

Effects of Roflumilast in Hospitalized COPD on Mortality and Re-hospitalization

Submitted by

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1. Research plan.

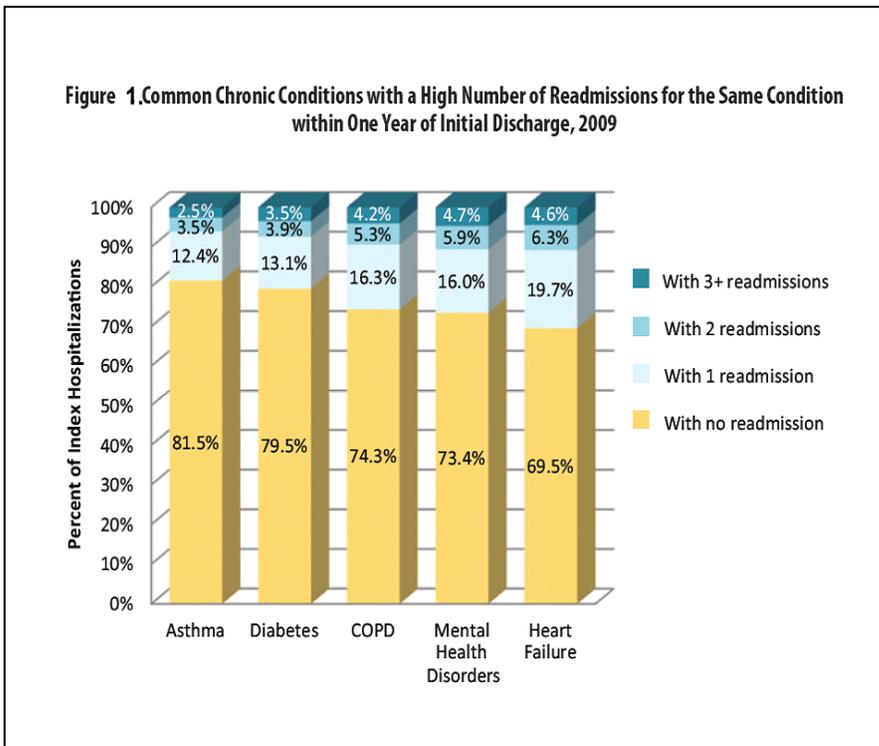
1.A. Background Chronic obstructive pulmonary disease (COPD) currently afflicts 24 million US residents and is the 3th leading cause of death in the United States {{164 Anonymous 2001; 368 Mannino,D.M. 1997}}. Each year, 150,000 patients die from

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COPD in the U.S. or about one patient every 4 minutes. Health care costs for COPD patients in the US is approximately \$6.5 billion/ year, but its indirect costs probably double the costs of care when taking into account lost productivity and caretaker expenses {{465 Sin,D.D. 2002; 378 Miravittles,M. 2002; 473 Sullivan,S.D. 2000}}. COPD exacerbations add considerably to that burden because they: (1) cause frequent hospital admissions and relapses or readmissions, (2) contribute directly to the death of many patients, either during hospitalization or shortly thereafter, (3) cause patients significant stress, prolonged physical discomfort, disability and dramatically reduced quality of life, (4) consume the majority of the resources available to manage this chronic condition, (5) frequently progress to a severe stage warranting hospitalization before any abortive treatment is instituted, and (6) may hasten the progressive loss of lung function, a steady decline that is a cardinal feature of COPD itself {{280 Garcia-Aymerich,J. 2003; 452 Seemungal,T.A. 1998}}. Two studies have estimated that acute exacerbations of COPD (AECOPD) account for 31 to 68% of the total costs of COPD care in the US {{378 Miravittles,M. 2002; 294 Halpern,M.T. 2003}}. Hence treatment that could prevent or ameliorate frequent or severe AECOPD could significantly lessen COPD morbidity and mortality as well as costs.

Hospitalized exacerbations are particularly relevant in COPD patients. Hospitalized exacerbations result in a profound impact on patient survival, function, symptoms and health status as well as costs Hospitalizations accounted for a significant component of COPD related costs. Re-hospitalization in COPD is frequent and associated with a particularly negative impact. Patients discharged from the hospital after a COPD exacerbation have a high mortality and are frequently readmitted with recurrent exacerbations. The cause of these high rates of readmission is not well understood. Although a number of pharmacologic and behavioral interventions have been used to decrease exacerbations in COPD, it is not clear that these same interventions are successful in reducing hospital admission rates or re-admission rates. Except for the use of noninvasive ventilation in patients that present in acute respiratory failure during COPD hospitalization, no new therapies have been discovered in the last 3 decades. Hence, investigations of new therapies to treat COPD patients who are hospitalized with a severe exacerbation are desperately needed.

1. B. Why survival and readmission rates are desirable outcomes for studying interventions in hospitalized acute COPD exacerbations. The Pennsylvania Cost Containment Council (PHC4) reported in 2012 using a statewide database of several million Pennsylvania residents hospitalized in FY 2009 that COPD was the third most common cause of readmission within the year post discharge (25.7%) and that 9.5% of patients were readmitted more than once in the year following hospitalization. In fact, 4.2 % of the COPD patients were readmitted 3+ times in the year post hospitalization. (Figure 1).



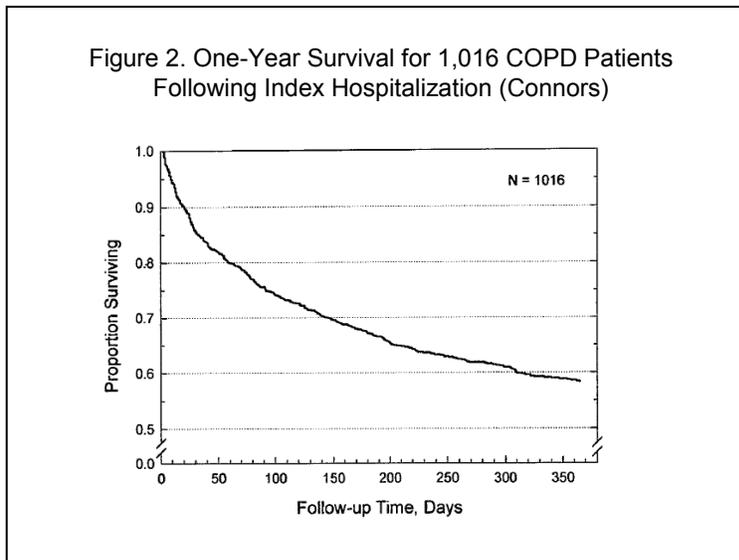
Others have reported similarly high readmission rates following COPD hospitalization as enumerated in the Table 1:

Table 1. Readmission rates following COPD Hospitalization

Citation	Study Structure	30 day readmission	60 day readmission	90 day readmission
Lau et al 2001	Retrospective	~15%	~25%	~30%
Almagro et al 2002	Prospective	~5%	~10%	~13%
Roberts et al 2002	Retrospective	34% 14% respiratory	NA	NA
Westert et al 2002	Retrospective	USA 4.1-1.3% Europe 8.5-13.4%	NA	NA
Almagro et al 2005	Prospective	16%	~25%	35%
Schiotz et al 2011	Retrospective	21.4 in 2007	NA	NA
Nansupawat et al 2012	Retrospective	13.6%	NA	NA
Moullec et al 2012	Prospective	~20%	~30%	~40%

Dinesen et al 2012	Prospective	~5%	~6%	~10%
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The rate of 30 day readmissions in these reports varies from 5-34% with the majority of studies suggesting that approximately 15% suffer such an event in that time frame. This readmission rate increases further by 60 days (6-30%) and 90 days (10-40%). Data from the AHRQ using the HCUP database found that 20.5% of U.S. COPD patients were readmitted within 30 days and that 85% of these included COPD as the primary or secondary diagnosis).{{1159 Elixhauser,A. 2011}} A summary of published literature suggests that readmissions are frequently seen in this population.



Survival is another extremely relevant endpoint to study the effect of an intervention during COPD hospitalization. The Study to Understand Prognosis and Preferences for Outcomes and Rates of Treatment (SUPPORT enrolled patients admitted with COPD exacerbations and reported an in-hospital mortality of 11% in patients presenting with acute hypercapneic respiratory failure.{{1152 Connors,A.F.,Jr 1996}} The 180 day mortality was 33% and the 2-year mortality was 49%. Several

other studies have reported in-hospital mortality rates of 11-24% and 22-35% after one and two year's follow-up respectively (Figure 2). The 2010 PHC4 dataset reports the average inpatient mortality rate for COPD readmissions to be 3.9%, but ranges from non-complicated readmission mortality rates of 1.45 % to 5.0% if pneumonia is the cause of readmission in a patient initially admitted with COPD or even higher at 11.6% mortality if respiratory failure is the cause for readmission.

These data demonstrate that readmission and survival following COPD hospitalization are clinical relevant and meaningful endpoints that occur with increased frequency following the index hospitalization.

2. Research plan.

2.A. What drives the increased morbidity and mortality associated with hospitalized acute COPD exacerbations? The inflammation associated with COPD, especially the heightened inflammation that occurs during hospitalized AECOPD, may contribute to the higher rates of morbidity and mortality. Data that demonstrates that inflammation is heightened in stable COPD and especially during an exacerbation is robust and identifies multiple potential responsible inflammatory cell lines, chemokines and cytokines. Elevations in serum systemic inflammatory mediators correlate with the

presence or worsening of COPD. A meta-analysis of 14 studies with data on systemic inflammation markers (e.g., CRP, fibrinogen, IL-6) and COPD confirmed the association between reduced lung function and systemic inflammation {{278 Gan,W.Q. 2004}}. COPD exacerbations are linked to increased signals of systemic oxidative stress (e.g., higher serum lipid peroxidation byproducts; up-regulated CD11/CD18 neutrophil adhesion molecules; increased cytochrome oxidase activity in lymphocytes and skeletal muscle {{420 Rahman,I. 1996; 395 Noguera,A. 1998; 442 Sauleda,J. 1998}}. CRP can induce pro-inflammatory cytokine and chemokine expression and the up-regulation of adhesion molecules that may promote lung inflammation {{411 Pepys,M.B. 2003}}. CRP levels appear to correlate with the future risk of morbidity and mortality in COPD {{364 Man,S.F. 2006; 239 Dahl,M. 2007}}. One group has reported that CRP is the biomarker that correlates best with the presence of a symptomatic exacerbation {{311 Hurst,J.R. 2006}}. Acute exacerbations that are slow to resolve are associated with higher CRP levels. A large population based study confirmed a significantly increased risk of hospitalization in COPD patients with CRP levels above 3 mg/L {{412 Perera,W.R. 2007; 239 Dahl,M. 2007}}.

IL-6 is also considered part of the acute phase response and appears to be a potent inducer of CRP expression in the liver. IL-6 levels are increased in sputum, bronchoalveolar lavage fluid and blood in COPD and are even further increased during periods of exacerbation {{364 Man,S.F. 2006; 198 Bhowmik,A. 2000; 207 Bucchioni,E. 2003; 518 Wedzicha,J.A. 2000}}. As with CRP, elevated IL-6 levels are associated with decreases in pulmonary function and a higher number of exacerbations per year {{260 Donaldson,G.C. 2005; 517 Wedzicha,J.A. 2002}}.

Additional pro-inflammatory mediators may be implicated based on published evidence of a correlation in the elevations in these biomarkers with disease severity {{208 Calikoglu,M. 2004;311 Hurst,J.R. 2006; 518 Wedzicha,J.A. 2000; 205 Bozinovski,S. 2008 }}. Activated bronchial epithelial cells, macrophages and neutrophils produce IL-8 {{471 Spruit,M.A. 2003;518 Wedzicha,J.A. 2000; 205 Bozinovski,S. 2008; }} , which is a potent chemoattractant for neutrophils and monocytes and likely contributes to the mobilization of these cells into the lung. IL-8 levels are increased in the sputum of COPD patients and correlate with the intensity of the neutrophilic response. TNF- α is a critical pro-inflammatory molecule that is believed to primarily work by inducing a broad spectrum of pro-inflammatory cytokines, chemokines, proteases and adhesion molecules by activating transcriptional activating factor p38 mitogen-activated protein kinases and nuclear factor (NF)- κ B in macrophages and epithelial cells {{205 Bozinovski,S. 2008;471 Spruit,M.A. 2003; 518 Wedzicha,J.A. 2000; 529 Yamamoto,C. 1997; 235 Crooks,S.W. 2000; 288 Gompertz,S. 2001 }}. IL-15 is important for the function of CD8 positive cells {{346 Ku,C.C. 2000; }} , an important inflammatory cell population in the lung in COPD. IL-17 is now understood to be a very critical pro-inflammatory cytokine, produced by highly inflammatory CD4 and CD8 T cells, which promotes the recruitment of numerous inflammatory leukocyte populations, and induces the expression of mucin by lung epithelial cells{{538 Oda,N. 2005;539 Chen,Y. 2003; 540 Hashimoto,K. 2004; }}. IL-18 is produced by inflammatory macrophages, and induces the production of chemoattractant factors including IL-8 (which attracts neutrophils and monocytes/macrophages to the site of inflammation){541 Imaoka,H. 2008;542 Petersen,A.M. 2007; 543 Hoshino,T. 2007; }} , and the expression of additional pro-inflammatory mediators such as GM-CSF and IL-6. Pulmonary and activation-regulated chemokine (PARC/CCL18) is produced at high levels in the lung,

and is up-regulated with activation of alveolar macrophages, and strongly recruits both CD4 and CD8 T cells to sites of inflammation. MIP1-1 (CCL23) is produced by activated macrophages, and acts to attract both monocytes and T cells to inflamed tissue, including the lung. Eotaxin-2 (CCL24) is produced in part by alveolar epithelial cells in response to pro-inflammatory cytokines such as TNF α , and may reflect the degree of both the local inflammatory status, and the state of epithelial cell activation in the diseased lung. Surfactant protein D (SP-D) is a large hydrophilic protein that is a member of the collagen containing C-type lectins or collectins and plays a role in protecting against viral infection, in clearing bacteria, fungi and apoptotic cells, and in resolving inflammation{{337 Kierstein,S. 2006;342 Kishore,U. 2006; }}. Serum SP-D is elevated in COPD compared to current and former smokers with airflow limitation and that serum SP-D levels greater than the 95th percentile of non-smokers are at increased risk for exacerbation in the subsequent 12 months {{357 Lomas,D.A. 2009; }}.

Inflammation associated with COPD exacerbation may be responsible for some of the non-pulmonary manifestations of an acute exacerbation and may explain some of the morbidity and mortality associated with these events. Cardiac events (e.g., arrhythmias, acute myocardial infarction, and unstable angina) are a major cause of morbidity and mortality in COPD and vascular dysfunction is commonly observed in even stable COPD {{374 McAllister,D.A. 2007; 532 Zureik,M. 2001; 483 Tillie-Leblond,I. 2006; 319 Janssens,J.P. 2001; 237 Curkendall,S.M. 2006; 297 Hansell,A.L. 2003; 262 Engstrom,G. 2002; 263 Engstrom,G. 2001; 372 Marcus,E.B. 1989; 413 Persson,C. 1986; 274 Friedman,G.D. 1976}}. Donaldson et al has recently reported that acute myocardial infarctions and cerebrovascular accidents more likely to occur 1-2 months following an acute exacerbation and correlate with increases in IL-6 and fibrinogen.{{1163 Donaldson,G.C. 2010}} Brekke and colleagues have shown that elevations of cardiac specific troponin are frequently present in acute COPD exacerbations and levels inversely correlate with long-term survival. {{1162 Brekke,P.H. 2009}}Flow mediated dilation (e.g., measure of vascular function) is impaired in stable COPD outpatients and is more impaired with worsened airflow obstruction or increased systemic inflammation {{536 Eickhoff,P. 2008; 187 Barr,R.Graham 2007; 537 Moro,L. 2008}}. We have recently reported that during an acute hospitalized exacerbation, COPD patients have markedly impaired flow mediated dilation that is worse than that reported in other disorders associated with severe systemic inflammation such as rheumatoid arthritis. {{1153 Marchetti,N. 2011}}

Increased thrombotic events are also found in COPD and those experiencing an acute hospitalized exacerbation.{{1144 Rizkallah,J. 2009}} An autopsy study in 46 patients who died within 24 hours of AECOPD hospitalization suggested cause of death as CHF in 37% and VTE in 21% {{534 Zvezdin,B. 2009}}. VTE occurs 5 times more commonly in COPD and can be an unexpected cause of AECOPD in up to 25% of hospitalized patients {{483 Tillie-Leblond,I. 2006}}. We have shown that COPD patients have increased levels of procoagulant factor VII levels and thrombin anti-thrombin products that indicate a heightened state of thrombosis.{{361 Vaidyula,V.R. 2009}} Others have reported denser fibrin complexes in COPD compared to age-matched controls. {{1161 Undas,A. 2009}} COPD, cardiac disease and VTE are closely coupled by shared risk factors (older age and smoking) as well as common pathophysiological features (presence of acute inflammation). Inflammation is present in all COPD stages and a variety of systemic inflammatory markers elevated in cardiovascular disease and VTE (IL-6, IL1 - β , TNF- α , MMP-9, MCP-1 and CRP) are also elevated in COPD{{278

Gan,W.Q. 2004; 260 Donaldson,G.C. 2005; 455 Shapiro,S.D. 2001; 426 Retamales,I. 2001; 434 Rutter,M.K. 2004; 340 Kinlay,S. 2003; 247 de Torres,J.P. 2008; 219 Churg,A. 2004; 528 Yamada,S. 2001}}

As described below, therapies such as PDE-4 inhibitors like roflumilast that can decrease lung and systemic inflammation may be useful in decreasing the acute pulmonary as well as non-pulmonary morbidity and mortality that is associated with severe COPD exacerbations that require hospitalization.

2.B. PDE-4 Inhibitors and specifically Roflumilast may have beneficial anti-inflammatory effects in the lung that may be relevant for COPD patients during an acute severe hospitalized exacerbation.

PDE-4 inhibition has consistently demonstrated a reduction in activity of several pro-inflammatory cells that are involved in the pathophysiology of COPD. There is some evidence that PDE-4 inhibition can suppress pro-inflammatory cell recruitment and chemokine and cytokine production.

Clinical data has supported the notion that roflumilast via its PDE-4 inhibition pathway may have important effects on decreasing exacerbation in COPD. Calverley has shown in COPD patients with moderate to severe disease that roflumilast for one year results in a small but significant improvement in FEV₁ and moderate reduction in exacerbation rate {{ Calverley, AJRCCM, 2007}} Fabbri et al has reported the effects of roflumilast vs. placebo in addition to salmeterol or tiotropium and found that roflumilast use was associated with sustained improvements in FEV₁ and reductions in exacerbations rates.{{ Fabbri, 2009, Lancet}} Calverley again reported in two additional studies that roflumilast use compared to placebo was associated with significant but modest increase in FEV₁, but more impressively, substantial reduction in both moderate and severe exacerbations. {{Calverley, Lancet, 2009}}. These data highlight the potential importance that roflumilast may have on decreasing the intensity of symptoms around the time of an acute severe exacerbation that requires hospitalization and its potential benefit on reducing mortality and the need for readmission.

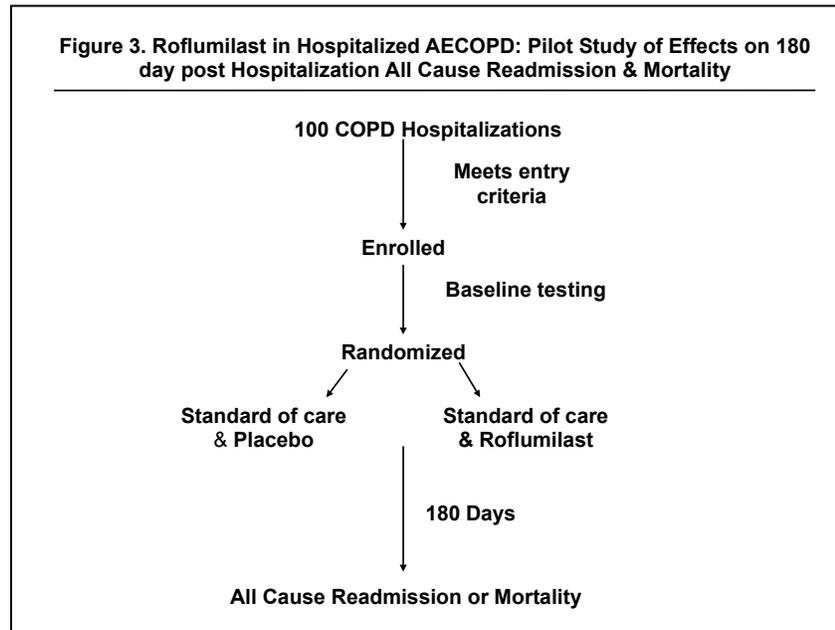
3. Study design

3. A. Specific Aims: In this **pilot** proposal, we will test the feasibility of roflumilast to decrease all cause readmission and mortality 180 days after hospitalization for acute COPD exacerbation. We propose to conduct this study in 100 patients at three centers to assess the tolerance and treatment effect of roflumilast in order to power an appropriate definitive phase III multicenter trial.

Our ultimate specific aims in the **phase III trial** will be to assess: **1) Primary aim:** Evaluate the effects of roflumilast 500 ug daily in hospitalized AECOPD patients on time to all-cause mortality or re-hospitalization during the 180 days following randomization and; **2) Secondary aims:** Evaluate the effects roflumilast 500 ug daily in this population on the following outcomes: respiratory-related death or re-hospitalization during the 180 days following randomization; rate of death or readmission during the 30 days post-discharge; treatment failure (see definition below); change in health status, FEV₁, and dyspnea during the 180 days following randomization; post-randomization ICU admission, need for non-invasive or invasive mechanical ventilation, and length of

hospital stay during the index hospitalization and; **3) Other:** Assess tolerance of roflumilast vs. placebo in hospitalized AECOPD

3.B. Study Design and Synopsis: Parallel-group, prospective, randomized, double blind, placebo-controlled trial of roflumilast 500 ug daily vs. placebo in approximately 100 hospitalized AECOPD patients (Figure 3). Both groups will receive GOLD guideline-recommended care.



Outcomes:

In this pilot proposal, 100 patients at three centers will be evaluated to test the feasibility of conducting this study and to assess the treatment effect of roflumilast to be able to power an appropriate definitive phase III multicenter trial. Our ultimate aims of the phase III trial will be:

1) Primary: time to all-cause mortality or re-hospitalization during the 180 days post-randomization. **2) Secondary:** respiratory death or respiratory re-hospitalization during the 180 days post-randomization; rate of death or readmission during the 30 days post-discharge; treatment failure (see definition below); change in health status, FEV₁, and dyspnea during the 180 days post-randomization; length of hospital stay during the index hospitalization. **3) Other:** assess tolerance of roflumilast vs. placebo in hospitalized AECOPD.

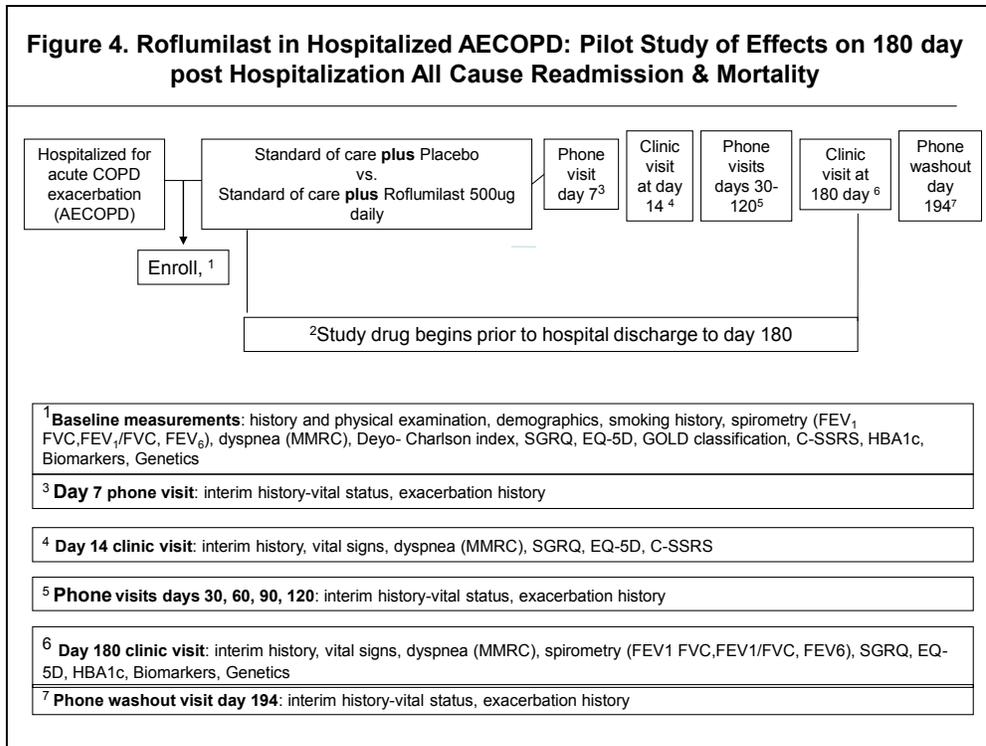


Figure 4 shows the overall study design, proposed study measurements, and time points for assessment. Patients hospitalized with AECOPD will be eligible for enrollment during their hospitalization with treatment being started before discharge from the hospital.. Demographics, blood tests, health related quality of life, comorbidity (Deyo-Charlson index), post bronchodilator spirometry, vital signs, dyspnea measured by MMRC, SaO₂ and amount of inspired O₂ to maintain SaO₂> 90% at rest, serum fibrinogen levels, HBA1c, Biomarkers and Genetics will be obtained after enrollment and then patients will be randomized to standard AECOPD care plus roflumilast 500 ug daily vs. placebo. Patients will begin roflumilast or placebo ≤ 12 hours of hospitalization for a total period of 180 days post enrollment. On discharge day (approximately day 3-4 after admission based on the recent COPD CRN zileuton study of hospitalized AECOPD), the measurements will be repeated as indicated (baseline measurements), with follow-up phone assessments at days 7, 30, 60, 90, 120 and 194 days post enrollment. An in-person clinical visit will be conducted at days 14 and 180 post randomization.

4. Methods:

4. A. Study Population. 100 AECOPD patients hospitalized at Temple University Hospital, Temple University Episcopal Hospital Campus, Jeanes Hospital and St Mary's Hospital.

Inclusion Criteria. Primary diagnosis of AECOPD defined as acute increase in dyspnea, sputum volume, and/or sputum purulence without other identified cause; consent obtained and treatment started before discharge from the hospital ; patient age >40, < 80 years old; cigarette smoking ≥ 10 pack-years; informed written consent.

Exclusion Criteria. Prior diagnosis or high suspicion for asthma based on investigator judgment; pulmonary edema, pneumonia, interstitial lung disease or significant

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bronchiectasis based on admission chest x-ray; intubated and mechanically ventilated at the time of evaluation; active liver disease, or transaminase elevations ($\geq 3 \times \text{ULN}$); history of alcoholism or heavy ethanol use; history of suicidal behavior ≤ 2 years or suicidal ideation ≤ 6 months prior to enrollment; pregnant or lactating females. Those on the following excluded medications: P450 inducers (e.g., rifampicin, phenobarbital, carbamazepine, and phenytoin) and CYP3A4 inhibitors or dual inhibitors that inhibit both CYP3A4 and CYP1A2 simultaneously (e.g., erythromycin, ketoconazole, fluvoxamine, enoxacin, cimetidine).

4. B. Study Procedure

Study will begin before patient's discharge from the hospital and last for 194 days.

Baseline: Will occur during the duration of admission stay in the hospital. Patients will have a medical history taken as well as smoking history. Patients will also be given a physical exam including vital signs. A spirometry test will also be performed. Women with the potential to become pregnant will be given a pregnancy test. Data will also be collected in regards to dyspnea scales, Deyo-Charlson index, and GOLD classification. Patients will complete a Columbia Suicide Severity Rating Scale to exclude patients with a history of suicidal behavior ≤ 2 years or suicidal ideation ≤ 6 months prior to enrollment

Randomization: Patients who are eligible to enroll in this study will be randomized to one of two treatment groups. Randomization will be done using a randomized block design stratified by treatment center. One group will receive roflumilast 500 mcg (Daliresp®) and the other will receive a placebo tablet. Patients will begin one of these treatment arms prior to hospital discharge for a total period of 180 days post enrollment.

Day of hospital discharge: A spirometry test will be performed and an adverse event assessment will be completed.

Day 7: Phone Visit. Data to be collected includes: interim history, adverse events assessments, vital status, and exacerbation history. During this telephone visit the study doctor or nurse coordinator will ask how the patient is feeling and if they have needed to visit the doctor or go to the emergency room or hospital since the last time they were contacted. They will be asked how well they are tolerating the study drug.

Day 14: Clinic Visit. At this visit vital signs, C-SSRS test, interim history, adverse event assessment, vital status, and exacerbation history will be recorded. The following questionnaires will given: EQ-5D, the St. George's Respiratory Questionnaire (SGRQ), and the Modified Medical Research Council dyspnea score (MMRC).

Days 30, 60, 90, & 120: Phone Visit. Data to be collected includes: interim history, adverse events assessments, vital status, and exacerbation history. During each of these telephone visits the study doctor or nurse coordinator will ask how the patient is feeling and if they have needed to visit the doctor or go to the emergency room or hospital since the last time they were contacted. They will be asked how well they are tolerating the study drug.

Day 180: Clinic Visit. At this visit vital signs, C-SSRS test, interim history, adverse event assessment, vital status, and exacerbation history will be recorded. A spirometry test will be performed as well during this visit. The following questionnaires will given: EQ-5D, the St. George's Respiratory Questionnaire (SGRQ), and the Modified Medical Research Council dyspnea score (MMRC).

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Day 194: Phone Visit. This phone call covers the wash out period for the study treatments. Data to be collected includes: interim history, adverse events assessments, vital status, and exacerbation history. During this telephone visits the study doctor or nurse coordinator will ask how the patient is feeling and if they have needed to visit the doctor or go to the emergency room or hospital since the last time they were contacted.

4. C. Demographics and Medical History. Age, gender, body mass index (BMI), smoking history (debut, amount, duration), presence of comorbidities, current medical therapy (bronchodilators, steroids, oxygen), history of pulmonary rehabilitation, influenza and pneumococcal vaccination, emergency room visits and hospitalizations during last year, number of prior exacerbations in prior year, history of coronary artery disease, stroke, transient ischemic attacks, and peripheral vascular disease. The Deyo-Charlson index, a standard and validated measure of co-morbidity {{252 Deyo,R.A. 1992; }}, will be used to assess the impact of other chronic illnesses on outcome.

4. D. Spirometry. Spirometry will be performed (post bronchodilator administration) at enrollment (baseline) or as soon as the subject is able to perform it after hospitalization, at the day of discharge and then 180 days post randomization according to ATS guidelines. {{174 Anonymous American Thoracic Society. 1995}} We realize that all subjects may not be able to perform spirometry initially upon presentation, but Niewoehner has reported that spirometry was able to be performed in a multicenter trial in acute hospitalized COPD patients 96% of the time at some point during the initial hospitalization.{{1143 Niewoehner,D.E. 2000}}Data will be presented in absolute numbers and as percent of reference predicted values, using prediction equations from Crapo {{233 Crapo,R.O. 1981}} for whites and by multiplying Crapo's predicted values by 0.88 to correct for blacks' smaller lung volumes. Airflow obstruction will be defined by postbronchodilator measured $FEV_1/FVC < 70\%$ and $FEV_1 < 70\%$ predicted at time of inclusion and will be used to define GOLD Grades.{{154 Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines.}}

4. E. Measurement of Hospitalization Duration.

Hospitalization duration will be measured both as the actual days of hospitalization, and as the number of days from admission to the day when patients meet criteria for discharge as outlined by others {{1028 Stevenson,N.J. 2005;415 Pinto-Plata,V.M. 2007; 90 Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008;}}. These criteria include: 1) stable vital signs (respiratory rate < 25 breath/minute, heart rate below 100 beats/minute, oral body temperature between 97 to 100°F) 2) ability to walk 50 feet without disabling dyspnea; 3) $SpO_2 > 90\%$ with ≤ 4 LPM oxygen at rest; 4) use of β -agonist therapy $>$ every 4 hours; 5) no escalation of care within the previous 12 to 24 hours. We plan to use these criteria to define when patients can be discharged to calculate hospital duration rather than the number of hospitalization days to avoid the social and non-clinical obstacles that are inadvertently wrapped into length of stay (e.g., getting a ride home, too late in the day, need other support services, etc.).

4. F. Measurement of Treatment Failure During Initial Hospitalization.

We have modified the definition of Niewoehner {{387 Niewoehner,D.E. 1999}}as follows: treatment failure during the initial hospitalization will include all-cause death, need for intubation and mechanical ventilation, post-randomization use of noninvasive ventilation, or intensification of pharmacologic treatment. Intensification of pharmacologic treatment

will be defined as an increased dose of systemic steroids (higher dose or longer duration), increased use of antibiotics (change in antibiotic or longer course), addition of theophylline, increased liter flow of supplemental oxygen > 2 at rest, and increase in frequency or dose of inhaled bronchodilators.

4. G. Quality of Life and Functional Status. Patients will complete a general and disease-specific self-administered quality of life and functional questionnaires. We will use EQ-5D and the St. George's Respiratory Questionnaire (SGRQ). Both are validated tools extensively used in COPD.{{1167 Meguro,M. 2007}}{{1166 Pickard,A.S. 2008}}

4. H. Columbia–Suicide Severity Rating Scale (C-SSRS). The Columbia–Suicide Severity Rating Scale (C-SSRS) is a 2-page questionnaire that

prospectively assesses suicidal ideation and behavior using a structured interview face to face for patient responses. The C-SSRS will be performed during each evaluation visit according to the assessment schedule on Figure 4. Suicidal ideation is classified on a 5-item scale: 1 (wish to be dead), 2 (nonspecific active suicidal thoughts), 3 (active suicidal ideation with any methods [not plan] without intent to act), 4 (active suicidal ideation with some intent to act, without specific plan), and 5 (active suicidal ideation with specific plan and intent). The C-SSRS also captures information about the intensity of ideation, specifically the frequency, duration, controllability, deterrents, and reasons for the most severe types of ideation. Suicidal behavior is classified on a 5-item scale: 0 (no suicidal behavior), 1 (preparatory acts or behavior), 2 (aborted attempt), 3 (interrupted attempt), and 4 (actual attempt). The C-SSRS will be performed during each evaluation visit according to the assessment schedule (see Figure 4). During visit, patients will complete the assessment with the health professional. Patients who do not have suicidal behavior or ideation will answer a limited number of questions and will usually complete the assessment in approximately 3 minutes. Patients with significant suicidal ideation and/or behavior may require up to 10 minutes to answer all relevant questions.

The patient should not be released from the evaluation site until it is confirmed

that the patient is not considered to be at any suicidal risk and all responses negative to the C-SSRS.

Positive reports are generated for any of the following findings:

- Suicidal ideation with intention to act (score of “4”)
- Suicidal ideation with specific plan and intent (score of “5”)
- Made suicide attempt
- Interrupted suicide attempt
- Aborted suicide attempt
- Preparatory behaviors for making a suicide attempt

At Screening (Visit 1), the C-SSRS will be completed for the patient's lifetime history of suicidal ideation and behavior. At all other visits, the C-SSRS will be completed for

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ideation and behavior since the previous visit. The patient's responses should remain negative throughout the entire trial. If a possible exclusion alert is triggered

by a positive response at Screening (Visit 1), 2 additional questions at the bottom of the report will need to be asked to determine whether exclusion criteria 9a and/or 9b have

been met. A response of "Yes" to either one or both of the follow-up questions will exclude the patient from the trial and necessitate immediate psychiatric referral of the patient on the same date as the screening visit. For all subsequent visits after Screening (Visit 1), if the patient's response generates a change in the findings report from negative to positive, the patient should be referred for immediate psychiatric evaluation on the same date as the site visit and the PI will be contacted. Positive suicidality indication findings reports after the Screening Visit (Visit 1), if confirmed by the *Investigator*, should be considered to be an AE. SAEs must be *reported* as outlined in Section 6. Ultimately, the determination of suicidality and risk is up to the *Investigator*'s clinical judgment.

4. I. Measurements of Dyspnea. Dyspnea will be measured by use of the modified Medical Research Council Dyspnea score. This is a well-known, validated and easy to use scale that is a reliable and sensitive tool specific to COPD patients that will allow detection of a response to therapy as well as comparison to other studies reporting dyspnea as an outcome in other COPD trials.

4. J. Description of Optimized Standard Care for COPD Exacerbations. All patients will receive standardized, optimized care for AECOPD {{387 Niewoehner,D.E. 1999}}. Nebulized albuterol (2.5 mg in 0.5 ml, dilute to 3 ml NSS administered every 4-6 hours) and ipratropium bromide (0.5 mg in 0.5 ml, diluted with 2.5 ml of saline administered every 6 hours) will be given. A 14-day course of systemic steroids consisting of intravenous methylprednisolone 125 mg every 6 hours for up to 72 hours, followed by once daily oral prednisone 60mg/day for days 4-7, 40 mg prednisone days 8-11 and 20 mg prednisone days 12-14 will be provided. A 7-day course of antibiotics will be selected based on patient's medical allergy history and relevant culture data if available. Supplemental oxygen will be provided by route and dose to achieve maximum patient comfort and compliance to maintain a SaO₂ > 92%. Noninvasive positive pressure ventilation will be utilized at the discretion of the treating physicians but will follow accepted guidelines {{133 Keenan,S.P. 2003; 136 Lightowler,J.V. 2003}}.

4. K. Intervention with Roflumilast. An IND for using roflumilast will not be required because this is an FDA approved medication for oral use and we are not seeking a change in label indications.

4. L. Drug/Placebo supply. Roflumilast and matching placebo will be provided by Forest Laboratories and stored by the Investigational Pharmacy Unit at Temple University Hospital.

4. M. Pharmacokinetics of Roflumilast. Following oral administration, roflumilast is rapidly absorbed with a t_{max} of about 1 hour and an 80% bioavailability. Roflumilast shows linear dose-proportional pharmacokinetics over a dose range of 250-1000 ug and plasma disposition half-life is about 10-20 hours making once daily dosing a feasible option. roflumilast is metabolized by CYP3A4 and CYP1A2 enzymes with N-oxide being the principal major metabolite. N-oxide has selectivity for the PDE4 isoenzyme and

mainly accounts for roflumilast's in vivo bioactivity. No major interactions have been reported between roflumilast and other COPD medications.

4. N. Contraindications and side effects to the use of Roflumilast.

1. Contraindications: Roflumilast is contraindicated in patients with moderate or severe liver impairment (Child-Pugh class B and C)

2. Side effects: Adverse events experienced by > 3% of patients in clinical trials of roflumilast included diarrhea (9.5%), weight loss (7.5%), nausea (4.7%), headache (4.4%) and back pain (3.2%). Patients discontinued therapy due to adverse effects more frequently with roflumilast (14.8%) compared to placebo (9.9%). Diarrhea and nausea were the most common adverse reactions leading to drug discontinuation in prior trials. Serious adverse events that occurred more frequently with roflumilast compared to placebo included diarrhea, atrial fibrillation, lung cancer, prostate cancer, acute pancreatitis acute renal failure. Less common adverse events, (1-2%) of patients that were more frequently reported with roflumilast use as compared to placebo included abdominal pain, anxiety, heartburn, depression, gastritis, rhinitis, sinusitis, muscle spasms, tremor, urinary tract infection, and vomiting. Roflumilast carries a warning for increased psychiatric adverse events and suicidality. Patient should be monitored for changes in mood including worsening insomnia, anxiety or depression or suicidal ideations. Roflumilast also carries a warning for weight loss. 20% of patients may experience moderate weight loss between 5 to 10% of ideal body weight compared to 7% with placebo.

Roflumilast side effects reported in ≥ 2% of recipients from 8 clinical trials as listed in the manufacturer's package insert are listed in the table below.

Treated with Roflumilast 500 mcg daily and Greater Than Placebo Treatment		
Adverse Reactions	Roflumilast	Placebo
(Preferred Term)	(N=4438)	(N=4192)
	n (%)	n (%)
Diarrhea	420 (9.5)	113 (2.7)
Weight decreased	331 (7.5)	89 (2.1)
Nausea	209 (4.7)	60 (1.4)
Headache	195 (4.4)	87 (2.1)
Back pain	142 (3.2)	92 (2.2)
Influenza	124 (2.8)	112 (2.7)
Insomnia	105 (2.4)	41 (1.0)
Dizziness	92 (2.1)	45 (1.1)

Decreased appetite	91 (2.1)	15 (0.4)
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Other side effects reported in 1- 2% of recipients include: Gastrointestinal disorders (abdominal pain, dyspepsia, gastritis, vomiting); Infections and infestations (rhinitis, sinusitis, urinary tract infection); Musculoskeletal and connective tissue disorders (muscle spasms); Nervous system disorders (tremor); and Psychiatric disorders (anxiety, depression).

4. O. Drug Interactions with Roflumilast.

1. Drugs That Induce Cytochrome P450 (CYP) Enzymes

Strong cytochrome P450 enzyme inducers decrease systemic exposure to roflumilast and may reduce the therapeutic effectiveness of roflumilast. Therefore the use of strong cytochrome P450 inducers (e.g., rifampicin, phenobarbital, carbamazepine, and phenytoin) with roflumilast is not recommended and these medications will be excluded from use in the trial.

2. Drugs That Inhibit Cytochrome P450 (CYP) Enzymes

The co-administration of roflumilast (500 mcg) with CYP3A4 inhibitors or dual inhibitors that inhibit both CYP3A4 and CYP1A2 simultaneously (e.g., erythromycin, ketoconazole, fluvoxamine, enoxacin, cimetidine) may increase roflumilast systemic exposure and may result in increased adverse reactions.

Prior studies have shown that the overall pattern of adverse events in patients with or without roflumilast and long acting beta agonists use is similar. There's no indication from prior studies that roflumilast increases long acting beta agonist related adverse events such as tachycardia or cardiovascular events, and that concomitant long acting beta agonist use does not increase the frequency of events associated with roflumilast treatment alone.

4. P. Assessment of Safety

1. Definition of adverse event.

An adverse event is any untoward medical experience occurring in a subject doing the trial whether or not is related to study drug. An adverse event includes a concurrent illness, injury, or any other aspect of the subject's health, as well as abnormal laboratory findings found to be of clinical significance. An adverse event includes worsening of an existing symptom or condition or post treatment events that occur as a result of protocol – mandated procedures.

An adverse event may be a disease, a set of related symptoms or signs, or single symptom or sign including an abnormal laboratory value.

For the purposes of this trial, all adverse events will be collected including adverse events that occurred subjects were permanently off of study drug but who continue to be followed for outcomes.

A serious adverse event is an adverse event occurring any does that results in any of the following, whether or not it is related to study drug: death, immediately life-

threatening circumstance, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability.

Life-threatening means that the subject in the view of the investigator was thought to be at immediate risk of death from the event as it occurred.

Serious events will be collected, including serious adverse events occurring when subjects were permanently off study drug but who continue to be follow-up for outcomes.

2. Unanticipated problems.

An unanticipated problem is any incident, experience, or outcome involving risk to subjects that meets all of the following criteria: unexpected in nature, severity or frequency; related or possibly related to participation in the research; and suggests that the research places subjects or others at a greater risk of harm.

3. Relatedness or causality.

Causality is defined as definitely, probably, possibly or not related to the investigational product:

- Not related, event clearly related to other factors
- Possibly related, sequence of events is compatible with study procedure but could have been produced by other factors
- Probably related, sequences of events that is compatible with study procedure that cannot be explained by other factors without much doubt
- Definitely related, sequence of events that is compatible with study related procedure and cannot be explained by other factors beyond a reasonable doubt.

4. Severity or intensity definition.

Severity describes the intensity of a specific event and is not related to whether or not it is a serious adverse event. Severity is defined as:

- Mild: symptoms barely noticeable or does not make the subject uncomfortable.
- Moderate: symptom of sufficient severity to make the subject very uncomfortable.
- Life-threatening: symptom of a sufficient severity to cause the subject to be in immediate risk of death. Treatment for the symptom may be given.

5. Documentation of adverse events.

Any adverse event, serious adverse event, or unanticipated problem occurring during the study must be documented in the subject source documents on the appropriate case report form page. Information relating to the adverse event such as onset, cessation date and times, intensity, seriousness, relationship to study drug and outcome is also documented in the case report form.

All adverse events will be followed until resolution, stabilization or until they are judged by the investigator to be no longer be clinically significant. All serious adverse events will be followed until resolution, stabilization, death, or subject withdrawal. Case report forms will be updated with any new or additional information as appropriate.

6. Reporting responsibilities of the investigator.

All serious adverse events, regardless of expectedness or causality will be reported by the site immediately (no later than 24 hours after occurrence to the sponsor, Temple IRB in accordance with IRB regulations and guidelines of the Temple IRB.

Reporting of Serious Adverse Events to Forest Research Institute (FRI)

Serious Adverse Event (SAE)

A serious adverse event is one that:

- Results in death
- Is an immediate threat to life
- Requires inpatient hospitalization, or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect.

Causality Assessment

For all AEs, the Investigator must provide an assessment of causal relationship to the IP. Causal relationship must be assessed by answering the following question:

Is there a reasonable possibility the IP caused the event?

Yes: There is evidence to suggest a causal relationship between the drug and the adverse event (ie, there is a reasonable temporal relationship, and/or the events are unlikely to be attributable to underlying disease, other drugs, or other factors). Dechallenge and/or rechallenge (if available) is positive

No: The relationship is unlikely or nonexistent (ie, there is no reasonable temporal relationship and/or the events are likely to be manifestations of underlying diseases, or commonly occur in the study population independent of drug exposure or other drugs/factors provide plausible explanations for the events), or the patient did not take the investigational product.

The Sponsor (Principal Investigator) is required to inform Forest Global Drug Safety of all SAEs. Forest Global Drug Safety must be notified immediately regarding any SAE that occurs after informed consent is obtained.

The Principal Investigator must report the event within 24 hours of first knowledge of any AE that meets one of the criteria for an SAE, to Forest Global Drug Safety on an SAE report form. If, during follow-up, any non-serious AE worsens and eventually meets the criteria for an SAE, that AE should be recorded as a new SAE.

The study center must transmit the SAE report form to the SAE fax number **(631) 858-7906** within 24 hours of first awareness of the event at the study center.

Supplemental information shall be submitted as soon as available and may include laboratory results, radiology reports, progress notes, hospital admission and emergency room notes, holding and observation notes, discharge summaries, autopsy reports, and death certificates.

Reporting of Pregnancy to Forest Research Institute

Study center personnel must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of first awareness of the pregnancy to the pregnancy fax number, **(631) 858-7906**), even if no AE has occurred, and follow it to term. If, however, the pregnancy is associated with an SAE (eg, if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed.

Fax all relevant SAE or pregnancy information to Forest Global Drug Safety. Contact information for Forest Global Drug Safety, personnel is as follows:

FOREST GLOBAL DRUG SAFETY DEPARTMENT

Fax: (631) 858-7906

4. Q. Assessment of effectiveness.

The present proposal is a pilot study designed to test the feasibility of a future project to be conducted in a multicenter trial in hospitalized patients, to determine the tolerance of roflumilast in hospitalized COPD patients, and to determine the treatment effect of roflumilast to decrease all-cause mortality and all cause readmission rate as add-on therapy. The specific aims will be:

1. Determine the tolerance of roflumilast in hospitalized COPD patients
2. Determine the treatment effect of roflumilast as add-on therapy to decrease all cause readmission and mortality as a composite endpoint 180 days after randomization to roflumilast or matching placebo \leq 12 hours after hospitalization for acute COPD exacerbation.

4. R. Statistical analysis plan.

The primary analysis for all study outcomes will be performed on an intention to treat basis. In the intention to treat approach, all subjects are analyzed in the group to which they were randomized, regardless of whether the assigned treatment was adhered to and regardless of whether there were any eligibility violations.

1. Analysis of baseline characteristics.

Univariate descriptive statistics will be calculated for baseline parameters overall and by treatment group. For categorical outcomes, these will include the number percentage of subjects in each category. For continuous variables, these will include the mean and standard deviation for variables with approximately normal distributions and the median and 25th and 75% percentiles for variables with skewed distributions.

2. Analysis of the primary endpoint. The primary outcome is time from randomization to a composite outcome of all cause re-hospitalization and all-cause mortality, whichever comes first. The primary analysis will be a log-ranked test and associated Kaplan-Meier plot, unadjusted for any covariates. Censoring will occur at the earliest date of any of the following occurrences, unless the subject has already experienced the primary outcome event:

- Lost to follow-up despite intensive efforts

- Subject or healthcare proxy withdrawal of consent
- 180 days after randomization.

3. Analysis of secondary outcomes.

Primary analysis of other time to event outcomes will be performed using unadjusted log rank tests that have associated Kaplan-Meier plots, similar to the primary analysis of the primary study outcome. Secondary analysis of these outcomes will use Cox regression to adjust for baseline subject factors (covariates) that are known to be associated with the outcome.

The primary analyses of changes in continuous measures such as absolute and percent changes in spirometry and dyspnea scores, for example, will be analyzed using an analysis of covariance where change from baseline to a particular time period is the dependent variable.

4. Assessment of treatment effect

In this pilot proposal, the treatment effect of roflumilast vs. placebo to prolong the time to event of a combined endpoint of death or re-hospitalization will be assessed in an intention to treat manner. In a similar manner, the treatment effect of roflumilast vs. placebo to effect all of the secondary endpoints proposed to be measured in the pivotal phase III trial will also be measured (time to respiratory death or respiratory re-hospitalization during the 180 days post-randomization; rate of death or readmission at 30 days post-discharge; treatment failure [see definition below]; change in health status, change in FEV₁, and dyspnea during the 180 days post-randomization; length of hospital stay during the index hospitalization; and the tolerance of roflumilast vs. placebo in hospitalized AECOPD).

Because this is a pilot study the intent is to see if there is a signal that would justify a larger clinical trial. Therefore the significance level has been set to 0.1 and the power has been set at 0.7. A total of 100 patients will enter this two treatment parallel-design study. The probability is 70 percent that the study will detect a treatment difference at a two sided 10.0 percent significance level, if the true hazard ratio is 1.654. This is based on the assumption that the accrual period will be 36 months and the follow up period will be 6 months and the median time to event is 8 months. The total number of events will be 73.

5. Strategy for Efficient Recruitment Strategies in Effects of Roflumilast in Hospitalized COPD on Mortality and Re-hospitalization

To ensure participation of a broad cross-section of patients and timely enrollment in this study, we will employ an aggressive and well-tested Temple recruitment strategy and we will collaborate with the other clinical centers. The Temple Lung Center has a strong record of clinical study recruitment and retention in the Philadelphia area, the greater Delaware Valley, throughout Pennsylvania, and from the entire mid-Atlantic region. Because we admit more AECOPD patients than any other hospital in Pennsylvania, we are especially well suited to serve as the recruitment anchor for the proposed study. We

currently maintain an IRB-approved clinical research registry with over 2500 COPD patients interested in clinical trials. Approximately one third of our COPD patients are black and over half are women. We have consistently succeeded in translating our high patient volumes and rich patient diversity into productive enrollment for national clinical trials. For COPD inpatient trials, potential research participants are identified during rounds on the pulmonary units, the emergency room, and on the pulmonary consult service. Internal medicine teams and case managers are contacted on a daily basis during the week. A listing of respiratory admissions is reviewed each day. Pulmonary section faculty members are regularly invited to local and regional meetings as well as hospital grand rounds where our research activities are showcased.

Because the proposed study seeks to enroll hospitalized COPD patients, we believe recruitment for this project will be challenging but achievable. This is why we propose a consortium of hospitals to achieve the recruitment endpoints. Temple Lung Center already has built a highly productive consortium of hospitals to participate in COPD trials such as the NHLBI long-term oxygen treatment trial (LOTT), simvastatin to prevent COPD exacerbations (STATCOPE), and our recently submitted NIH application for NIH RFP NHLBI-ECB-HR-2013-20-SS Engine for Fast Execution of Clinical Trials (EFFECT - Engine for Fast Execution of Clinical Trials). In both LOTT and STATCOPE, Temple leads recruitment of COPD subjects across all centers. In EFFECT, the planned focus is on hospitalized patients. For this study, we propose to use three of our consortium members from LOTT, STATSCOPE, and EFFECT.

St. Mary Medical Center in Langhorne, Pennsylvania, is licensed for 327 beds and is part of Catholic Health East, the second largest regional healthcare system in the Delaware Valley. Its staff of over 500 physicians provides care for more than 20,000 inpatients, 42,000 emergency department patients, and 150,000 outpatients annually. Over the past decade, St. Mary has participated in more than 50 sophisticated studies in cancer (e.g., Phase III trials of new treatments, pain management studies, and early detection studies), cardiology (e.g., protocols in acute coronary syndrome, unstable angina, myocardial infarction, and drug-eluting stents), osteoporosis, and diabetes. Drs. Lee and Serpico direct the largest pulmonology practices in the area and have associate full-time pulmonologists who staff St. Mary and maintain substantial in- and out-patient COPD practices. Drs. Lee and Serpico will serve as co-PIs at the St. Mary site.

Jeanes Hospital is a 176-bed, full-service acute-care hospital located in Northeast Philadelphia. Jeanes offers a complete range of medical and surgical care to the surrounding community. In 1996 Jeanes became a member of Temple University Health System and through this relationship it offers high-acuity medical services found at academic medical centers within the comfort of a local neighborhood hospital. Dr. Panetta, a recent graduate of the Temple Lung Center pulmonary and critical care fellowship program will serve as the PI at the Jeanes site. A set of recruitment tools and strategies will be produced and distributed for investigator and coordinator use. Temple University Hospital - Episcopal Campus is a full-service Emergency Department that provides high-quality care for 45,000 patients each year in North Philadelphia and is 3.5 geographic miles away from Temple University Main Campus. The Emergency department treats patients who have serious injuries as well as patients with more minor illnesses and injuries. The Emergency department is open 24 hours a day, 365 days a year and is staffed by board-certified Temple doctors and specially trained nurses. There is also a 21-bed inpatient medical/telemetry unit at Temple University Hospital -
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Episcopal Campus for patients who require a short hospital stay. Full-time registered nurses and hospitalists staff this modern unit. If required, the Emergency Department or 21-bed inpatient telemetry unit can provide patients with fast access to specialized doctors and services at Temple University Hospital. All COPD patients seen at the Episcopal Campus who need inpatient care will be transferred as inpatients to Temple University Hospital Main Campus for their COPD hospitalization.

Appendix:

Biomarker Blood

Biomarker specimens will be collected at baseline, every 6 months a participant is in the study, and at the last pre-washout visit.

Preparation

1. Label all vacutainers and cryo-vials before the participant arrives. Use white labels for vacutainer tubes and colored cryo-labels for vials. Your sheet of white labels includes labels for the routine lab tubes and genetics tubes which you should also label at this time. It is recommended to assemble all the labeled tubes and vials in a large zip-lock bag with a white label on it to reduce errors.
2. Confirm that the participant has read, understood, and signed a consent form.
4. Determine whether the participant has ever had a bleeding disorder. If yes, check with the local study investigator regarding safety of proceeding.

Blood Collection and Processing

1. Blood samples will be collected in one 10 ml purple top K2EDTA tubes
2. Tubes should be promptly mixed after being drawn by gentle inversion 8-10 times (do not shake).
3. *Centrifuge tubes at 1,100 RCF for 10 minutes.

*Note: You need to put a small spacer at the bottom of the sleeve when centrifuging lavender top tubes. Don't use something that will get stuck in the sleeve or stuck on the tube (a cap from another tube, solid side up, has been suggested). The reason for the spacer is that the design of the lavender cap doesn't allow it to sit at the bottom of the sleeve and the force of the centrifuging will separate the tube from its top. Be sure that the centrifuge is balanced. Always have equivalent tubes opposite each other in order to

achieve the balance. If a tube does not have an equivalent tube use a similar type of tube filled with water to achieve the balance.

4. After centrifugation of the lavender top tube(s), carefully remove the hemogard. Using a plastic transfer pipette, and being careful not to disturb the red or white cell layers, transfer the clear plasma into lavender-labeled vials (1 ml in each vial). Note that 1 ml is about half the vial. It is imperative for analysis that the first tube of plasma be harvested from the very top of the plasma layer. You must aliquot sequentially. The last aliquot may be less than 1 ml (depending on what volume is remaining in the lavender top tube), but that aliquot should also be saved. Thus, the initial vials will contain 1 ml of plasma, the last vial may contain less than 1 ml, and some vials may remain empty and can be discarded. Never 'top off' the last vial as contents will expand and squeeze out the cap as they freeze. Always use a different pipette for every source tube. Remember that it's more important to get a pure sample than to get every last drop of plasma .

5. When filling vials, do not try to spread the contents equally across all available vials. Fill each vial to at least the 1 ml mark and put the remainder (< 1 ml) in the last vial. In most cases you will fill fewer vials than you have labeled.

6. If the plasma or serum has a slight pinkish color, hemolysis has occurred. Hemolysis results from the escape of hemoglobin from inside the red cells into the serum or plasma. Try to avoid the following things which cause hemolysis:

- Using too small a needle gauge
- Shaking the tubes
- Drawing blood from a hematoma
- Centrifuging the blood before it is completely clotted

7. The plasma must be isolated and aliquoted within 60 minutes of the draw.

8. Dispose of remaining blood products into red biohazard bag.

9. Seal cryo-vials firmly and place upright in tube rack or box. Place tube rack or box into -80°C freezer immediately. After the vials are frozen, the vials can be transferred into the participant's zip-lock bag awaiting shipment to the DCC. If it is more convenient for your lab, vials may remain in cell-divided freezer boxes for shipping to the DCC as long as vials remain in order by ID number and there is a separate packing list for each

ID. Boxes should be numbered (Box 1, Box 2, etc.) and the starting corner should be marked.

- I. All vials should be in the -70°C freezer within 2 hours of phlebotomy.

Storage and Shipment of Vials

1. Vials should remain at -70°C until shipment.

PAXGENE TUBES:

Obtain specimen in Pax Gene Tube

Specimen remains at room temperature for for 2-2.5 hrs

Freeze at -20 for 24 hours

After 24 hours freeze at -70 until shipment

Columbia Suicide Severity Rating Scale

The Columbia–Suicide Severity Rating Scale (C-SSRS) is a 2-page questionnaire that prospectively assesses suicidal ideation and behavior using a structured interview face to face for patient responses. Suicidal ideation is classified on a 5-item scale: 1 (wish to be dead), 2 (nonspecific active suicidal thoughts), 3 (active suicidal ideation with any methods [not plan] without intent to act), 4 (active suicidal ideation with some intent to act, without specific plan), and 5 (active suicidal ideation with specific plan and intent).

The C-SSRS also captures information about the intensity of ideation, specifically the frequency, duration, controllability, deterrents, and reasons for the most severe types of ideation. Suicidal behavior is classified on a 5-item scale: 0 (no suicidal behavior),

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1 (preparatory acts or behavior), 2 (aborted attempt), 3 (interrupted attempt), and 4 (actual attempt).

The C-SSRS will be performed during each evaluation visit according to the assessment schedule (see Figure 4). During visit, patients will complete the assessment with the health professional. Patients who do not have suicidal behavior or ideation will answer a limited number of questions and will usually complete the assessment in approximately 3 minutes. Patients with significant suicidal ideation and/or behavior may require up to 10 minutes to answer all relevant questions.

The patient should not be released from the evaluation site until it is confirmed that the patient is not considered to be at any suicidal risk and all responses negative to the C-SSRS.

Positive reports are generated for any of the following findings:

- Suicidal ideation with intention to act (score of “4”)
- Suicidal ideation with specific plan and intent (score of “5”)
- Made suicide attempt
- Interrupted suicide attempt
- Aborted suicide attempt
- Preparatory behaviors for making a suicide attempt

At Screening (Visit 1), the C-SSRS will be completed for the patient’s lifetime history of suicidal ideation and behavior. At all other visits, the C-SSRS will be completed for ideation and behavior since the previous visit. The patient’s responses should remain negative throughout the entire trial. If a possible exclusion alert is triggered

by a positive response at Screening (Visit 1), 2 additional questions at the bottom of the report will need to be asked to determine whether exclusion criteria 9a and/or 9b have

been met. A response of “Yes” to either one or both of the follow-up questions will exclude the patient from the trial and necessitate immediate psychiatric referral of the patient on the same date as the screening visit. For all subsequent visits after Screening (Visit 1), if the patient’s response generates a change in the findings report from negative to positive, the patient should be referred for immediate psychiatric evaluation on the same date as the site visit and the PI will be contacted. Positive suicidality indication findings reports after the Screening Visit (Visit 1), if confirmed by the *Investigator*, should be considered to be an AE. Ultimately, the determination of suicidality and risk is up to the *Investigator*’s clinical judgment.

Columbia –Suicide Severity Rating Scale Process

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Forms:

Baseline/Screening Version

Since Last Visit Version

1. Screening/Baseline Visit: Patient must not have a history of suicidal behavior ≤ 2 years or suicidal ideation ≤ 6 months prior to enrollment. The Screening/Baseline Version will be used to screen patients. During screening, if there is a positive response to questions 1 thru 5 do not enroll patient and notify the PI for possible psychiatric referral.

2. Clinic Visit 14 or Clinic Visit 180
 - During study patient's follow up clinic visit, the coordinator will assess for suicide risk utilizing the Since Last Visit version. A positive answer to question 4 or 5 indicating presence of ideation with at least some intent to die suggests a clear need for further evaluation or clinical management. Discuss with PI while patient still at visit C-SSRS score. Patient's scoring a 4 or a 5 should be discontinued from study and sent to Temple ER or to Episcopal ER for immediate evaluation.

Suicide Ideation

Level	MANAGEMENT PROTOCOL	
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Level	Severity	
0	Low Risk	ROUTINE Behavioral Health Referral at physician discretion
1 & 2	Mild	ROUTINE Behavioral Health Referral at physician discretion
3	Moderate	Review by physician—Consider safety precautions and telephone consult with Behavioral Health
4 & 5	Serious	EMERGENT ACTION NECESSARY: Behavioral Health Consultation and Patient Safety Monitor/Procedures

