase; FSH, follicle-stimulating hormone; HRCT, high-resolution computed tomography; DLco, diffusing capacity of the lung for carbon monoxide; AE, adverse event; HAD, Hospital Anxiety and Depression; ICE CAP, Investigating Choice Experiments for Preferences of Older People; UCSD SOBQ, University of California at San Diego Shortness of Breath Questionnaire; SGRQ, St. George's Respiratory Questionnaire

1If the enrollment visit occurs within 14 days of the screening visit, these procedures do not need to be repeated.

2 There will be interim blood draws for blood cell counts and serum chemistries. These may be drawn at the clinical center or a laboratory closer to subject's home.

3Final study kit will be provided to allow tapering of PRED/PL.

4Female subjects only.

IV. Primary Endpoints

Treatment with AZA-PRED-NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC. Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.

V.Secondary Endpoints

Secondary goals of this study are to assess differences between treatment groups for the following:

1. Mortality
2. Time to death
3. Frequency of acute exacerbations (AExs)
4. Frequency of maintained FVC response
5. Time to disease-progression
6. Change in DL_{CO}
7. Change in Composite Physiologic Index (CPI)
8. Change in resting alveolar-arterial oxygen gradient
9. Change in 6-minute walk test (6MWT) distance
10. Change in 6MWT oxygen saturation area under the curve
11. Change in 6MWT distance to desaturation < 88%
12. Change in 6MWT minutes walked
13. Changes in health status as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ)
14. Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ)
15. Frequency and types of adverse events (AEs)
16. Frequency and types of respiratory complications, both infectious and noninfectious
17. Frequency of hospitalizations, both all-cause and respiratory-related

VI. Endpoint Descriptions

vii. Primary Endpoint

Primary Endpoint: FVCs are values of the PFTs measured at enrollment and serially until study completion at 60 weeks.

FVC response (primary endpoint) will be defined as the post-bronchodilator FVC measurements. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of FVC measurements across the 3 treatment groups. Response variables are values of the FVC measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. A 2-step Fisher's least significant difference (LSD) procedure will be applied to control the experiment-wise error rate at 0.05. The first step of this testing procedure will be based on a 2-degree-of-freedom omnibus test. If the first test is statistically significant at the 0.05 level, then each of the 3 pairwise comparisons will be tested at the 0.05 level. The 3 pairwise comparisons are: NAC vs. PL, AZA-PRED-NAC vs. PL, and NAC vs. AZA-PRED-NAC. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing FVC data will not be imputed for the primary analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. A careful examination of reasons for study discontinuation will be conducted to assess the validity of MCAR. Sensitivity analyses will be used to examine the untestable assumption that the observed data violate the MAR assumption. The MMRM models will be implemented using PROC MIXED in SAS.

viii. Secondary Endpoint #1 and 2

Secondary Endpoints #1 and 2: All-cause mortality and time to death including cause of death measured during the time period of patient follow in the trial.

Mortality and cause of death will be tabulated by treatment. Time of death (any cause death) will be calculated as the difference between date of death or last follow-up and date of randomization. Kaplan-meier event rates will be calculated at representative intervals by treatment group and the log rank test will be used to make pairwise group comparisons. Time to event plots will be generated also. Other specific cause of death "time to event" analyses maybe generated as deemed necessary.

ix. Secondary Endpoint #3

Secondary Endpoints #3: Acute exacerbations will be identified and adjudicated during the following-up period of the trial.

An acute exacerbation (AEx) will be identified by either the site PI or DCC medical monitor and then adjudicated by the IPF adjudication committee. If AEx is suspected, the case will be sent to the IPFnet Adjudication Committee, which will assign a final diagnosis. If there is disagreement among members, the majority opinion will be recorded. The final diagnosis is defined as the following:

1. **Definite acute exacerbation** - All criteria met; no alternative etiology
2. **Unclassifiable acute worsening** - Insufficient data to evaluate all criteria; no alternative etiology
3. **Not acute exacerbation** - Alternative etiology identified that explains acute worsening

The number of patients with one or move AExs will be tabulated along with the total number of distinct AEx. Comparison of the treatments groups will be done with a chi-square test, i.e. the number of patient with one or more events.

x. Secondary Endpoint #4

Secondary Endpoint #4: Maintained FVC response at follow-up visits during the course of the trial, using the percent predicted FVC values.

To calculated maintained FVC response at each visit in the percent predicted FVC will be computed and each follow-up measurement be compared to baseline value. Patients with a percent predicted FVC value at or above the baseline percent predicted FVC value will be classified as having maintained FVC response. Patients with reduced percent predicted FVC values or missing for any reason, including death or medical frailty, will be classified as having not maintained FVC response. The number of patients with one or move maintained FVC response s will be tabulated along with the total number of distinct maintained FVC responses. Comparison of the treatments groups will be done with a chi-square test.

xi. Secondary Endpoint #5

Secondary Endpoint #5: Time to disease-progression is the composite of time to death and/or 10% decline in FVC.

The time to death or a 10% decline in FVC will be defined as the time to disease-progression. A 10% decline in FVC from enrollment will be confirmed with an additional visit within 6 to 8 weeks yielding a second 10% decline. For patients with a confirmed 10% decline in FVC, the time to disease-progression will be defined as the difference between the initial visit date with a 10% FVC decline and date of randomization. For patients without a confirmed 10% decline in FVC the time to disease-progression will be defined as the difference between date of death or last follow-up and date of randomization. Kaplan-meier event rates will be calculated at representative intervals by treatment group and the log rank test will be used to make pairwise group comparisons. Time to event plots will generated also.

xii. Secondary Endpoint #6

Secondary Endpoint #6: Change in DL_{CO} at enrollment, 30, and 60 weeks of the trial.

DL_{CO} response will be defined as the hemoglobin corrected DL_{CO} measurements. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of DL_{CO} measurements across the 3 treatment groups. Response variables are values of the DL_{CO} measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing DL_{CO} data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xiii. Secondary Endpoint #7

Secondary Endpoint #7: Change in Composite Physiologic Index (CPI) at enrollment, 30, and 60 weeks of the trial.

CPI response will be defined as by the following formula from components of DLCO and PFTs:

CPI=91.0-(percent predicted DL_{CO})-(0.53*percent predicted FVC) +(0.34*percentage predicted FEV1)

A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of CPI measurements across the 3 treatment groups. Response variables are values of the CPI measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing CPI data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xiv. Secondary Endpoint #8

Secondary Endpoint #8: Change in alveolar-arterial oxygen gradient (A-a gradient) at the 60 week visit.

The A-a gradient can be calculated by the following formula:

P_B = Barometric Pressure

PaO_2 = arterial PO_2

FIO_2 (Friction of inspired oxygen)= 0.21

PIO_2 (Partial pressure of inspired oxygen) = FIO_2*(P_B-47)

PAO_2 (alveolar PO_2) = PIO_2-$PaCO_2$/0.8

A-a gradient = PAO_2- PaO_2

Change in A-a gradient will be assessed with a linear regression model with difference from baseline to the 60 week measurement as the outcome. Variables in the model will include treatments, age, sex, race, and height. Contrast estimates of differences in treatment means (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures.

xv. Secondary Endpoint #9

Secondary Endpoint #9: Change in 6-minute walk test (6MWT) distance at enrollment, 30, and 60 weeks of the trial.

6MWT distance response will be defined as the distance covered during the 6-minute walk test. For situations where patient attended the visit but was unable to walk, the 6MWT distance will imputed as 0. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT distance measurements across the 3 treatment groups. Response variables are values of the 6MWT distance measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT distance data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xvi. Secondary Endpoint #10

Secondary Endpoint #10: Change in 6MWT oxygen saturation area under the curve at enrollment (oxygen saturation AUC), 30, and 60 weeks of the trial.

Oxygen saturation AUC will be calculated at each visit using the SpO^2 levels recorded at set intervals during each 6MWT. The trapezoidal rule will be used to estimate the area under the curve between each SpO^2 measurement and then summed to calculate oxygen saturation AUC. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of oxygen saturation AUC measurements across the 3 treatment groups. Response variables are values of the oxygen saturation AUC measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing oxygen saturation AUC data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xvii. Secondary Endpoint #11

Secondary Endpoint #11: Change in 6MWT distance to desaturation < 88% at enrollment, 30, and 60 weeks of the trial.

6MWT distance to desaturation < 88% response will be defined as the distance covered during the 6-minute walk test before reaching the desaturation limit of 88%. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT distance to desaturation <

88% measurements across the 3 treatment groups. Response variables are values of the 6MWT distance to desaturation < 88% measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT distance to desaturation < 88% data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xviii. Secondary Endpoint #12

Secondary Endpoint #12: Change in 6MWT minutes walked at enrollment, 30, and 60 weeks of the trial.

The 6MWT minutes walked response will be defined as the number of minutes the patient walked during the assessment. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT minutes walked measurements across the 3 treatment groups. Response variables are values of the 6MWT distance to desaturation < 88%measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT minutes walked data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xix. Secondary Endpoint #13

Secondary Endpoint #13: Changes in health status/quality of life (QoL) as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ) at enrollment, 30, and 60 weeks of the trial.

For SF-36 two summary scores, SF-36 Aggregate Physical and SF-36 Aggregate Mental, will be derived and used in the analysis. For EuroQol two summary scores, EuroQol score and EuroQol Thermometer Response, will be derived and used in the analysis. For St. Georges' four summary scores; St. Georges' Symptoms Score, St. Georges' Activity Score, St. Georges' Impacts Score, and St. Georges' Total Score; will be derived and used in the analysis. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of each QoL measurement across the 3 treatment groups. Response variables are values of the QoL measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing QoL data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xx. Secondary Endpoint #14

Secondary Endpoint #14: Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ) at enrollment, 30, and 60 weeks of the trial.

For UCSD SOBQ a total score will be derived and used in the analysis. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of UCSD SOBQ total across the 3 treatment groups. Response variables are values of the UCSD SOBQ total at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing UCSD SOBQ total data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xxi. Secondary Endpoint #15

Secondary Endpoint #15: Frequency and types of adverse events (AEs) during the following-up period of the trial.

AEs will be identified by the site PI and coded into the MEDRA medical dictionary. The number of patients with one or move AEs will be tabulated along with the total number of distinct AEs. Comparison of the treatments groups will be done with a chi-square test, i.e. the number of patient with one or more events. Serious adverse events (SAEs) will be tabulated and tested in the same manner as AEs. Other groupings of AEs maybe generated as deemed necessary.

xxii. Secondary Endpoint #16

Secondary Endpoint #16: Frequency and types of respiratory complications, both infectious and noninfectious during the following-up period of the trial.

Respiratory complications will be identified by the site PI and coded into the MEDRA medical dictionary. The number of patients with one or move respiratory complications will be tabulated along with the total number of distinct respiratory complications. Comparison of the treatments groups will be done with a chi-square test, i.e. the number of patient with one or more events.

xxiii. Secondary Endpoint #17

<u>Secondary Endpoint #17:</u> Frequency of hospitalizations, both all-cause and respiratory-related during the following-up period of the trial.

Hospitalizations will be identified by the site PI. The number of patients with one or move hospitalizations will be tabulated along with the total number of distinct hospitalizations. Comparison of the treatments groups will be done with a chi-square test, i.e. the number of patient with one or more events.

VII. Analyses of Demographic and Baseline Data

Patient demography such as age, weight, sex, ethnicity, and race will be summarized. The patient medical history and conditions such as duration of IPF, smoking status, coronary artery disease, acute MI, valvular heart disease, HF, atrial fibrillation, diabetes, lung cancer, clubbing, etc…will be summarized. Measurements related to primary and secondary endpoints at baseline such as 6MWT, spirometry, lung diffusion testing, ABBs, lung volume, and quality of life measures will be summarized. Treatment group differences in select demographic and clinical characteristics at baseline will be examined.

VIII. Pre-Specified Subgroups of Interest

Treatment effects will be estimated and compared within key subgroups:

- higher enrollment FVC
- typical vs. atypical HRCT reading at baseline
- a recent vs. more remote diagnosis (time from initial diagnosis of IPF ≤ 1 year and > 1 year)
- lower CPI score at enrollment
- use of medical therapy for gastroesophageal reflux
- ethnic background
- sex
- smoking history (current/ex-smoker vs. never smoker), given potential impact on oxidant status
- presence of emphysema > 25% on high-resolution computed tomography (HRCT)

IX. Interim Analyses

One interim analysis is planned to be conducted at approximately 0.50 information time. To conserve the overall type I error rate of 0.05 the O'Brien-Fleming Spending Function will be used to allow for stopping if large treatment effects are observed while allowing the final significance level to be conserved at the nominal level (Lan and DeMets 1983). Information time will be defined in two dimensions, the first is number of enrolled patients and the second is the 60 week follow-up. So the calculation of information time will be a summation or integration of a two dimensional space or area. To calculate information time the first step is to determine the number of weeks of possible follow-up for each enrolled patient at the time of the interim analysis. For example if a patient has completed 60 weeks then 60 would be their results or if a patient was randomized 21 days ago then their result would be 3. The second step is to add up number of weeks of possible follow-up from the first step and divide by 60 and total number of planned patients. For the planned interim analysis or any interim analysis the primary efficacy analysis will not be assessed at the alpha of 0.05 in the testing procedure descripted in "Primary Endpoint" section. Instead the interim significance level alpha determined from the spending function will be used in the testing procedure.

O'Brien-Fleming Group Sequential Boundaries

One Interim Analysis and a Final Analysis		
Information Time	**Bound for |Z| Statistic**	**Cumulative Alpha**
0.50	2.9626	0.00305
1.00	1.9686	0.05000

I. References

Lan KKG, DeMets, DL. Discrete sequential boundaries for clinical trials. Biometrika. 1983;70:659-663.

Addendum to the PANTHER-IPF Analysis Plan

Eric Yow, Lead Statistician

22OCT2011

On 14OCT2011 the DCC received a memorandum from the Executive Secretary of IPFnet DSMB regarding the recommendations for investigators of the PANTHER-IPF study from DSMB meeting 12OCT2011 conference call. The recommendations indicated as "Effective Immediately" were the following:

- Randomization into the prednisone, azathioprine, and N-acetylcysteine (triple therapy) arm of the study should stop.
- All sites should be notified that prednisone, azathioprine, and N-acetylcysteine should be discontinued as soon as possible for participants in the triple therapy arm. Prednisone may be discontinued according to the tapering dose schedule outlined in the study protocol, as per the treating physician's discretion.
- Participants in the triple therapy arm should be immediately notified with instructions for discontinuation of study drugs.
- The PANTHER-IPF trial should continue with double blind randomization into the N-acetylcysteine and placebo arms.
- Participants randomized to the prednisone, azathioprine, and N-acetylcysteine arm should continue to be followed in the study for health outcomes, vital status, and adverse events. Participants in this arm should not be re-randomized into the other study arms.
- The study investigators may modify the follow-up visit and data collection schedules as appropriate for safety monitoring and collection of follow-up data.

These changes to the conduct and design of the PANTHER-IPF require deviations from the prescribed statistical analysis plan. The impact to general public health requires immediate reporting of the interim results describing and comparing of the AZA-PRED-NAC and PL arms of the trial in an interim fashion using the best data available in the PANTHER-IPF database. We plan to generate an analysis of enrollment, baseline, efficacy, and safety results (summary data only) of the AZA-PRED-NAC and PL arms for the protocol PIs who subsequently will submit a peer reviewed journal article for publication.

PANTHER-IPF Trial
Statistical Analysis Plan

Final Analysis Version

Prepared by

Duke Clinical Research Institute

Table of Contents

I. Study Summary

PREDNISONE, AZATHIOPRINE, AND N-ACETYLCYSTEINE: A STUDY THAT EVALUATES RESPONSE IN IDIOPATHIC PULMONARY FIBROSIS

There are currently no drug therapies that have proven to be effective in the treatment of idiopathic pulmonary fibrosis (IPF). Previous clinical drug trials have been difficult to interpret due to lack of true placebo (PL) controls or other methodological concerns. Clinical efficacy of immunosuppressive therapies and agents that reduce oxidative stress remains controversial. The IPF Clinical Research Network (IPFnet) will conduct a randomized, double-blind, placebo-controlled trial designed to assess the safety and efficacy of N-acetylcysteine (NAC) as monotherapy and in combination with azathioprine (AZA) and prednisone (PRED) in subjects with mild or moderate IPF. Approximately 390 subjects who have mild to moderate IPF (defined as forced vital capacity percent predicted [FVC%pred] ≥ 50% and diffusing capacity of the lung for carbon monoxide percent predicted [DLco%pred] ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Each subject will be treated up to a maximum of 60 weeks, followed by a tapering of PRED/ NAC and a 4-week period for safety evaluation.

The primary endpoint is the change in longitudinal measurements of FVC over the 60-week treatment period. The primary goal of this study is to establish an evidence-based standard of care and clarify myths from facts for pharmacotherapy of IPF.

On 14OCT2011 the DCC received a memorandum from the Executive Secretary of IPFnet DSMB regarding the recommendations for investigators of the PANTHER-IPF study from DSMB meeting 12OCT2011 conference call. The protocol was rewritten such that the study would continue with double blind randomization into the N-acetylcysteine and placebo arms. Changes to the conduct of the trial were made to the protocol.

Further explanation can be found in the PANTHER-IPF protocol, MOOP, and DSMB charter.

II. Study Design

i. Overview

This study will be a randomized, double-blind, PL-controlled trial designed to assess the safety and efficacy of NAC and the combination of AZA-PRED-NAC in subjects with newly diagnosed IPF. The 3 treatment regimens are:

1) AZA-PRED-NAC

2) NAC

3) PL

Approximately 390 subjects with mild to moderate IPF (defined as FVC%pred ≥ 50% and DLco%pred ≥ 30%) diagnosed within the past 48 months will be enrolled. The study will employ a 3-arm design with 1:1:1 randomization to NAC, AZA-PRED-NAC, and PL. Once enrolled, subjects will visit the clinical center at 15-week intervals thereafter. Between visits, subjects will visit local blood-draw centers or the clinical center for monitoring of blood counts and serum chemistries on a predefined schedule. Each subject will be treated and followed for a maximum of 60 weeks.

After the 60-week visit, subjects will be taken off all study agents except for PRED/PL and will be placed on a tapering dose for up to 3 weeks. Four weeks after the final dose of PRED/PL is taken, subjects will return for a final safety checkup.

Since the AZA-PRED-NAC group was stop and reported due to safety reasons the final analysis will only report the NAC and PL groups

ii. Randomization

Patients are randomized in a 1:1:1 ratio to one of 3 treatment regimes. The study is double blinded with regard to the treatment regime. The randomization scheme consists of a permuted block design with stratification by clinical site. With the dropping of the AZA-PRED-NAC group the randomization ratio is 1:1 of the 2 remaining treatment regimes, either NAC or PL

iii. Data Sources

A database of case report form and lab data will be created in the Inform, a web based data entry system. The database will be converted SAS for statistical analysis. The randomization treatment assignment and kit information will be provided though ALMAC nightly transfer to the DCRI server and converted to SAS.

iv. Analysis Population and Missing Data

All randomized patients in the NAC and PL will be included in the analysis population for assessing the primary and secondary endpoints.

v. General Methodology

Definition of Statistical Significance: The statistical plan will test non-directional hypotheses, i.e., all tests will be 2-sided. The level of significance for all efficacy and safety analyses will be 0.05.

Statistical Tests: For situations were one observation per patient is observed, like safety comparisons at individual time points, a general analysis convention will be used unless otherwise specified. For continuous and pseudo-continuous variables, treatment group differences will be tested using the Wilcoxon rank-sum test for two groups and Kruskal-Wallis one-way analysis of variance for comparisons of more than two groups. For censored data, like time to event, treatment group differences will be tested using the log rank test. For discrete variables, treatment group differences will be tested using the chi-square test. In the situation of low cell counts the treatment group differences will be tested using Fisher's exact method.

Descriptive Statistics: For continuous and pseudo-continuous variables the number of observations, number of missing values, mean, standard deviation, median, twenty-fifth percentile, and seventy-fifth percentile will be given. For yes/no, categorical, and/or ordinal variables a simple count and percent or tally will be given. Other statistics may be considered if necessary.

Descriptive Plots: Descriptive plots may replace or produced in addition to descriptive statistics if deemed appropriate. If deemed necessary plots of descriptive statistics such as spaghetti, mosaic, box, cumulative distribution, loess, and etc… will be provided.

Study Listings: Study data will be listed by treatment group, visit if applicable, and patient where appropriate.

Software and Validation Procedures: All data presented in interim and final analyses will be generated and validated under the guidance of the DCRI Statistical SOPs.

vi. Common Variable Definitions

Common pulmonary studies predicted values will be calculated with the following equations:

- **FVC Prediction Equations**

  Males:

  $$FVC(L) = 0.060(height(cm)) - 0.0214(age) - 4.650$$

  Females:

  $$FVC(L) = 0.0491(height(cm)) - 0.0261(age) - 3.590$$

  African-Americans: use scaling factor of 0.88 in addition to gender equations

- **FEV_1 Prediction Equations**

  Males:

  $$FEV_1 = 0.0414(height(cm)) - 0.0244(age) - 2.190$$

  Females:

  $$FEV_1 = 0.0342(height(cm)) - 0.255(age) - 1.578$$

  African Americans: use scaling factor of 0.88 in addition to gender equations

- **DLCO Prediction Equations**

  Males:

  $$DLCO(\mathrm{mL/min/mmHg}) = 0.416(height(cm)) - 0.219(age) - 26.34$$

  Females:

  $$DLCO(\mathrm{mL/min/mmHg}) = 0.256(height(cm)) - 0.144(age) - 8.36$$

- **DLCO Correction Equations**

  Altitude Correction:

  National Jewish Center, Denver Colorado

  Salt Lake City, Utah

  $$DLCO_c = DLCO_{measured}[1.0 + 0.0035(PAO_2 - 120)$$

  Hemoglobin (Hgb) Correction:

  a=0.7

  Hgb=g/dL

  $$DLCO_c = (DLCO_{measured}(14.6a + Hgb))/((1 + a)Hgb)$$

III. Schedule of Assessments

Procedure	Screening Visit 0	Enrollment Visit 1	Wk 4 Visit 2	Wk 15 Visit 3	Wk 30 Visit 4	Wk 45 Visit 5	Wk 60 Visit 6	Final Safety Visit 7
Informed consent	X							
Medical history	X							
Inclusion/exclusion criteria	X							
Pregnancy test (if applicable)	X			X	X	X		
Review of lung biopsy	X							
ABG	X						X	
6MWT		X			X		X	
Physical examination	X		X		X		X	X
Vital signs with oximetry	X	X	X	X	X	X	X	X
Body height and weight	X	X	X	X	X	X	X	X
CBC[2]	X	X[1]	X	X	X	X	X	
Chemistry panel[2]	X	X[1]	X	X	X	X	X	
Urinalysis	X							
Research blood draw and urine collection (if consent granted)		X		X	X	X	X	
TPMT measurement (if not already done)	X							
FSH (if applicable)	X							
HRCT (if necessary)	X							
Spirometry	X	X		X	X	X	X	
DLCO	X				X		X	
Lung volumes	X						X	
Evaluate for acute exacerbation		X	X	X	X	X	X	X
Review AEs		X	X	X	X	X	X	X
Review concomitant meds		X	X	X	X	X	X	
Dispense subject diary		X		X	X	X	X	
Review subject diary		X	X	X	X	X	X	
Dispense study treatment		X		X	X	X	X[3]	
Gender Substudy questionnaire[4]		X						
HAD Scale	X	X[1]	X	X	X	X	X	
EuroQol		X			X		X	
ICE CAP		X			X		X	
UCSD SOBQ		X			X		X	
SGRQ		X			X		X	
SF-36		X			X		X	

Abbreviations: ABG, arterial blood gas; 6MWT, 6-minute walk test; CBC, complete blood count; TPMT, thiopurine methyl transferase; FSH, follicle-stimulating hormone; HRCT, high-resolution computed tomography; DLco, diffusing capacity of the lung for carbon monoxide; AE, adverse event; HAD, Hospital Anxiety and Depression; ICE CAP, Investigating Choice Experiments for Preferences of Older People; UCSD SOBQ, University of California at San Diego Shortness of Breath Questionnaire; SGRQ, St. George's Respiratory Questionnaire

1If the enrollment visit occurs within 14 days of the screening visit, these procedures do not need to be repeated.

2 There will be interim blood draws for blood cell counts and serum chemistries. These may be drawn at the clinical center or a laboratory closer to subject's home.

3Final study kit will be provided to allow tapering of PRED/PL.

4Female subjects only.

IV. Primary Endpoints

Treatment with AZA-PRED-NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC. Treatment with NAC will provide the same efficacy as PL, as measured by longitudinal changes in FVC.

V. Secondary Endpoints

Secondary goals of this study are to assess differences between treatment groups for the following:

1. Mortality
2. Time to death
3. Frequency of acute exacerbations (AExs)
4. Frequency of maintained FVC response
5. Time to disease-progression
6. Change in DL_{CO}
7. Change in Composite Physiologic Index (CPI)
8. Change in resting alveolar-arterial oxygen gradient
9. Change in 6-minute walk test (6MWT) distance
10. Change in 6MWT oxygen saturation area under the curve
11. Change in 6MWT distance to desaturation < 88%
12. Change in 6MWT minutes walked
13. Changes in health status as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ)
14. Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ)
15. Frequency and types of adverse events (AEs)
16. Frequency and types of respiratory complications, both infectious and noninfectious
17. Frequency of hospitalizations, both all-cause and respiratory-related

VI. Endpoint Descriptions

vii. Primary Endpoint

<u>Primary Endpoint:</u> FVCs are values of the PFTs measured at enrollment and serially until study completion at 60 weeks.

FVC response (primary endpoint) will be defined as the post-bronchodilator FVC measurements. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of FVC measurements across the 3 treatment groups. Response variables are values of the FVC measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing FVC data will not be imputed for the primary analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. A careful examination of reasons for study discontinuation will be conducted to assess the validity of MCAR. Sensitivity analyses will be used to examine the untestable assumption that the observed data violate the MAR assumption. The MMRM models will be implemented using PROC MIXED in SAS.

viii. Secondary Endpoint #1 and 2

<u>Secondary Endpoints #1 and 2:</u> All-cause mortality and time to death including cause of death measured during the time period of patient follow in the trial.

Mortality and cause of death will be tabulated by treatment. Time of death (any cause death) will be calculated as the difference between date of death or last follow-up and date of randomization. Kaplan-meier event rates will be calculated at representative intervals by treatment group and the log rank test will be used to make the comparison of NAC and PL. Time to event plots will be generated also. Other specific cause of death "time to event" analyses maybe generated as deemed necessary.

ix. Secondary Endpoint #3

<u>Secondary Endpoints #3:</u> Acute exacerbations will be identified and adjudicated during the following-up period of the trial.

An acute exacerbation (AEx) will be identified by either the site PI or DCC medical monitor and then adjudicated by the IPF adjudication committee. If AEx is suspected, the case will be sent to the IPFnet Adjudication Committee, which will assign a final diagnosis. If there is disagreement among members, the majority opinion will be recorded. The final diagnosis is defined as the following:

1. **Definite acute exacerbation** - All criteria met; no alternative etiology
2. **Unclassifiable acute worsening** - Insufficient data to evaluate all criteria; no alternative etiology
3. **Not acute exacerbation** - Alternative etiology identified that explains acute worsening

The number of patients with one or move AExs will be tabulated along with the total number of distinct AEx. Comparison of NAC and PL groups will be done with a chi-square test, i.e. the number of patient with one or more events.

x. Secondary Endpoint #4

Secondary Endpoint #4: Maintained FVC response at follow-up visits during the course of the trial, using the percent predicted FVC values.

To calculated maintained FVC response at each visit in the percent predicted FVC will be computed and each follow-up measurement be compared to baseline value. Patients with a percent predicted FVC value at or above the baseline percent predicted FVC value will be classified as having maintained FVC response. Patients with reduced percent predicted FVC values or missing for any reason, including death or medical frailty, will be classified as having not maintained FVC response. The number of patients with one or move maintained FVC response will be tabulated along with the total number of distinct maintained FVC responses. Comparison of NAC and PL groups will be done with a chi-square test.

xi. Secondary Endpoint #5

Secondary Endpoint #5: Time to disease-progression is the composite of time to death and/or 10% decline in FVC.

The time to death or a 10% decline in FVC will be defined as the time to disease-progression. A 10% decline in FVC from enrollment will be confirmed with an additional visit within 6 to 8 weeks yielding a second 10% decline. For patients with a confirmed 10% decline in FVC, the time to disease-progression will be defined as the difference between the initial visit date with a 10% FVC decline and date of randomization. For patients without a confirmed 10% decline in FVC the time to disease-progression will be defined as the difference between date of death or last follow-up and date of randomization. Kaplan-meier event rates will be calculated at representative intervals by treatment group and the log rank test will be used to make the comparison of NAC and PL groups. Time to event plots will generated also.

xii. Secondary Endpoint #6

Secondary Endpoint #6: Change in DL_{CO} at enrollment, 30, and 60 weeks of the trial.

DL_{CO} response will be defined as the hemoglobin corrected DL_{CO} measurements. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of DL_{CO} measurements across the 3 treatment groups. Response variables are values of the DL_{CO} measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing DL_{CO} data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xiii. Secondary Endpoint #7

Secondary Endpoint #7: Change in Composite Physiologic Index (CPI) at enrollment, 30, and 60 weeks of the trial.

CPI response will be defined as by the following formula from components of DLCO and PFTs:

$CPI=91.0-(\text{percent predicted } DL_{CO})-(0.53*\text{percent predicted FVC}) +(0.34*\text{percentage predicted FEV1})$

A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of CPI measurements across the 3 treatment groups. Response variables are values of the CPI measured at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing CPI data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xiv. Secondary Endpoint #8

Secondary Endpoint #8: Change in alveolar-arterial oxygen gradient (A-a gradient) at the 60 week visit.

The A-a gradient can be calculated by the following formula:

P_B = Barometric Pressure

PaO_2 = arterial PO_2

FIO_2 (Friction of inspired oxygen)= 0.21

PIO_2 (Partial pressure of inspired oxygen) = $FIO_2*(P_B-47)$

PAO_2 (alveolar PO_2) = $PIO_2-PaCO_2/0.8$

A-a gradient = $PAO_2- PaO_2$

Change in A-a gradient will be assessed with a linear regression model with difference from baseline to the 60 week measurement as the outcome. Variables in the model will include treatments, age, sex, race, and height. Contrast estimate of differences in NAC and PL means (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures.

xv. Secondary Endpoint #9

Secondary Endpoint #9: Change in 6-minute walk test (6MWT) distance at enrollment, 30, and 60 weeks of the trial.

6MWT distance response will be defined as the distance covered during the 6-minute walk test. For situations where patient attended the visit but was unable to walk, the 6MWT distance will imputed as 0. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT distance measurements across the 3 treatment groups. Response variables are values of the 6MWT distance measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT distance data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xvi. Secondary Endpoint #10

Secondary Endpoint #10: Change in 6MWT oxygen saturation area under the curve at enrollment (oxygen saturation AUC), 30, and 60 weeks of the trial.

Oxygen saturation AUC will be calculated at each visit using the SpO^2 levels recorded at set intervals during each 6MWT. The trapezoidal rule will be used to estimate the area under the curve between each SpO^2 measurement and then summed to calculate oxygen saturation AUC. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of oxygen saturation AUC measurements across the 3 treatment groups. Response variables are values of the oxygen saturation AUC measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing oxygen saturation AUC data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xvii. Secondary Endpoint #11

Secondary Endpoint #11: Change in 6MWT distance to desaturation < 88% at enrollment, 30, and 60 weeks of the trial.

6MWT distance to desaturation < 88% response will be defined as the distance covered during the 6-minute walk test before reaching the desaturation limit of 88%. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT distance to desaturation < 88% measurements across the 3 treatment groups. Response variables are values of the 6MWT distance to desaturation < 88% measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height.

Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT distance to desaturation < 88% data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xviii. Secondary Endpoint #12

Secondary Endpoint #12: Change in 6MWT minutes walked at enrollment, 30, and 60 weeks of the trial.

The 6MWT minutes walked response will be defined as the number of minutes the patient walked during the assessment. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of 6MWT minutes walked measurements across the 3 treatment groups. Response variables are values of the 6MWT distance to desaturation < 88%measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing 6MWT minutes walked data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xix. Secondary Endpoint #13

Secondary Endpoint #13: Changes in health status/quality of life (QoL) as measured by the SF-36, EuroQol, and St. George's Respiratory Questionnaire (SGRQ) at enrollment, 30, and 60 weeks of the trial.

For SF-36 two summary scores, SF-36 Aggregate Physical and SF-36 Aggregate Mental, will be derived and used in the analysis. For EuroQol two summary scores, EuroQol score and EuroQol Thermometer Response, will be derived and used in the analysis. For St. Georges' four summary scores; St. Georges' Symptoms Score, St. Georges' Activity Score, St. Georges' Impacts Score, and St. Georges' Total Score; will be derived and used in the analysis. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of each QoL measurement across the 3 treatment groups. Response variables are values of the QoL measured at baseline and every 30 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing QoL data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability.

For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xx. Secondary Endpoint #14

Secondary Endpoint #14: Changes in dyspnea as measured by the University of California at San Diego Shortness of Breath Questionnaire (UCSD SOBQ) at enrollment, 30, and 60 weeks of the trial.

For UCSD SOBQ a total score will be derived and used in the analysis. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of UCSD SOBQ total across the 3 treatment groups. Response variables are values of the UCSD SOBQ total at baseline and every 15 weeks until study completion at 60 weeks. Variables in the model will include treatment; time; and time by treatment, age, sex, race, and height. Contrast estimate of differences in NAC and PL slopes by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing UCSD SOBQ total data will not be imputed for the analysis. The correlation structure involves multiple pieces, including measurement errors, random variation, and inter-individual variability. For the longitudinal data analyses, an unstructured correlation matrix for within-subject errors will be assumed. Other correlation structures, including compound symmetry, will be examined as needed. The MMRM models will be implemented using PROC MIXED in SAS.

xxi. Secondary Endpoint #15

Secondary Endpoint #15: Frequency and types of adverse events (AEs) during the following-up period of the trial.

AEs will be identified by the site PI and coded into the MEDRA medical dictionary. The number of patients with one or move AEs will be tabulated along with the total number of distinct AEs. Comparison of the NAC and PL groups will be done with a chi-square test, i.e. the number of patient with one or more events. Serious adverse events (SAEs) will be tabulated and tested in the same manner as AEs. Other groupings of AEs maybe generated as deemed necessary.

xxii. Secondary Endpoint #16

Secondary Endpoint #16: Frequency and types of respiratory complications, both infectious and noninfectious during the following-up period of the trial.

Respiratory complications will be identified by the site PI and coded into the MEDRA medical dictionary. The number of patients with one or move respiratory complications will be tabulated along with the total number of distinct respiratory complications. Comparison of the NAC and PL groups will be done with a chi-square test, i.e. the number of patient with one or more events.

xxiii. Secondary Endpoint #17

Secondary Endpoint #17: Frequency of hospitalizations, both all-cause and respiratory-related during the following-up period of the trial.

Hospitalizations will be identified by the site PI. The number of patients with one or move hospitalizations will be tabulated along with the total number of distinct hospitalizations. Comparison of the NAC and PL groups will be done with a chi-square test, i.e. the number of patient with one or more events.

VII. Analyses of Demographic and Baseline Data

Patient demography such as age, weight, sex, ethnicity, and race will be summarized. The patient medical history and conditions such as duration of IPF, smoking status, coronary artery disease, acute MI, valvular heart disease, HF, atrial fibrillation, diabetes, lung cancer, clubbing, etc…will be summarized. Measurements related to primary and secondary endpoints at baseline such as 6MWT, spirometry, lung diffusion testing, ABBs, lung volume, and quality of life measures will be summarized. NAC and PL group differences in select demographic and clinical characteristics at baseline will be examined.

VIII. Pre-Specified Subgroups of Interest

Treatment effects will be estimated and compared within key subgroups:

- higher enrollment FVC
- typical vs. atypical HRCT reading at baseline
- a recent vs. more remote diagnosis (time from initial diagnosis of IPF $\leq$ 1 year and > 1 year)
- lower CPI score at enrollment
- use of medical therapy for gastroesophageal reflux
- ethnic background
- sex
- smoking history (current/ex-smoker vs. never smoker), given potential impact on oxidant status
- presence of emphysema > 25% on high-resolution computed tomography (HRCT)

IX. Interim Analyses

One interim analysis is planned to be conducted at approximately 0.50 information time. To conserve the overall type I error rate of 0.05 the O'Brien-Fleming Spending Function will be used to allow for stopping if large treatment effects are observed while allowing the final significance level to be conserved at the nominal level (Lan and DeMets 1983). Information time will be defined in two dimensions, the first is number of enrolled patients and the second is the 60 week follow-up. So the calculation of information time will be a summation or integration of a two dimensional space or area. To calculate information time the first step is to determine the number of weeks of possible follow-up for each enrolled patient at the time of the interim analysis. For example if a patient has completed 60 weeks then 60 would be their results or if a patient was randomized 21 days ago then their result would be 3. The second step is to add up number of weeks of possible follow-up from the first step and divide by 60 and total number of planned patients. For the planned interim analysis or any interim analysis the primary efficacy analysis will not be assessed at the alpha of 0.05 in the testing procedure descripted in “Primary Endpoint” section. Instead the interim significance level alpha determined from the spending function will be used in the testing procedure.

O’Brien-Fleming Group Sequential Boundaries

One Interim Analysis and a Final Analysis		
Information Time	**Bound for |Z| Statistic**	**Cumulative Alpha**
0.50	2.9626	0.00305
1.00	1.9686	0.05000

I. References

Lan KKG, DeMets, DL. Discrete sequential boundaries for clinical trials. Biometrika. 1983;70:659-663.

PANTHER SAP Changes

On 14OCT2011 the DCC received a memorandum from the Executive Secretary of IPFnet DSMB regarding the recommendations for investigators of the PANTHER-IPF study from DSMB meeting 12OCT2011 conference call. The protocol was rewritten such that the study would continue with double blind randomization into the N-acetylcysteine and placebo arms. Changes to the conduct of the trial were made to the protocol.

Summary of changes

- Analysis population is change from all randomized patients to all randomized pateints to either the N-acetylcysteine and placebo arms.
- The primary analysis was modified from a 3 treatment group analysis to a 2 treatment group analysis. The same model and assumptions was used. The multistage LSD procedure was removed. Only the N-acetylcysteine and placebo arms were compared.
- The secondary analyses for longitudinal mixed models were modified in the same manner as the primary analysis. Only the N-acetylcysteine and placebo arms were compared.
- The remaining secondary analyses and safety analyses were originally written as pairwise comparisons of the treatment groups. These sections were modified to only compare the N-acetylcysteine and placebo arms.