TITLE: Treatment of Pneumocystis jirovecii in COPD (The TOPIC Study)

NCT 05418777

Approval Date: 6/8/2022

Protocol and Statistical Analysis Plan

Treatment of *Pneumocystis jirovecii* in COPD (The TOPIC study)

Project Period: March 1st, 2022 – February 28th, 2023

Co-Principal Investigator: Carmen DeMarco, MD
Infectious Diseases Faculty, Beaumont Health
Assistant Professor of Internal Medicine, OUWB School of Medicine
3535 West 13 Mile Road, Suite 605, Royal Oak, MI 48073
Tel: 248 832-2255 E-Mail: Carmen.Demarco@Beaumont.Org

Co-Principal Investigator: Matthew D. Sims, MD PhD, FACP, FIDSA
Director of Infectious Diseases Research, Beaumont Health
Professor of Internal Medicine and Foundational Medical Studies, OUWB School of Medicine
3811 West 13 Mile Road, Suite 401, Royal Oak, MI 48073
Tel: 248 551-0027 E-Mail: Matthew.Sims@Beaumont.Org

Co-Investigator: Enrique Calvo-Ayala, MD, MSc
Medical Director of Pulmonary Rehabilitation, Beaumont Health
Assistant Professor of Internal Medicine, OUWB School of Medicine
3535 West 13 Mile Road, Suite 344, Royal Oak, MI 48073
Tel: 248 551-0497 E-Mail: Enrique.Calvo-Ayala@Beaumont.Org

Co-Investigator: Daniel Ortiz, PhD
System Director, Microbiology and Molecular Pathology, Beaumont Health
Assistant Professor of Pathology, OUWB School of Medicine
3811 West 13 Mile Road, Royal Oak, MI 48073
Tel: 248 551-4749 E-Mail: Daniel.Ortiz@Beaumont.Org

Co-Investigator: Sandeep Sohal, MD
Infectious Diseases Fellow, PGY4
3601 West 13 Mile Road, Royal Oak, MI 48073
Tel: 929 302-5394 E-Mail: Sandeep.Sohal@Beaumont.Org

Co-Investigator: Jeremy Steinbruck, MD
Infectious Diseases Fellow, PGY4
3601 West 13 Mile Road, Royal Oak, MI 48073
Tel: 781 366-1391 E-Mail: Jeremy.Steinbruck@Beaumont.Org

Co-Investigator: Ralph Akiki, MD
Internal Medicine Resident, PGY 1
3601 West 13 Mile Road, Royal Oak, MI 48073
Tel: 248 551-3000 E-Mail: Ralph.Akiki@Beaumont.Org

Co-Investigator: Alexa Giovanatti, MD
Internal Medicine Resident, PGY 2
3601 West 13 Mile Road, Royal Oak, MI 48073
Tel: 248 551-3000 E-Mail: Alexa.Giovanatti@Beaumont.Org

Co-Investigator: Gloria Hong, MD
Pulmonary Critical Care Fellow, PGY 5
3535 West 13 Mile Road, Suite 344, Royal Oak, MI 48073
Tel: 248 551-0497 E-Mail: Gloria.Hong@Beaumont.Org

Abstract

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease associated with a chronic inflammatory response in airways and lung parenchyma, resulting in significant morbidity and mortality worldwide. Smoking is the primary risk factor for development of COPD and progression of the disease is associated with acute exacerbations of COPD (AECOPD) that can be triggered by acute bacterial or viral airway infections or can occur independently of infection. AECOPD can lead to hospitalization, progression of the disease, and mortality. COPD affects an estimated 11.7% of the world population and was the third leading cause of death worldwide in 2019.

The causes of progression of COPD, especially in the absence of continued tobacco use, are incompletely understood and a significant area of need. One proposed trigger for progression and increased AECOPD is colonization with *Pneumocystis jirovecii* (PJ), a fungal pathogen best known for causing pneumonia in patients with HIV or other forms of immunosuppression. It has been found to be more prevalent in those with severe COPD, particularly AECOPD, but as a colonizer, not a cause of acute pneumonia. Several studies have linked this pathogen with progression of COPD, showing that PJ perpetuates an inflammatory and lung remodeling response, contributing to development of airway obstruction, emphysema and accelerating the disease course.

Despite the overwhelming evidence that PJ contributes to the progression of COPD and to AECOPD, there have been no clinical trials to date on treating PJ in the context of COPD in review of the literature or documented on clinicaltrials.gov. The most likely explanation for this lack is the limited ability to detect PJ and the dependence on an invasive procedure, bronchoalveolar lavage, to obtain an appropriate sample. Currently we are studying the prevalence of PJ in this population of patients using a non-invasive, PCR based, sputum test for detection which will allow us to identify potential patients for a clinical trial. Our study aims to conduct a pilot prospective, randomized, double-blind, placebo-controlled treatment arm in hospitalized patients with AECOPD found to have PJ in their sputum, and without radiographic evidence of pneumonia. We will compare standard of care treatment plus trimethoprim-sulfamethoxazole vs placebo. Outcomes to be assessed are time to return to baseline oxygen, length of stay, need for mechanical ventilation, AECOPD free interval, readmissions to the hospital within 3 months, and clearance of PJ from end of treatment through 3 months.

Data obtained from this pilot study can be utilized to design a larger future clinical trial for the treatment of AECOPD as well as a larger prophylactic study on prevention or COPD progression and AECOPD using the paradigm of prophylaxis of PJ which has been so successful in the HIV population. This study addresses the gaps in current knowledge on the clinical significance of PJ in COPD patients and the management of COPD could potentially be revolutionized if treatment or prevention of PJ proves successful.

SPECIFIC AIMS

According to the World Health Organization (WHO), chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, causing 3.23 million deaths in 2019 .[1] The most common root cause of COPD in the United States is cigarette smoke and COPD affects more than 15 million Americans. There are approximately 1.5 million emergency department visits, 700,000 hospitalizations, and 150,000 deaths per year in the United States due to COPD. It is the fourth leading cause of hospital readmission with 20-25% of cases lead to readmission within 30 days of discharge.[2–5] Despite significant decreases in mortality over the last 20 years due to reduction of smoking, it was still the fourth leading cause of death in US in 2019.[4,6] The data on the impact of COPD for 2020 and 2021 is somewhat skewed due to the impact of COVID-19 but for both years it is considered the 6th leading cause of death in the US.[7,8]

A key factor in the high morbidity and mortality of COPD is that it is a progressive and irreversible disease characterized by a limitation of airflow due to a combination of damage to the small airways (bronchiolitis) and parenchyma of the lung (emphysema) due to chronic inflammation. Progression of the disease and the irreversible damage to the lower respiratory tract leads to increasing episodes of acute exacerbations of COPD (AECOPD) during which there is a sustained increase in cough, sputum production, and shortness of breath. AECOPD can be self-limited but can also lead to progressive respiratory failure.

One cause of COPD progression is *Pneumocystis jirovecii* (PJ), a fungal pathogen best known for causing pneumonia in patients with HIV or other forms of immunosuppression. PJ is not typically found in the respiratory tract of healthy adults but is highly prevalent in those with severe COPD, particularly those with AECOPD, and the inflammation caused by PJ has been shown to lead to progression of COPD.

Detection of PJ generally requires a bronchoalveolar lavage (BAL) to sample the lower respiratory tract and then use direct florescent antibodies (DFA) to stain the sample for the presence of PJ. In the COPD population, a BAL is often too invasive for their tenuous respiratory status and DFA has very low sensitivity for PJ (~20%).

The standard treatment for PJ is trimethoprim-sulfamethoxazole (TMP-SMX), an FDA approved, inexpensive antibiotic with a well-established safety profile. Despite the large body of evidence pointing toward PJ as a cause of COPD progression and a factor which leads to AECOPD, there has never been a trial of therapy for PJ in the COPD population. Such studies are difficult due to the invasive nature and low sensitivity of standard testing for PJ in this population. Using a highly sensitive PCR based assay, we can easily and safely test the sputum of COPD patients to identify carriers of PJ in order to include them in a clinical trial.

We hypothesize that adding TMP-SMX to standard of care treatment of AECOPD in patients who are colonized with PJ will improve the clinical outcome for the patient. **We further hypothesize** that TMP-SMX can eradicate colonization with PJ. This proposal seeks to confirm our hypothesis through the following two specific aims:

Aim 1: Determine if there is clinical improvement in COPD patients colonized with PJ treated with standard of care plus TMP-SMX vs standard of care plus placebo.

Rationale: For each participant we will determine time to return to baseline oxygen needs, need for mechanical ventilation during the index admission, length of stay, COPD assessment test, time to next admission for AECOPD and number of readmissions for AECOPD in the following 3 months.

Aim 2: Determine the persistence of colonization with PJ in AECOPD patients treated with standard of care plus TMP-SMX vs standard of care plus placebo.

2.1 Determine if TMP-SMX eliminates PJ at end of TMP-SMX therapy and the durability of clearance

2.2 Determine the duration of PJ colonization after standard of care treatment for AECOPD

Rationale: For each participant we will assay for the presence of PJ using PCR on sputum at end of therapy, 1 month and 3 months.

This study is a pilot which will serve as ***proof of concept*** that screening for PJ in the AECOPD population and treating it with the commonly available, safe, and inexpensive antibiotic TMP-SMX will be an effective strategy. This study is ***innovative*** as it utilizes a new technology for screening of patients for PJ and for the first time attempts to improve the treatment of AECOPD by targeting PJ as a causative agent. This study is ***significant*** as it will aid in the design and implementation of future large-scale studies aimed at both treatment and prevention of AECOPD with TMP-SMX. Following the paradigm of prevention of PJ with daily prophylactic TMP-SMX established in the HIV population, we are also planning future studies to prevent acquisition of PJ in the COPD population in order to prevent progression of COPD using daily TMP-SMX prophylaxis. If these future large-scale studies prove successful, it has the potential to be ***transformative*** in the care of one of the

most significant diseases in the world, reduce hospitalizations, readmissions, and overall morbidity and mortality.

Significance

The Size and Overall Impact of the Clinical Condition:

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. Prior to COVID, the World Health Organization (WHO) ranked COPD as the third leading cause of death worldwide, causing 3.23 million deaths in 2019 with over 80% of these deaths occurring in low- and middle-income countries.[1] The most common root cause of COPD in the United States is cigarette smoke but in certain regions other pollutants such as wood smoke can also be a major cause. **COPD affects more than 12 million Americans; there are approximately 1.5 million emergency department visits, 700,000 hospitalizations, and 150,000 deaths per year in the United States due to COPD. It is the fourth leading cause of hospital readmission with 20-25% of cases lead to readmission within 30 days of discharge**.[2,3] Despite significant decreases in mortality over the last 20 years due to reduction of smoking it was still the fourth leading cause of death in US in 2019.[4,6] The data on the impact of COPD for 2020 and 2021 is somewhat skewed due to the impact of COVID-19 but for both years it is considered the 6th leading cause of death in the US.[7,8]

The high morbidity and mortality of COPD stems from the fact that it is a progressive and irreversible disease characterized by a limitation of airflow due to a combination of damage to the small airways (bronchiolitis) and parenchyma of the lung (emphysema) due to chronic inflammation. Progression of the disease and the irreversible damage to the lower respiratory tract leads to increasing episodes of acute exacerbations of COPD (AECOPD) during which there is a sustained increase in cough, sputum production, and shortness of breath. AECOPD can be self-limited but can also lead to progressive respiratory failure.

The Target of This Study:

AECOPD can be triggered by acute bacterial or viral airway infections or can occur independently of infection. In a significant subset of COPD patients, progression appears to be related to a specific pathogen which can cause inflammation in the lower respiratory tract when colonizing a patient without establishing a typical symptomatic infection. *Pneumocystis jirovecii* (PJ) is a ubiquitous, unicellular fungal pathogen with lung tropism. It is an obligate extracellular organism and exists in vegetative and cyst forms.[9] It is **an opportunistic pathogen, not typically found in the respiratory tract of healthy adults**. PJ is known for causing pneumonia in patients with human immunodeficiency virus (HIV) or other forms of immunosuppression. The presentation of PJ varies depending on the underlying co-morbidities; in HIV patients, PJ presents as a sub-acute pneumonia, but in transplant patients, it presents as an acute fulminant pneumonia; both have high mortality associated with the active infection. [10] **In COPD patients, PJ colonizes the airways without causing an active infection**.

Despite not causing an active infection, **PJ been shown in animal and human studies to serve a significant role in COPD pathogenesis and progression**. The cell wall of PJ contains β-D-glucans which cause pulmonary tissue damage by stimulating the release of reactive oxidants from macrophages and triggering the release of TNF-α, IL-6, IL-8 and MIP-2.[11–13] In both humans and animal models, this leads to a deterioration of lung function as PJ perpetuates an inflammatory and lung remodeling response, contributing to development of airway obstruction, emphysema and accelerating the disease course.[14–17] Treatment with corticosteroids, which is often employed in COPD patients, increases the risk of PJ colonization and infection.[9,18,19]

PJ colonization has been described with high prevalence in patients with COPD, especially during episodes of AECOPD.[20] Studies vary as to the exact percentage of COPD patients colonized with PJ, colonization correlates with severity of disease, ranging from 16% to 67%; the methodology of detection can also impact sensitivity of detection.[21] PJ has been shown in animal models to induce alveolar macrophage activation, pro-inflammatory interleukin elevation, and changes in pulmonary surfactant, and multiple studies suggest a possible pathogenic link with severity of airflow obstruction and COPD development and progression.[14,17]

The Intervention

The current standard of care for detection of PJ has significant limitations in COPD patients. There is no reliable *in vitro* culture system and traditional detection methods are based on histochemical staining with direct fluorescent antibodies (DFA) that target the cyst form of the organism isolated from bronchoalveolar lavage (BAL) fluid. BAL is an invasive diagnostic procedure that often cannot be performed safely in patients with AECOPD due to associated risk of worsening respiratory failure and intubation. In non-HIV patients, PJ is at a lower bioburden and generally exists in the vegetative form rather than the cyst form. The lower PJ cyst

burden leads to a lower sensitivity of DFA (20%) which specifically targets the cyst form. This in turn leads to difficulty in identifying PJ in the non-HIV population including transplant and COPD patients. This has led to a lack of clinical trials for potential therapeutic interventions targeting PJ to treat AECOPD or to prevent progression of COPD.[22]

We are currently collecting and analyzing sputum from AECOPD patients under the EPIC study (Evaluation of *Pneumocystis* in COPD), IRB# 2022-016, to determine prevalence of PJ in our AECOPD population. The EPIC study employs a novel method for detecting PJ in COPD patients using a non-invasive sample collection of sputum analyzed with the Unyvero system which is a novel nucleic acid amplification testing (NAAT) based assay demonstrated to have high sensitivity for PJ. The system is currently FDA approved for detection of PJ in BAL samples but, in Europe, sputum is routinely used. The ability to routinely identify the subgroup of patients colonized with PJ among the hospitalized patients with AECOPD is a necessary step toward selection of the most appropriate potentially treatable patients to include in this pilot study designed to treat AECOPD. The EPIC study will identify colonized patients and refer them to screening for this study in which we will treat them for PJ in addition to the standard of care treatment for AECOPD to determine if it improves outcomes.

Trimethoprim-sulfamethoxazole (TMP-SMX) is the standard of care for treating PJ pneumonia in HIV patients, and has been shown in prior studies to have high clinical cure rates.[23] Our intervention is adding TMP-SMX to the treatment regimen for patients hospitalized with AECOPD and colonized with PJ.

The goal of this study is to determine if treating PJ in AECOPD with confirmed PJ colonization has a beneficial clinical impact. As a secondary goal of the study, we will determine if TMP-SMX can decolonize these patients and if the decolonization is durable for at least 3 months.

Clinical Relevance

This study addresses an important problem as the burden of COPD on patients is immense contributing to a significant number of hospitalizations and premature deaths. The economic burden on patients and the healthcare system is tremendous with the direct medical costs of COPD over the next 20 years estimated to be nearly $1 trillion dollars in the United States alone.[3]

Multiple studies have confirmed high rates of PJ colonization in COPD patients, and the highest rates are seen in patients with AECOPD.[20] During the clinical trial of the Unyvero system for lower respiratory infections at Beaumont, we found 8 patients out of nearly 500 total patients in the study who were positive for PJ. Of note, HIV patients, who are at the highest risk for PJP, were excluded from that study. Two of the 8 patients had other classic risk factors for PJ pneumonia (transplant and leukemia), the remaining 6 patients all had severe COPD and two of them were so severe that they entered hospice. These findings were what led us to conclude that the association of PJ with COPD was clinically significant and led to the design of this trial.

Through this study we hypothesize that we will be able to show that the simple addition of TMP-SMX to the standard treatment regimen for AECOPD will lead to clinical benefit. If our hypothesis is confirmed larger trials of therapy designed to treat AECOPD and to prevent acceleration of the disease process would be possible. TMP-SMX is a proven, safe, and inexpensive antibiotic, the addition of TMP-SMX as a treatment for AECOPD would be both safe and cost-effective. More importantly, using the paradigm of AIDS patients, prophylaxis with a single daily tablet of TMP-SMX can nearly eliminate the incidence of PJ pneumonia.[24] Further clinical trials could compare if daily TMP-SMX could significantly reduce AECOPD. A reduction in AECOPD of even 5% would represent a huge improvement in clinical care and significant savings to the cost of healthcare.[25] The low cost, safety profile, and ease of availability of TMP-SMX makes this a therapy that could be applied worldwide even in the lowest resource areas.

Innovation

Our study has multiple innovative aspects. It will employ a **novel, rapid diagnostic method, using a non-invasively collected sample** to make a microbiological diagnosis. The Unyvero system is a multiplex PCR platform which can detect 20 respiratory pathogens including PJ, and it is currently the only FDA approved NAAT assay for PJ. In the United States, it is only approved to be run on BAL samples, though in Europe the system is routinely used for testing sputum and still shows excellent sensitivity and specificity.[26,27] By using this novel diagnostic platform, our study will rapidly identify if PJ is present in the sputum, thus allowing us to select appropriate candidates for treatment with TMP-SMX. It is **the first study** that will investigate if a treatment course with TMP-SMX added to standard of care in AECOPD patients colonized with PJ has clinical benefit. We could only find a single study in the literature and via a search of clinicaltrials.gov investigating TMP-SMX for the treatment of AECOPD but it did not stratify by PJ colonization and only enrolled end stage AECOPD patients already on mechanical ventilation. TMP-SMX was found to be non-inferior to

ciprofloxacin.[28] If we would be able to show that identifying PJ colonization will allow selection of the most appropriate patients for treatment with TMP-SMX, and that clearance of the colonization leads to improved outcomes, we could add a significant treatment option in the management of AECOPD and modifying the course of illness.

It is **the first study** that will investigate the duration of PJ colonization for 3 months following an episode of AECOPD, **all prior studies of PJ in COPD are point prevalence studies** with no follow up. Lastly, it is **the first study** that will investigate if treatment with TMP-SMX is able to clear colonization with PJ from COPD patients, and it will longitudinally assess the durability of the decolonization over 3 months. **No previous studies determined microbiological clearance of PJ or the durability of clearance**.

Study design – The TOPIC Study (**T**reatment **o**f ***P**neumocystis jirovecii* **i**n **C**OPD)

Objective/Hypothesis

We hypothesize that adding TMP-SMX to standard of care treatment of AECOPD in patients who are colonized with PJ will improve the clinical outcome for the patient. **We also hypothesize** that TMP-SMX will be able to microbiologically eradicate colonization with PJ in patients hospitalized with AECOPD.

Patients

Patients admitted with AECOPD are currently being identified daily for the EPIC study, IRB#2022-016, which is determining the prevalence of PJ colonization in our AECOPD patient population. This is accomplished using a search built in the EPIC EMR which identifies these patients. We will use the EPIC study to refer patients who are colonized with PJ to screening of inclusion/exclusion criteria for the TOPIC study (Table 1). To minimize potential for bias, the TOPIC study is randomized, double-blinded, and placebo controlled.

Inclusion	Exclusion
• Caries the diagnosis of COPD and admitted for and admitted with AECOPD to Beaumont, Royal Oak • AECOPD requires increased cough, increased sputum production, and shortness of breath +/- increased oxygen needs from baseline • Able to produce a sputum sample • Men or women, age ≥40 and <90 • Previously enrolled in the EPIC study and PJ detected in their sputum • Currently treated with steroids • Kidney function not severely impaired (CrCl≥60) • AST and ALT ≤ 5x Upper limit of normal • Willing and able to provide consent to the study	• Currently diagnosed with pneumonia or COVID • Allergy or hypersensitivity to TMP-SMX • Current ICU admission or mechanical ventilation • Active cancer or chemotherapy (except non-melanoma skin cancer) • Other potentially confounding pulmonary diagnosis • HIV, leukopenia, neutropenia or other immunosuppressive condition or current use of immunosuppressive medications • Presence of gastrointestinal tract abnormalities that would prevent absorption of medications • Patients with concomitant infection requiring antibiotics active against PJ • Concomitant use of coumadin, phenytoin, pioglitazone, repaglinide, rosiglitazone, glipizide or glyburide • Megaloblastic anemia due to folate deficiency • Pregnancy • Expected life span less than 3 months

Table 1: Inclusion/Exclusion Criteria

If eligible by inclusion/exclusion patients will be approached for consent for the TOPIC study, full consent will be used as this is an interventional study. If a potential patient is a woman of childbearing potential then a pregnancy test will be obtained to confirm that they are not pregnant (after consent since it will be a study procedure) and if positive the patient will be screen failed. See Appendix A for examples of specific exclusion criteria. Key personnel include 3 attending physicians, 3 fellows, and 2 residents available to consent. In addition, a nurse coordinator experienced at consenting will be available to consent or assist the physicians with consenting in order to further educate physician trainees who are sub-investigators on this study. Given physician time commitments we expect the nurse may perform a majority of the consenting herself. Patients who consent for participation will be randomized in a 1:1 ratio to one of two groups.

Group #1 – Fifteen patients will receive a suspension with the equivalent of one DS TMP-SMX by mouth every 12 hours.

Group #2 – Fifteen patients will receive a suspension with placebo by mouth every 12 hours.

Randomization will be generated by biostats and sealed envelopes will be held by Dr. Koerber in the Research Pharmacy to be opened when a patient is recruited. Dr. Koerber is the only one who will be unblinded and, in case of an emergency, he can break the blind at the request of one of the attending physician investigators.

The Intervention

The typical treatment of PJ pneumonia is TMP-SMX is 2 DS tablets every 8 hours for 21 days. Higher concentrations of TMP-SMX are needed to treat the cyst form found in PJ pneumonia. For colonized patients who carry the vegetative form of PJ, a standard dose of TMP-SMX is more appropriate and less likely to cause side effects and potential discontinuation. As such the dose to be used for this study is 1 DS Q12H for 10 days. TMP-SMX is a large tablet which is difficult to over-encapsulate for blinding purposes. As such, the Research Pharmacy will supply a TMP-SMX suspension in individual dose syringes or a matching placebo in individual dose syringes for this study. A single syringe for TMP-SMX will contain 160 mg of trimethoprim and 800 mg of sulfamethoxazole, the same amount found in one DS tablet of TMP-SMX. If the patient is discharged prior to completing the 10-day course of the medication, they will be sent home with the remaining days of study medication and a medication diary which will be collected at the end of therapy visit. Standard operating

procedures used for all clinical trials within the Infectious Diseases Clinical Trials Unit will be followed at discharge including appropriate instruction of the patient on medication use, filling out the medication diary, when to contact the study staff, and making appointments for follow up.

When a patient with PJ pneumonia is hypoxic, the treatment of the PJ in addition to TMP-SMX includes steroids. This is done to minimize the inflammation from the β-D-glucans of PJ that are released when PJ is killed by TMP-SMX. It is unclear if steroids would be needed with the lower expected levels of PJ in the AECOPD patients but, since most patients with AECOPD are treated with steroids in any case, we feel it is safer to only include patients receiving steroids, hence the inclusion requirement of steroid use.

Evaluations

Upon consent patients will be given the COPD Assessment Test (CAT) (https://www.mdcalc.com/copd-assessment-test-cat), a validated scoring system used in COPD patients to assess progression, functional status, and effectiveness of pulmonary rehabilitation.[29–32] Participants will be monitored daily while in the hospital for highest oxygen need, need for non-invasive or invasive mechanical ventilation, and adverse events, daily monitoring will be carried out by the nurse coordinator. Medications used for the treatment of COPD and antibiotics used will be collected retrospectively for each patient. If the patient remains hospitalized at end of therapy, 30 days, or 90 days then follow up will occur in the hospital. Otherwise, the patient will be asked to come to the Infectious Diseases Clinical Research Office for follow up visits at end of therapy (+0-3 days to allow for schedule issues and end of therapy falling on a weekend), 30 days +/-5 days, and 90 days +/-10 days. A $30 stipend will be paid to the participants after each follow up visit. At the end of treatment visit, the medication diary will be collected. At each follow up visit, the nurse coordinator will review the patient's need for oxygen, their medications for the treatment of COPD, any need to see a physician or go to an urgent care or an emergency room for COPD, and any admission to the hospital for COPD. The CAT will be administered at each follow up visit. An expectorated sputum sample will be collected at each follow up visit which will be transported to the Infectious Diseases Research Laboratory for analysis; sputum samples will be processed and analyzed with the Unyvero LRT system for presence of PJ by PCR. The schedule of events for

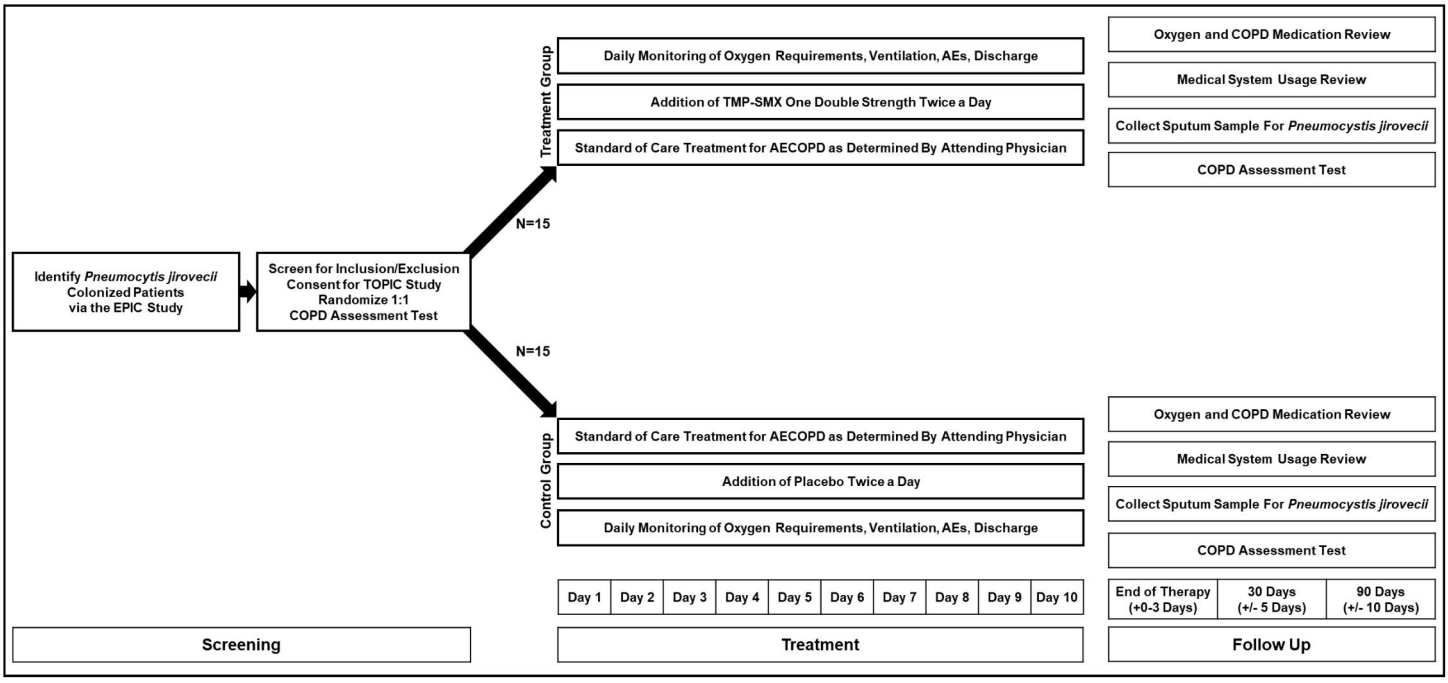

Figure 1: Schedule of Events

the study is shown in figure 1.

Statistical analysis

No formal power analysis was performed, the sample size of 15 patients per arm is felt to be appropriate for a pilot study and appropriate to the funding for this application. We expect to find enough cases of PJ colonization to identify and recruit the 30 patients to be included in the study from the pool of patients for the EPIC study.

Time to return to baseline O2 requirements, and time to next admission for exacerbation will be analyzed using cox proportional hazards regression or other appropriate time-to-event analysis method. Need for mechanical

ventilation will be analyzed using logistic regression. LOS will be analyzed using quantile regression. The number of urgent care/emergency room visits and number of hospitalizations for AECOPD within 3 months and mortality will be analyzed using Poisson regression (or other appropriate method if there is evidence of overdispersion). Durability of decolonization, change in medications for COPD, and CAT score will be a longitudinal analysis. This analysis plan was developed by Julie George in Biostatistics who will perform the analysis.

Any participants who withdraw prior to the EOT assessment will be dropped from the study and replaced. Any participants who withdraw after completing the EOT assessment will be analyzed in a modified intent to treat population and not replaced. Only participants who complete all assessments will be analyzed as per protocol.

Anticipated Outcomes

We anticipate treatment of PJ colonized AECOPD patients with standard of care plus TMP-SMX will improve symptoms, decrease LOS and increase the interval free of acute exacerbations above standard of care alone. Using the CAT, we expect to have a quantifiable score which can demonstrate a difference in outcomes between the two groups.

We also anticipate that patients with AECOPD who are colonized with PJ and treated with standard of care alone will maintain colonization over the 3-month period of follow up. Patients who are treated with TMP-SMX in addition to standard of care are expected to have microbiological eradication of PJ colonization and we expect that eradication to last through the 3-month follow up visit.

Potential problem areas and alternative tactics

This study is relatively simple and straight forward, we do not anticipate any issues with identifying or recruiting patients for the study but recognize that recruitment can be lower than expected for any study.

By identifying patients colonized with PJ in the EPIC study, we will carry the cost of screening for the TOPIC study in the EPIC study, which allows the funding for this study to bear other needed expenses and make it feasible. The key personnel for the EPIC study overlap completely with the TOPIC study. We aim to enroll 30 patients but if we will have difficulties in recruiting, we may need to decrease the number of patients in the treatment group to 10 in each arm. Having a stipend for follow up visits will help with recruitment as will having Dr. Calvo-Ayala, the director of Pulmonary Rehabilitation, as an investigator.

It is possible that we may see clinical improvement in the TMP-SMX group without microbiologic eradication. This could be due to TMP-SMX incompletely killing the PJ, we could have chosen a sub-optimal dose for the study, or it could be that the high sensitivity of PCR and the ability to detect dead organisms will work against us and we could still detect the organisms even if all were killed. Checking for colonization again at 30 days and at 90 days may be able to reveal this as dead organisms are cleared from the respiratory tract. It may be that it will take a longer trial to actually show microbiologic clearance even in the setting of clinical benefit.

This is a pilot study and, since we will have a small number of patients, we may not be able to detect statistically significant differences between the two groups, however by selecting the known PJ colonized patients for the study we are increasing the chances of being able to show a difference between the groups.

Potential for continuing this study with additional extramural funding

If our pilot study shows that there are potential clinical improvements in COPD patients decolonized from PJ, we plan to design a larger, adequately powered, clinical trial that will investigate our hypothesis further in order to provide further knowledge that will help in the treatment and prevention of AECOPD. If our hypothesis is proven to be correct, this has the potential to revolutionize COPD treatment and impact millions of people. An AECOPD reduction of even 5% would represent a huge improvement in clinical care and significant savings to the cost of healthcare.[18] The cost and ease of availability of TMP-SMX makes this a therapy that could be applied worldwide even in the lowest resource areas. Extrapolating the success of TMP-SMX as a prophylactic agent against PJ in HIV, we could consider a larger trial of prophylaxis with TMP-SMX against PJ in COPD patients.

Once preliminary data is available, we plan to apply for 2 separate R01s for the October 2023 deadline, under PA-20-183, Research Project Grant (Parent R01 Clinical Trial Required), through NIH-NHLBI. One R01 is for treatment and one for prophylaxis. If other more specific RFAs for COPD are announced, we will consider applying for those. We have examined the possibility of applying for foundation awards. However, the awards through the American Lung Association and the Chest Foundation have limited funding and are too short-term to support a large-scale study but could potentially support a similar study to this one with a somewhat larger population if additional data is needed before applying for an R01. The COPD Foundation, while supporting

recruitment of studies and collaboration among COPD researchers does not offer its own grant funding. One very tempting funding opportunity is through PCORI and the RFA for Phased Large Awards for Comparative Effectiveness Research. This opportunity allows for a well-funded 18-month discovery phase and a 5-year full-scale clinical trial with significantly better funding than the R01 mechanism which would allow for a large multi-center study. Dr. Sims has collaborators at many large academic research institutions in both Infectious Diseases and Pulmonary Critical Care who can be tapped as co-investigators for such an opportunity. In addition, this would be a place where the resources of the COPD foundation for supporting the recruitment of studies could be utilized. For a PCORI opportunity, a stake-holders group consisting of patients, care takers, and physicians would be assembled to help guide the research. Since the intervention to be studied consists of a medication which is already approved by the FDA with a long safety record and proven uses for prophylaxis in other conditions, this could be well positioned as an intervention in comparative effectiveness research.

Timeline of milestones

The entire study will take place over a single year. We already secured funding for the EPIC study and are expecting IRB approval, we should be enrolling patients in EPIC by early February 2022. We have secured a Unyvero system for use in the EPIC study and the TOPIC study and it has already been installed.

We have prepared the SRC and IRB applications for this study and will submit them as soon as funding is awarded. We expect to be able to begin recruitment one month after funding approval. Enrollment and data collection will be over 8 months with final follow up visits within the last 3 months of the study. Final data analysis will take place in the final month of funding after the last patient visit. Preparation of presentations, and preparation of a publication will begin immediately after data analysis is complete.

Resources

The Infectious Diseases Research Program of Beaumont Health is based at Beaumont Hospital - Royal Oak and consists of the Infectious Diseases Research Laboratory (IDRL) and the Infectious Diseases Clinical Trials Unit (IDCTU).

The IDRL is approximately 1,000 square feet housed within the Beaumont Health Research Institute Building. The laboratory is dedicated to the culturing and investigation of microbial pathogens, with wet-laboratory bench space consistent with that of any modern university-based research space. Extensive molecular biology facilities to manipulate and characterize microbial organisms are in use within the research laboratory including alarmed refrigerators and freezers (-20 º, -70 º, -86 º) all on backup generator which is where any samples will be stored. Adjacent to the research laboratory are additional research facilities, including a large stand-alone cold room, a 37°C warm room, a microscopy room (including a Nikon Eclipse 90i brightfield/fluorescent microscope, and a confocal Nikon Eclipse Ti), and glassware preparation room (including an autoclave and glassware washing and drying equipment). In addition to Dr. Sims, there is a fulltime PhD research associate, a fulltime technician, and a fulltime research assistant (50% dedicated to laboratory and 50% as Dr. Sims' admin) staffing the IDRL. Dr. Sims' office is attached to the IDRL and is ~150 square feet and includes meeting space.

In the IDRL we currently have 3 Unyvero systems, 2 systems composed of 1 cockpit, 1 lysator, and 2 analyzers each; they are currently in use for another clinical trial. The third system has 1 cockpit, 1 lysator, and 1 analyzer and is dedicated to the EPIC and TOPIC studies. This system can easily handle 4 samples analyzed per day and 6 samples depending on the availability of staff to start a third run, this is more than sufficient to handle all but the busiest recruitment days without needing to refrigerate the samples overnight. Dr. Sims has had ongoing collaborations with OpGen, the manufacturer of the Unyvero system, and they have committed the extra system to this project (see letter of support from OpGen).

The IDCTU has a research suite of approximately 750 square feet housed in the Beaumont Hospital – Royal Oak – Medical Office Building, which is located on the hospital campus directly across from the main entrance to the hospital. The space consists of 2 offices and a large common office for the staff, an examining room for follow-up visits, a waiting room for patients, a laboratory for processing of research samples containing a tabletop centrifuge, a refrigerator, a -20º freezer, and a blood-drawing station. There is an additional 100-square-foot storage room for study supplies located in the same building. Dr. DeMarco's office sits within the IDCTU.

Kaleigh Belanger, RN, will be the study coordinator for this trial and is based within the IDCTU. Follow up visits will take place at the IDCTU and be scheduled as convenient for the participant.

Dr. Sims and Dr. DeMarco both serve as primary investigators for research studies run by the IDCTU, and 4 additional Infectious Diseases physicians serve as sub-investigators for most trials to supply backup for when Drs. Sims and DeMarco are unavailable. In addition to committed physician researchers, to assist with study management and participant recruitment, research support staff includes 5 highly trained research nurses and a fulltime research coordinator. The research department consists of 1 clinical research nurse manager who oversees and manages the daily operations of currently active clinical trials. She is responsible for the supervision of the 5 nurses and 1 coordinator who are all extensively trained in screening procedures, obtaining informed consent, case report form completion, and data collection and research paperwork completion, including regulatory documents.

Research departments are provided with state-of-the-art computers, tablets, and printers, which are supported by Beaumont's Information Systems Department. A 24-hour help line is available for staff to resolve computer issues. Computer upgrades are regularly performed by Beaumont's Information Systems Department. All staff have private offices with locking file cabinets to secure participant files; the ID Research Program maintains its own copy machines and secure faxes. There is adequate space to support the clinical research coordinators for this study.

The likelyhood of success in both the study and the educational aspect for the trainiees involved in the research is increased based on the key personnel for this study. It is a highly experienced, multidisciplinary group representing 3 separate disciplines that synergize for this project.

Dr. Matthew Sims, MD PhD (Co-PI), the Director of Infectious Diseases Research, has run multiple sponsored and investigator-initiated trials using the Unyvero system and presented/published numerous aspects of this work. He has been an investigator on clinical trials of the Unyvero system for pneumonia, joint infection, and now urinary tract infections as well as a clinical content expert at the FDA meeting which

reviewed the Unyvero LRT cartridge that led to its approval (all studies and consulting were managed through Beaumont Research Institute and all funds were paid to Beaumont). He has mentored over 50 trainees (high school students, college students, medical students, residents, and fellows) and 5 junior faculty in research. He is a member of the key teaching faculty for both the Internal Medicine Residency and the Infectious Diseases Fellowship.

Dr. Carmen DeMarco, MD (Co-PI), is a newer attending within the Infectious Diseases Section at Royal Oak and has 50% dedicated time for research and is being mentored by Dr. Sims. She is already experienced as a sub-investigator in clinical trials and is now PI on 3 trials including the EPIC study and 2 sponsored trials. She is a member of the key teaching faculty for both the Internal Medicine Residency and the Infectious Diseases Fellowship.

Dr. Enrique Calvo-Ayala, MD MSc (Sub-I), the Medical Director of Pulmonary Rehabilitation, holds a Master of Science in Clinical Research and is highly experienced at treating COPD patients. He is a member of the key teaching faculty for both the Internal Medicine Residency and the Pulmonary Critical Care Fellowship.

Dr. Daniel Ortiz, PhD (Sub-I), the System Director of Microbiology and Molecular Pathology for Beaumont Health, adds additional expertise regarding NAAT testing to the team. He is a member of the key teaching faculty for the Pathology Residency.

There are a total of 5 trainees taking part in this study. Drs. Steinbrook and Sohal are first year Infectious Disease Fellows. Drs. Akiki and Giovanati are Internal Medicine Residents who have both expressed an interest in a career in Infectious Diseases, and Dr. Gloria Hong is a Pulmonary Critical Care Fellow. Their participation in this study will help train them in the identification and consent of patients as well as research design and data analysis.

Dr. Sims' laboratory is highly experienced at using the Unyvero system and was the highest recruiter in the initial clinical trials of the system which led to its FDA approval. Kim Powell, who will be performing the testing has extensive experience performing this specific assay.[26] OpGen, the manufacturer of the system, is very interested in supporting this study as well as other trials.

References

1. Chronic obstructive pulmonary disease (COPD). Available at: https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd). Accessed 31 January 2022.
2. Overview of Clinical Conditions With Frequent and Costly Hospital Readmissions by Payer, 2018 #278. Available at: https://www.hcup-us.ahrq.gov/reports/statbriefs/sb278-Conditions-Frequent-Readmissions-By-Payer-2018.jsp. Accessed 31 January 2022.
3. Zafari Z, Li S, Eakin MN, Bellanger M, Reed RM. Projecting Long-term Health and Economic Burden of COPD in the United States. Chest **2021**; 159:1400–1410. Available at: https://pubmed.ncbi.nlm.nih.gov/33011203/. Accessed 31 January 2022.
4. CDC - Data and Statistics - Chronic Obstructive Pulmonary Disease (COPD). Available at: https://www.cdc.gov/copd/data.html. Accessed 31 January 2022.
5. Heron M. National Vital Statistics Reports Volume 67, Number 5 July 26, 2018. Natl Vital Stat Reports **2018**; 67:1–15.
6. Vital Statistics Reports N. National Vital Statistics Reports Volume 70, Number 8 July 26, 2016 Deaths: Final Data 2019. **2021**; Available at: https://www.cdc.gov/nchs/products/index.htm. Accessed 31 January 2022.
7. Murphy SL, Kochanek KD, Xu J, Arias E. Mortality in the United States, 2020 Key findings Data from the National Vital Statistics System. **2020**; Available at: https://www.cdc.gov/nchs/products/index.htm. Accessed 31 January 2022.
8. COVID-19 preventable mortality and leading cause of death ranking - Peterson-KFF Health System Tracker. Available at: https://www.healthsystemtracker.org/brief/covid19-and-other-leading-causes-of-death-in-the-us/. Accessed 31 January 2022.
9. Morris A, Norris KA. Colonization by pneumocystis jirovecii and its role in disease. Clin Microbiol Rev **2012**; 25:297–317.
10. Lee Y-T, Chuang M-L. Pneumocystis jirovecii pneumonia in AIDS and non-AIDS immunocompromised patients – an update. J Infect Dev Ctries **2018**; 12:824–834. Available at: https://jidc.org/index.php/journal/article/view/10357. Accessed 29 September 2021.
11. Lebron F, Vassallo R, Puri V, Limper AH. Pneumocystis carinii cell wall beta-glucans initiate macrophage inflammatory responses through NF-kappaB activation. J Biol Chem **2003**; 278:25001–25008. Available at: https://pubmed.ncbi.nlm.nih.gov/12716885/. Accessed 31 January 2022.
12. Hahn PY, Evans SE, Kottom TJ, Standing JE, Pagano RE, Limper AH. Pneumocystis carinii cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. J Biol Chem **2003**; 278:2043–2050. Available at: https://pubmed.ncbi.nlm.nih.gov/12419803/. Accessed 31 January 2022.
13. Calderón EJ, Rivero L, Respaldiza N, et al. Systemic inflammation in patients with chronic obstructive pulmonary disease who are colonized with Pneumocystis jiroveci. Clin Infect Dis **2007**; 45:17–19.
14. Morris A, Sciurba FC, Norris KA. Pneumocystis: A novel pathogen in chronic obstructive pulmonary disease? COPD J Chronic Obstr Pulm Dis **2008**; 5:43–51.
15. Morris A, Sciurba FC, Lebedeva IP, et al. Association of chronic obstructive pulmonary disease severity and Pneumocystis colonization. Am J Respir Crit Care Med **2004**; 170:408–413.
16. Kling HM, Shipley TW, Patil SP, et al. Relationship of Pneumocystis jiroveci Humoral Immunity to Prevention of Colonization and Chronic Obstructive Pulmonary Disease in a Primate Model of HIV Infection. Infect Immun **2010**; 78:4320. Available at: /pmc/articles/PMC2950365/. Accessed 20 January 2022.
17. Shipley TW, Kling HM, Morris A, et al. Persistent Pneumocystis colonization leads to the development of chronic obstructive pulmonary disease in a nonhuman primate model of AIDS. J Infect Dis **2010**; 202:302–312. Available at: https://pubmed.ncbi.nlm.nih.gov/20533880/. Accessed 22 September 2021.

18. Calero-Bernal ML, Martin-Garrido I, Donazar-Ezcurra M, Limper AH, Carmona EM. Intermittent Courses of Corticosteroids Also Present a Risk for Pneumocystis Pneumonia in Non-HIV Patients. Can Respir J **2016**; 2016.

19. Msaad S, Yangui I, Bahloul N, et al. Do inhaled corticosteroids increase the risk of Pneumocystis pneumonia in people with lung cancer? World J Clin Cases WJCC **2015**; 3:843. Available at: /pmc/articles/PMC4568535/. Accessed 20 January 2022.

20. Xue T, Ma Z, Liu F, et al. Pneumocystis jirovecii colonization and its association with pulmonary diseases: A multicenter study based on a modified loop-mediated isothermal amplification assay. BMC Pulm Med **2020**; 20:1–13.

21. Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical significance of Pneumocystis colonization. J Infect Dis **2008**; 197:10–17.

22. Bateman M, Oladele R, Kolls JK. Diagnosing Pneumocystis jirovecii pneumonia: A review of current methods and novel approaches. Med. Mycol. 2020; 58:1015–1028. Available at: https://pubmed.ncbi.nlm.nih.gov/32400869/. Accessed 29 September 2021.

23. Bellamy RJ oh. HIV: treating Pneumocystis pneumonia (PCP). BMJ Clin Evid **2008**; 2008. Available at: /pmc/articles/PMC2907978/. Accessed 31 January 2022.

24. Weyant RB, Kabbani D, Doucette K, Lau C, Cervera C. Pneumocystis jirovecii: a review with a focus on prevention and treatment. Expert Opin Pharmacother **2021**; 22:1579–1592.

25. Iheanacho I, Zhang S, King D, Rizzo M, Ismaila AS. Economic burden of chronic obstructive pulmonary disease (COPD): A systematic literature review. Int J COPD **2020**; 15:439–460. Available at: https://pubmed.ncbi.nlm.nih.gov/32161455/. Accessed 29 September 2021.

26. Ozongwu C, Personne Y, Platt G, et al. The Unyvero P55 'sample-in, answer-out' pneumonia assay: A performance evaluation. Biomol Detect Quantif **2017**; 13:1. Available at: /pmc/articles/PMC5634815/. Accessed 29 September 2021.

27. Jamal W, Al Roomi E, AbdulAziz LR, Rotimi VO. Evaluation of curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. J Clin Microbiol **2014**; 52:2487–2492. Available at: https://europepmc.org/articles/PMC4097734. Accessed 29 September 2021.

28. Nouira S, Marghli S, Besbes L, et al. Standard versus Newer Antibacterial Agents in the Treatment of Severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease: A Randomized Trial of Trimethoprim-Sulfamethoxazole versus Ciprofloxacin. Antibiot Trial COPD Exacerbation • CID 2010:143. Available at: https://academic.oup.com/cid/article/51/2/143/301482. Accessed 18 January 2022.

29. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J **2009**; 34:648–654. Available at: https://pubmed.ncbi.nlm.nih.gov/19720809/. Accessed 31 January 2022.

30. Dodd JW, Hogg L, Nolan J, et al. The COPD assessment test (CAT): response to pulmonary rehabilitation. A multicentre, prospective study. Thorax **2011**; 66:425–429. Available at: https://pubmed.ncbi.nlm.nih.gov/21398686/. Accessed 31 January 2022.

31. Husebø G, Köll RM, Nielsen AF, Mestad K, Govertsen AK, Lærum BN. CAT-score is a predictor for mortality in COPD. Eur Respir J **2016**; 48:PA3106. Available at: https://erj.ersjournals.com/content/48/suppl_60/PA3106. Accessed 31 January 2022.

32. Jones PW, Tabberer M, Chen WH. Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores. BMC Pulm Med **2011**; 11. Available at: https://pubmed.ncbi.nlm.nih.gov/21835018/. Accessed 31 January 2022.

TOPIC Inclusion/Exclusion Checklist

Inclusion (all must be yes to enroll the subject)

Y	N	
☐	☐	Carries the diagnosis of COPD and admitted for and admitted with AECOPD to Beaumont, Royal Oak • AECOPD requires increased cough, increased sputum production, and shortness of breath +/- increased oxygen needs from baseline
☐	☐	Able to produce a sputum sample
☐	☐	Age ≥ 40 and < 90
☐	☐	Previously enrolled in the EPIC Study and PJ detected in their sputum
☐	☐	Currently treated with steroids
☐	☐	Kidney function not severely impaired (CrCl ≥ 60)
☐	☐	AST and ALT ≤ 5x upper limit of normal
☐	☐	Willing and able to consent to the study

Exclusion (all must be no to enroll the subject)

Y	N	
☐	☐	Currently diagnosed with pneumonia or COVID-19
☐	☐	Allergy or hypersensitivity to trimethoprim-sulfamethoxazole
☐	☐	Current ICU admission or mechanical ventilation
☐	☐	Active cancer or chemotherapy (except non-melanoma skin cancer)
☐	☐	Other potentially confounding pulmonary diagnosis
☐	☐	HIV, leukopenia, neutropenia or other immunosuppressive condition or current use of immunosuppressive medications
☐	☐	Presence of gastrointestinal tract abnormalities that would prevent absorption of medications
☐	☐	Patients with concomitant infection requiring antibiotics active against PJ
☐	☐	Concomitant use of coumadin, phenytoin, pioglitazone, repaglinide, rosiglitazone, glipizide or glyburide
☐	☐	Megaloblastic anemia due to folate deficiency
☐	☐	Pregnancy
☐	☐	Life expectancy less than 3 months

Form Completed By: : _____ _____ _____

(Printed name) (Signature) (Date/Time)

IRB NUMBER: 2022-102
IRB APPROVAL DATE: 06/08/2022

Principal Investigator: _____ _____ _____

(Printed name) (Signature) (Date/Time)

Appendix A

Examples of Exclusion Criteria

Confounding pulmonary diagnoses: Interstitial Lung Disease, Pulmonary Fibrosis, BOOP, severe uncontrolled asthma, any other pulmonary condition which in the opinion of the PI is potentially confounding.

Commonly used immunosuppressive agents: tacrolimus, cyclosporine, mycophenolate, sirolimus, everolimus, baricitinib, tofacitinib, upadacitinib, ruxolitinib, fedratinib, adalinmumab, anakinra, etanercept, infliximab, rituximab, ocilizumab, ustekinumab.

GI abnormalities which might prevent absorption of medications: roux-en-Y gastric bypass, duodenal switch (gastric sleeve maintains reasonable absorption). Other GI conditions which in the opinion of the PI potentially would prevent the absorption of TMP-SMX.