# BELIMUMAB TREATMENT OF Chronic Obstructive Pulmonary Disease (COPD) COPD PATIENTS WITH ANTI-GRP78 AUTOANTIBODIES

# (BOTEGA)

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# PROTOCOL SYNOPSIS

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| **Protocol Title:** | **Belimumab Treatment of COPDChronic Obstructive Pulmonary Disease (COPD) Patients with Anti-GRP78 Autoantibodies (BOTEGA)** |
| **Protocol Number:** | IRB-300000119 |
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| **Clinical Phase:** | Phase II clinical investigation |
| **Trial Site:** | University of Alabama at Birmingham |
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| **Participating Medical Center:** | University of Alabama at Birmingham (UAB) |
| **Funding Source:** | GlaxoSmithKline (GSK) |
| **Study Rationale:** | This is intended to be an initial "proof-of-concept" study to show feasibility, validate assays and approaches, and explore dosing and safety of belimumab in pulmonary COPD patients who have clinically relevant (and quantifiable) autoimmune responses. |
| **Study Objectives:** | The primary goal of clinical trial is to determine effects of belimumab on levels of autoantibodies against glucose regulated protein 78 (GRP78) among patients with pulmonary COPD attributable to cigarette smoking. |
| **Study Hypothesis:** | Our study hypothesis is that belimumab treatment will safely reduce circulating levels of autoantibodies that are associated with COPD, and comorbidities of this lung disease, including atherosclerosis. |

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| **Study Aims:** | **Specific Aim 1:** To conduct a double-blind, Phase II trial, in which 18 former smokers with COPD and circulating anti-GRP78 autoantibody levels >mean normal values (by ELISA), will be randomized 2:1 to belimumab *vs*. placebo. Subjects will receive 8 infusions of either belimumab or placebo over a 6 month interval. Plasma anti-GRP78 antibodies will be measured pre-treatment, and at 1, 3, 5, and 7 months. *We hypothesize belimumab therapy will more effectively reduce anti-GRP78 IgG autoantibodies, the primary endpoint of this trial, compared to placebo.*<br><br>**Specific Aim 2:** To determine effects of the belimumab therapy on secondary endpoints (at the times detailed for Aim 1) that include levels of pneumococcal-binding antibodies (by ELISA), circulating B-cell numbers and phenotypes (by flow cytometry), and the rate and severity of adverse events (AE) at any time during treatment. *We hypothesize belimumab will have dose-related effects on B-cell numbers and their differentiation, while minimally reducing host defense antibodies, and will also have an acceptable AE profile.* |
| **Study Design:** | Following baseline screening assessments, patients that meet all inclusion/exclusion criteria will be randomly assigned to receive one of the following treatments in a ratio of 2:1:<br><br>**1.) Arm 1: Belimumab (n = 12).** Subjects randomized to the experimental treatment arm will receive i.v. administrations of belimumab (10 mg/kg), consisting of three "loading" doses, two weeks apart, followed by five (5) more monthly infusions. The final dose is administered at month 6 with final assessment at month 7 and a safety visit at month 10.<br><br>**2.) Arm 2: Placebo Controls (n = 6)**. These subjects will be treated with identically appearing placebo i.v. on the same schedule as the experimental arm subjects (i.e., three "loading" doses, two weeks apart, followed by five more monthly infusions. Again, the final assessment will be performed at month 7 (210$\pm$10 days after treatment start). |
| **Planned Sample Size:** | A total of 18 subjects will be enrolled in this trial. In order to reach the needed sample size of 18, twenty-five subjects will be randomized to account for subjects who have been withdrawn early due to the current COVID-19 pandemic in order to comply with guidance regarding human subjects research conduct and also for medical reasons un-related to study drug or study procedures. |
| **Duration of Treatment:** | Six months |
| **Duration of Participation** | Ten months |
| **Inclusion Criteria:** | **1.)** A history of past tobacco smoking ($\geq$10 pack years), but quit for $\geq$6 months at the time of enrollment. Smoking cessation will be confirmed by serum cotinine assays.<br><br>**2.)** Clinical diagnosis of at least moderate COPD as defined by the Global Initiative for Obstructive Lung Disease (GOLD) criteria*:* |

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| | a. Post bronchodilator $FEV_1/FVC < 70\%$,<br>b. Post bronchodilator $FEV_1$ between 25% and 80% predicted, with or without chronic symptoms (i.e., cough, sputum production).<br>COPD<br>3.) Ability and willingness to give informed consent.<br><br>4.) Levels of plasma autoantibodies against a 15–mer peptide sequence within the GRP78 molecule (a.a. 246-260) that are in the upper three quartiles of values present in COPD patients. This epitope will henceforth be denoted as GRP78 Peptide 25. Levels of anti-GRP78 Peptide 25 autoantibodies are significantly correlated with carotid artery intimal medial thickness (IMT) among COPDpatients.<br><br>5.) Age 40-75 y.o. COPD is a disease of older individuals. |
| **Major Exclusion Criteria:** | 1.) History of more than one moderate (treated as outpatient) or severe (requiring hospitalization) acute exacerbation of COPD within the 12 months prior to screening or any moderate or severe exacerbation within the 4 months prior to screening. A past history of an acute exacerbation is the single biggest risk for recurrence.[36] Exclusion of subjects at highest risk will minimize drop-outs.<br><br>2.) Oral steroids or cellular immunosuppressant use (e.g., cyclophosphamide) within 6 months.<br><br>3.) History or clinical or laboratory evidence of other autoimmune syndromes (confirmed by self-report and medical record review).<br><br>4.) Inability or unwillingness to complete the treatment and surveillance protocols.<br><br>5.) Listed for lung transplant at time of enrollment. This exclusion will mitigate any potential, however slight, that a patient could be rejected for transplantation due to surgeon concerns about this novel therapy (and will also obviate early drop-outs due to transplantation).<br><br>6.) History of malignant neoplasm within the last 5 years.<br><br>7.) Evidence of serious suicide risk including any history of suicidal behavior in the last 6 months and/or any suicidal ideation in the last 2 months or those, in the investigator's judgment, pose a significant suicide risk.<br><br>8.) History of a primary immunodeficiency.<br><br>9.) Significant IgG deficiency (IgG level < 400 mg/dL).<br><br>10.) IgA deficiency (IgA level < 10 mg/dL).<br><br>11.) Currently on any suppressive therapy for a chronic infection (such as tuberculosis, pneumocystis, cytomegalovirus, herpes simplex virus, herpes zoster or atypical mycobacteria). |

12.) Hospitalization for treatment of infection within 60 days of screening.

13.) Use of parenteral (IV or IM) antibiotics (antibacterials, antivirals, anti-fungals, or anti-parasitic agents) within 60 days of screening.

14.) Current drug or alcohol abuse or dependence, or a history of drug or alcohol abuse or dependence within 365 days prior to Day 0 (for compliance and safety).

15.) History of a positive HIV test or positive screening test for HIV.

16.) Serologic evidence of current or past Hepatitis B (HB) or Hepatitis C (HC) infection based on positive tests for HBsAg or HBcAb, or HCAb.

17.) History of an anaphylactic reaction to parenteral administration of contrast agents, human or murine proteins or monoclonal antibodies.

18.) Any other clinically significant abnormal laboratory value in the opinion of the investigator.

19.) Any intercurrent significant medical or psychiatric illness that the investigator considers would make the candidate unsuitable for the study.

20.) Women of Child Bearing Potential (WCBP) must have a negative urine pregnancy test at screening, and agree to 1 of the following:

Complete abstinence from intercourse from 2 weeks prior to administration of the 1st dose of study agent until 16 weeks after the last dose of study agent (Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are <u>not</u> acceptable methods of contraception)

OR

Consistent and correct use of 1 of the following acceptable methods of birth control for 1 month prior to the start of the study agent, during the study, and 16 weeks after the last dose of study agent:

- Oral contraceptive, either combined or progestogen alone
- Injectable progestogen
- Implants of levonorgestrel or etonogestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches
- Intrauterine device (IUD) or intrauterine system (IUS) with <1% failure rate as stated in the product label

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| | ○ Male partner sterilization (vasectomy with documentation of azoospermia) prior to the female subject's entry into the study, and this male is the sole partner for that subject. For this definition, "documented" refers to the outcome of the investigator's/designee's medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records<br>○ Double barrier method: condom and occlusive cap (diaphragm or cervical/vault caps) plus spermicidal agent (foam/gel/film/cream/suppository)<br><br>21.) Use of Excluded Medications:<br>• Anti-B-cell therapy:<br>○ Wash out of 5 therapeutic half lives after prior B-cell therapy, or until pharmacodynamic effect would be minimal (e.g., 1 year following rituximab)<br>• 365 days Prior to Belimumab:<br>○ Any biologic investigational agent (e.g., abetimus sodium, anti CD40L antibody, BG9588/ IDEC 131)<br>▪ Investigational agent applies to any drug not approved for sale in the country in which it is being used<br>• 30 Days Prior to Belimumab (or 5 half lives, whichever is greater)<br>○ Any non-biologic investigational agent<br>▪ Investigational agent applies to any drug not approved for sale in the country in which it is being use<br>• Live vaccines within 30 days prior to baseline or concurrently with belimumab |
| **Study Endpoints:** | Primary Endpoint: The primary endpoint of this trial is a reduction of circulating autoantibodies against the GRP78 epitope most correlated with carotid atherosclerosis.<br><br>Secondary Endpoints**:** The secondary endpoints of this trial are: 1.) treatment effects on concentrations of pneumococcal polysaccharide-binding antibodies by ELISA. 2.) treatment effects on absolute numbers and phenotypes of circulating B-cells by flow cytometry, and 3.) Adverse event comparisons. |

# 1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

## 1.1 OBJECTIVE

The goal of this randomized blinded Phase IIa clinical trial is to begin to determine selected effects (and safety) of belimumab therapy in pulmonary COPD patients who have autoantibodies with specificities against glucose regulated protein 78 (GRP78).

The information gained in this trial will be invaluable, and enable us, and others, to credibly propose a subsequent larger and longer clinical trial, that could more definitively test **the effects of belimumab on the progression of COPD and atherosclerosis (and perhaps other related comorbidities)**.

## 1.2 SPECIFIC AIMS

**Hypothesis:**

Our specific study hypothesis is that belimumab treatment will safely reduce circulating levels of autoantibodies to GRP78 that have been associated with pulmonary COPD, and comorbidities of this lung disease, including atherosclerosis.

**Specific Aims:**

**Specific Aim 1:** To conduct a double-blind, Phase II trial, in which 18 former smokers with pulmonary COPD (per chest CT scans), and circulating anti-GRP78 autoantibody levels >mean normal values (by ELISA), will be randomized 2:1 to belimumab *vs*. placebo. Plasma anti-GRP78 will be measured pre-treatment, and at 1, 3, 5, and 7 months. *We hypothesize belimumab therapy will more effectively reduce anti-GRP78 IgG autoantibodies, the primary endpoint of this trial, compared to placebo.*

**Specific Aim 2:** To determine effects of the belimumab therapy on secondary endpoints that include levels of pneumococcal-binding antibodies (by ELISA), circulating B-cell numbers and phenotypes (by flow cytometry), and adverse events (AE). *We hypothesize belimumab will have dose-related effects on B-cell numbers and their differentiation, but will minimally reduce host defense antibodies, and will have an acceptable adverse event profile.*

## 1.3 BACKGROUND

Chronic obstructive pulmonary disease (COPD), which includes the COPD phenotype of COPD, is the third leading cause of death world-wide, and the prevalence (and impact) of these lung disorders are increasing. COPD/COPD directly cause considerable disability and premature deaths.[1-3] In addition, the systemic manifestations of COPD, such as osteoporosis and atherosclerosis, result in considerable additional morbidity and mortality.[4-6] Current treatments are nonspecific and have little impact on the natural histories of either the lung abnormalities or comorbidities.

"Chronic inflammation" has been implicated in the genesis of COPD and the several singular comorbidities of this disease, but the causal immunological mechanism(s) have remained enigmatic.

We, and others, have shown that chronic inflammation in COPD can be mediated by pathogenic autoantibodies, autoreactive T-cells, and other immunological abnormalities that parallel features of conventional autoimmune syndromes.[6,7] Moreover, **recent findings (to follow) implicate autoimmunity in the genesis and progression of COPD, and the osteoporosis and atherosclerosis that are often associated with this lung disease**:

## 1.4 PRELIMINARY DATA

We, and now others, have shown that antigen-antibody (immune) and complement complexes, which are known to cause severe tissue injuries in SLE and other autoimmune diseases, are also near ubiquitous in lungs of patients with severe COPD/COPD.[7] We have also shown circulating B-cells are highly differentiated in COPD, a consequence of repetitive antigen stimulation typically seen in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and other autoantibody-mediated disorders.[8]

We've also reported serum B-lymphocyte stimulating factor (BLyS, aka: B cell activating factor or BAFF) concentrations are also abnormally increased in COPD patients (Figure 1).[8] We were particularly interested in study of this mediator because belimumab, a specific anti-BLyS agent, is already available for treatment of SLE. **The adaptation of belimumab therapy for use in autoimmune COPD patients is the basis of the current proposal.** Our BLyS findings in COPD patients have since been corroborated by at least two other groups.[9,10] One of those studies also indicated anti-BLyS therapy had favorable effects in a preclinical mouse model of COPD.[9]

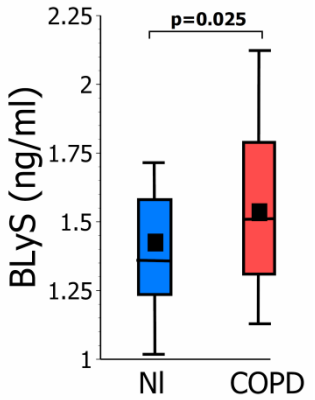


**Figure 1** (*right*). Plasma levels of BLyS are greater in COPD patients (n=90) than in healthy normal subjects (Nl) (n=53).[8]

We also recently discovered autoantibodies against glucose regulated protein 78 (GRP78) are prevalent in patients with COPD (Figure 2).[6] GRP78 is a heat shock protein, and an autoantigen in several other immune disorders.[11,12] Intrapulmonary GRP78 is upregulated by smoke exposure, viral infections, and various other injuries, and this molecule is over-expressed in COPDtous lungs.[6] The presence of anti-GRP78 was also highly associated with osteoporosis in smokers (Figure 3).[6]

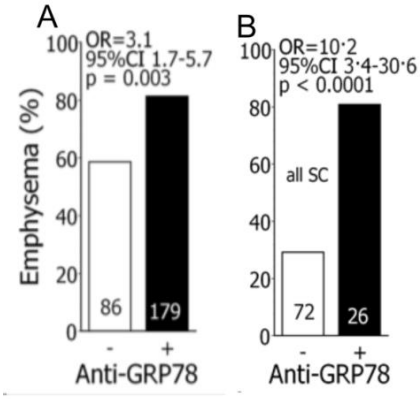


**Figure 2** (*left*). **A**) Anti-GRP78 autoantibodies detected by Western blots were more prevalent in smokers/former smokers with COPD, independently of COPD.[6] OR= odds ratio, 95%CI = 95% confidence interval. Numbers in columns denote subject "n". **B**.) The association between COPD and anti-GRP78 autoantibodies was especially strong among smoke controls (SC) who are heavy smokers (>30 pack-years) that do not have expiratory airflow obstruction on spirometry.[6]

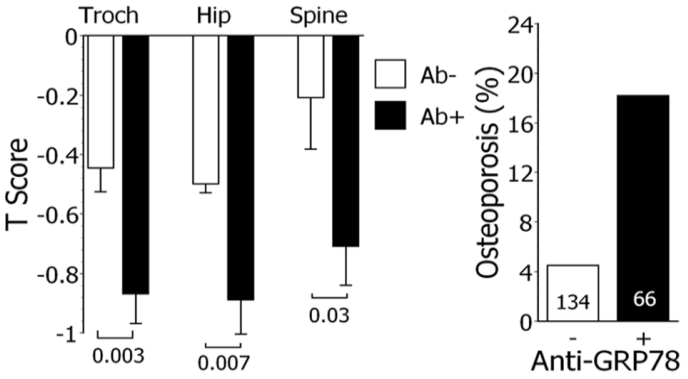


**Figure 3** (*right*). **GRP78** (left panel) and panel) are greater with anti-GRP78 densities (BMD) absorptiometry number of BMD young, gender means. T scores < osteoporosis.[5,6]

**Osteoporosis and Anti-Autoantibodies.** T scores osteoporosis prevalence (right among the smoking cohort autoreactivity.[6] Bone mineral were measured by dual X-ray (DXA). T scores are the standard deviations from and ethnic-specific reference -2.5 at any site defined

Other recent findings also show anti-GRP78 autoantibody levels are predictive of COPD progression (Figure 4).

The circulating anti-GRP78 autoantibodies in these patients have direct pathogeneic effects that can cause or contribute to lung, bone, and other diseases. In addition to function as an intracellular protein chaperone and key component of the unfolded protein response, GRP78 is extracellularly exported by stressed cells.[11] Extracellular GRP78 acts like a cytokine, binding to and stimulating various cell surface receptors that transduce signals on many different cell types. Studies by us, and others, show anti-GRP78 autoantibodies can **bind and crosslink these cell surface GRP78-receptor complexes on target cells, including macrophages and osteoclasts (the bone correlate of a macrophage), which then increase their productions of pro-inflammatory mediators implicated in lung injury and bone resorption**.[6,12] Anti-GRP78 in patients with RA and other immunological diseases also have similar injurious effects.[12]

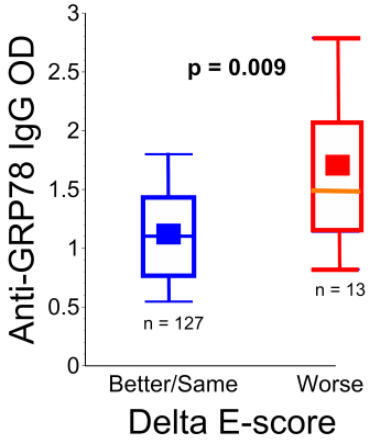


**Figure 4** (left)**. COPD Progression and Anti-GRP78 Autoantibodies.** Anti-GRP78 autoantibody levels (per ELISA Optical Density [OD]) are greater in subjects whose COPD worsens over the next 2 years, per serial CT COPD scores. Shown here are heavy smokers with COPD but normal spirometry (denoted as SC in Fig. 2). (Manuscript in preparation).

Additional p***reliminary (unpublished) data show anti-GRP78 autoantibodies from COPD patients bind to primary human endothelial cells that were stressed by culturing them in turbulent flow conditions, due to increased translocation of GRP78 to the cell surfaces (not shown).***

We have recently further refined our immunological studies by testing for autoreactivity against individual short peptide sequences within the GRP78 molecule. Thirty-one (31) different peptides within the GRP78 molecule were selected using an autoantibody epitope prediction algorithm. Candidate peptides were then synthesized *in vitro*, and identities were confirmed by mass spectroscopy prior to individual testing in ELISA. We have found associations between carotid artery IMT in COPD patients and circulating levels of autoantibodies with specificities against four particular linear epitopes. The strongest of these associations is with a 15–mer peptide sequence (GRP78 aa. 246-260) that we have denoted as anti-GRP78 Peptide 25 (Figure 5). The use of specific peptide

epitope targets in subsequent ELISA and other autoantibody detection assays will improve reproducibility, diagnostic sensitivities and specificities, potentiate development of assays using panels of atherogenic autoantigens, and eliminate confounding introduced by vagaries of tertiary structure (e.g., folding) that we have noted among different batches of intact, whole recombinant GRP78.

**How Does Autoimmunity Arise?** COPD and COPD probably do not start as autoimmune diseases. Autoimmunity usually develops subsequent to other injurious processes, such as infections, cancer, etc., by epitope spread or mimicry, or other unknown mechanisms.[14-19] Most "secondary" autoimmunities are probably clinically benign. In some cases, however, these "new" immune responses cause striking additional morbidity, e.g., carditis or nephritis following otherwise trivial *Streptococcal* infections, neurologic syndromes associated with cancer, and many others.[14-16]

The microbiome within smoke-damaged lungs is highly immunogenic, and the chronic immune responses that result are also likely conducive to the development of autoimmunity.[20] In addition, neoantigens generated by reactive constituents within tobacco smoke can also provoke autoimmune responses.[21]

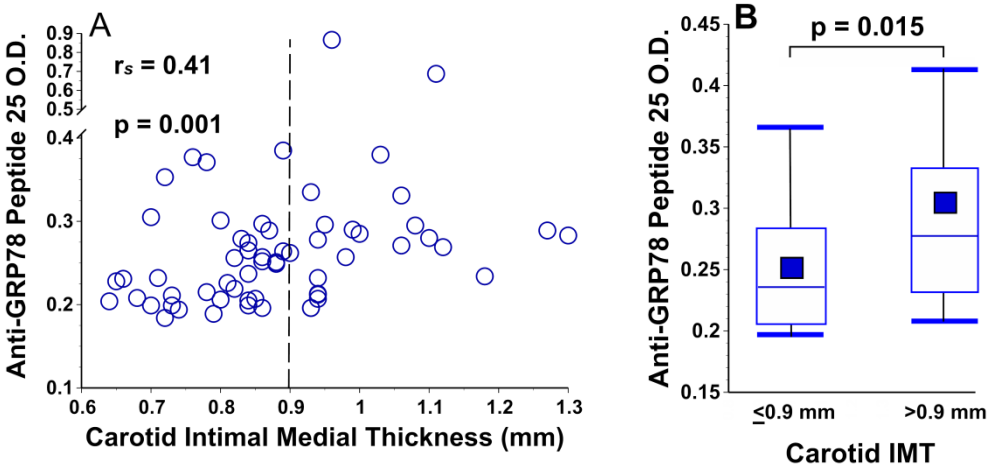


**Figure 5. A**) Correlation between levels of circulating autoantibodies against an internal GRP78 epitope (GRP78 Peptide 25) and carotid intimal thickness (IMT) in COPD patients. Horizontal dotted line denotes upper limit of normal for IMT.[13] O.D. = optical density of ELISA determinations. **B**) Anti-GRP78 Peptide 25 levels are greater in COPD patients with abnormally increased carotid IMT (n = 26) vs those with normal IMT (n = 38) per Mann-Whitney comparisons. Ascending horizontal lines successively denote 10$^{th}$, 25$^{th}$, 50$^{th}$, 75$^{th}$, and 90$^{th}$ percentiles. Solid squares denote mean. These carotid-autoantibody associations were less significant among subjects who did not have pulmonary COPD.

The involvement of autoimmune processes in the pathogenesis of smoking-associated lung disease could account for well-known, but heretofore enigmatic, observations that pulmonary inflammation among these patients often persists, and their respiratory function frequently continues to rapidly deteriorate, despite removal of the inciting injury (e.g., tobacco smoking).[22] Once autoimmunity develops, the process has a tendency to be self-perpetuating, or at least recurrent, since the autologous protein(s) [autoantigen] that is the target of this immune responses is continually being renewed.

Furthermore, an autoimmune pathogenesis can also help explain the resistance of these disorders to medical therapies, including aerosolized and systemic glucocorticoids. Many autoimmune diseases, and **autoantibody-mediated lung diseases in particular,** are also highly resistant to steroids and nonspecific agents. Among these examples, granulomatosis with polyangitis ("Wegener's syndrome"), Goodpasture's syndrome, acute interstitial lung diseases associated with

polymyositis, RA, or other classical autoimmune diseases, and lung transplant rejection due to alloantibodies, have very high rates of progression and mortality when treated primarily (or solely) with steroids. In contrast, targeted therapies that reduce autoantibodies or diminish their production can benefit patients afflicted with these syndromes.[23-34]

The findings described in the preceding section led us to posit: ***Autoantibodies play a causal role in smoking-related COPD, osteoporosis, and atherosclerosis in patients with this lung disease.*** A corollary of this paradigm is that autoantibody reduction therapy with belimumab may delay (or maybe even prevent) progression of these disorders better than current nonspecific therapies.

The foregoing series of studies (and others) provide a rationale to treat at-risk COPD patients with biological agents that were developed for therapy of other autoantibody-mediated diseases.[33,34] Our studies have also identified a mechanistic biomarker (anti-GRP78 IgG autoantibodies) that will be a useful surrogate endpoint in early phase clinical trials (as we intend to employ it here).[6]

We propose to **begin** to test our hypothesis in an unprecedented bench-to-bedside pilot trial in which we aim to decrease autoantibody concentrations in COPD patients by using belimumab, a commercially-available biological agent with activity against BLyS.

### 1.5 SIGNIFICANCE

If our central hypothesis is correct, targeted therapy to reduce pathogenic autoantibodies could ultimately result in significant benefit for a lung disease, and associated comorbidities, that have been inexorable.

# 2 RESEARCH DESIGN AND METHODS

## 2.1 CLASSIFICATION AND METHODOLOGICAL DESIGN

This is a randomized, double-blinded placebo-controlled Phase II clinical trial to explore the use of belimumab in patients with smoking-associated COPD and autoantibodies.

## 2.2 STUDY DESIGN

**Subjects**: Eighteen (18) subjects will be recruited for this project from among ambulatory patients in The Kirklin Clinic (TKC) or Lung Health Center (LHC) at the University of Alabama in Birmingham (UAB). The LHC is directed by Dr. Dransfield, a Co-PI of this proposal. Potential participants of both genders and all ethnicities will be eligible for this trial. No special populations will be studied.

**Recruitment Methods**: The recruitment process will begin via one of two possible pathways:

1) Referral of the prospective participant to the investigators/research coordinator by a clinic physician who has knowledge of the proposed research, and obtains patient consent for the research team to approach the patient.

2) Alternatively, individuals who have provided signed IRB-approved HIPPA compliant consent for participation in clinical trial research registries may be approached by a designated investigator in those registries. The LHC maintains a registry of more than 1000 COPD patients who could be screened for potential participation in the trial.

## 2.3 STUDY TREATMENTS

Following procurement of informed consent and completion of baseline screening assessments, patients that meet all inclusion/exclusion criteria will be randomly assigned to receive one of the two following treatments, starting on Day 1, in a ratio of 2:1.

**1.) <u>EXPERIMENTAL BELIMUMAB TREATMENT</u> (n=12).** Subjects randomized to the experimental treatment arm will receive i.v. administrations of belimumab (10 mg/kg), consisting of three "loading" doses, two weeks apart, followed by five (5) more monthly infusions. The final assessment will be performed at month 7 (see Table 1).

**2.) <u>PLACEBO CONTROLS</u> (n=6)**: These subjects will be treated with identically appearing placebo i.v. on the same schedule as the experimental arm subjects (i.e., three "loading" doses, two weeks apart, followed by five more monthly infusions. The final assessment will be performed at month 7 (see Table 1).

| | **Loading Doses** | | | **Maintenance Doses** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Days | 1 | 14 | 30 | 60 | 90 | 120 | 150 | 180 | 210 |
| Study Drug | x | x | x | x | x | x | x | x | Assessment only (final) |

**Table 1.** Schedule of study drug infusions (either belimumab or placebo) and final assessment

### 2.4 RANDOMIZATION

Randomization by random number generator and assignment to one of the two treatment arms will be effected by the Investigational Drug Service at UAB.

### 2.5. STUDY TREATMENT SCHEMATIC

The experimental interventions are outlined below in Table 2:

| **Study Day (month)** | **Screening*** | **1** | **14** | **30 (1)** | **60 (2)** | **90 (3)** | **120 (4)** | **150 (5)** | **180 (6)** | **210 (7)** | **240 (8)** | **300 (10)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Clinic Visit** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | | **x**† |
| **Physical Exam** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | | |
| **Experimental Treatment** | | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | | | |
| **Spirometry** | **X#** | | | **x** | | **x** | | **x** | | **X#** | | |
| **DLCO** | **X** | | | | | | | | | | | |
| **6 Minute Walk Test** | **x** | | | | | | | | | **x** | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experimental Immunologic Assays** | x | | | x | | x | | x | | x | | |
| **Carotid Ultrasound** | x | | | | | | | | | x | | |
| **IgA** | x | | | | | | | | | | | |
| **IgG** | x | | | x | x | x | x | x | x | x | | |
| **Viruses**‡ | x | | | | | | | | | | | |
| **CBC with platelets** | x | | x | x | x | x | x | x | x | x | | |
| **Basic Metabolic Panel** | x | | x | x | x | x | x | x | x | x | | |
| **Liver Function**** | x | | x | x | x | x | x | x | x | x | | |
| **Cotinine** | x | | | | | | | | | x | | |
| **Autoimmune Serologies**†† | x | | | | | | | | | | | |
| **Pregnancy Tests (if applicable)** | x | x | x | x | x | x | x | x | x | x | | x† |
| **Post-treatment Phone Checks** | | x | x | x | x | x | x | x | x | | x | x |

**Table 2. Outline of Study Procedures and Tests. Notes:** Telephone contacts will also be established on the days following experimental treatments (i.e., at day 2, 15, 31, 61, 91, 121, 151, and 181).

*Screening evaluations can occur over more than one day.

#Pre- & Post-bronchodilator Spirometry at screening and day 210 only, otherwise post- bronchodilator only

†Clinic visits and pregnancy tests at 10 months if applicable (female subjects with childbearing potential). Other subjects will have phone checks at 10 months.

‡Viruses include HIV, Hepatitis C antibody (HCAb), Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody (HBcAb)

**Routine liver function tests include alkaline phosphatase, total bilirubin, ALT, AST, and INR.

††Autoimmune serologies are ANA, RF, anti-CCP, anti-SSA, anti-Jo-1

## 2.6 STUDY PROCEDURES

Routine clinical care for patients with pulmonary COPD will be followed as ordered by the primary physician.

All subjects will have infusions of study medications (belimumab or placebo) in an expert, dedicated, continuously-monitored UAB infusion center at the intervals delineated in Table 1. The treatment protocol will last for 180 days (6 months), with a final observation and specimen collection at day 210 (7 months) (see Table 1). Patients removed from the study for unacceptable AE will be followed until resolution or stabilization of the problem(s). Telephone interviews to detect potential late complications and outcomes will be conducted by study coordinators at other intervals (see Table 2).

Patients will be monitored carefully while participating in this study for occurrences of adverse events, laboratory test abnormalities, and changes in vital signs. Adverse events will be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Belimumab will be administered IV over a minimum of 1 hour. The infusion rate may be slowed or interrupted if the subject develops an infusion reaction. In the event of a serious reaction, study agent administration must be discontinued immediately and the appropriate medical therapy administered.

In the post-marketing setting, delayed onset of symptoms of acute hypersensitivity reactions has been observed, especially in the first or second infusion. Subjects will remain under clinical supervision for 3 hours after completion of the first 2 infusions. Should symptoms of acute hypersensitivity occur, an extended period of monitoring may be appropriate, based on clinical judgement. This may include, but is not limited to, monitoring vital signs and observing any untoward reactions. Beyond the first 2 infusions, subjects should be monitored during infusion and for an appropriate period of time after infusion (this will generally be one hour, again based on investigator judgement).

Subjects should be made aware of the potential risk, the signs and symptoms of such reactions, and the importance of immediately seeking medical attention. For further information, see the belimumab IB.

Venous blood for experimental purposes (e.g., end-point measures), totaling 29 ml, and study-specific pulmonary function tests (i.e., spirometry and diffusing capacities), will be obtained at screening (baseline) and at 1, 3, 5, and 7 months after treatment started (Table 2). Additional venous phlebotomy specimens for safety monitoring tests and assays appropriate for the experimental use of belimumab will be obtained at these and other intervals as needed (specified below).

The results of the pulmonary function tests, clinical data, and safety studies will be logged in case report forms (CRF) as they are generated (in real time). The experimental laboratory (immunological) assays will be performed intermittently among accumulated batched specimens and results recorded as they are performed.

### 2.6.1 Screening

Screening: Following informed consent, patients will undergo screening assessments to determine that all inclusion/exclusion criteria are met prior to randomization or receiving the study drug treatments.
The following screening information will be collected and entered into the study case report forms (CRFs) and/or web-based data collection system.

1. Physician names and contact information, general medical and surgical histories, including suicidality, co-morbidities, concurrent medications, and allergies.
    a. ***Excluded Concomitant medications***
    - Anti-B-cell therapy:
        - Wash out of 5 therapeutic half lives after prior B-cell therapy, or until pharmacodynamic effect would be minimal (e.g., 1 year following rituximab)

- 365 days Prior to Belimumab:
    - Any biologic investigational agent (e.g., abetimus sodium, anti CD40L antibody, BG9588/ IDEC 131)
    - Investigational agent applies to any drug not approved for sale in the country in which it is being used
- 180 Days Prior to Belimumab:
    - Intravenous cyclophosphamide
        - If concomitant use, enhanced safety monitoring required.
            - Serum IgG levels should be measured monthly in this situation
            - Benlysta should be discontinued in subjects with serum IgG levels <250 mg/dL associated with a severe or serious infection
- 30 Days Prior to Belimumab (or 5 half-lives, whichever is greater)
    - Any non-biologic investigational agent
        - Investigational agent applies to any drug not approved for sale in the country in which it is being use
- Live vaccines within 30 days prior to baseline or concurrently with belimumab

2. Recording of vital signs and medication regimen.
3. Review of medications
4. Brief assessment of symptoms.
5. Recording of laboratory and clinical testing results.
6. Pulmonary function test results (pre/post bronchodilator (BD) spirometry and DLCO).
7. Serum cotinine
8. Chest CT, with measurement of $F_{950}$[35] within the preceding 2 years if available

The screening assessment may take place over more than one day. Eligible patients will then undergo the following tests and procedures prior to randomization:

- Detailed medical history and demographics
- Physical exam to include vital signs and blood pressure.
- Laboratory evaluations specific for Inclusion/Exclusion Criteria to include: CBC, BMP, Liver function tests, HIV, hepatitis B and C tests, IgA, ANA, RF, anti-SSA, anti-Jo-1, anti-CCP, cotinine, urine pregnancy test (if applicable),, IgA, IgG.

Patients who fulfil all entry/exclusion criteria, and agree to participate, will have remaining baseline assessments (spirometry, DLCO, 6MWD, and carotid ultrasounds), blood obtained for experimental immunology assays (see Primary and Secondary Endpoints, Section 2.8) and be randomized to receive one of the study treatments described above in Section 2.3. No more than 30 days should elapse between completion of the eligibility assessments and the first experimental treatment.

Subsequent events include:

### 2.6.2 Experimental procedures

Day 1 (Initiation of therapy):

Patients will report to a UAB infusion center for the following:

Day 1:

- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, (must be < 7 days prior to first dose).
- Adverse Event (AE) assessment.
- First infusion of study drug.

Day 2:

- Phone assessment by study personnel, including an AE assessment

Day 14:

- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable.
- Second infusion of study drug
- AE assessment

Day 15:

- Phone assessment by study personnel, including an AE assessment

Day 30 (Month 1):

- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), and IgG, pregnancy test, if applicable.
- Spirometry (post-BD)
- Third infusion of study drug
- AE assessment

Day 31:

- Phone assessment by study personnel, including an AE assessment

Day 60 (Month 2):

- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, and IgG.
- Fourth infusion of study drug

- AE assessment

Day 61:
- Phone assessment by study personnel, including an AE assessment

Day 90 (Month 3):
- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, and IgG.
- Spirometry (post-BD)
- Fifth infusion of study drug
- AE assessment

Day 91:
- Phone assessment by study personnel, including an AE assessment

Day 120 (Month 4):
- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, and IgG.
- Sixth infusion of study drug
- AE assessment

Day 121:
- Phone assessment by study personnel, including an AE assessment

Day 150 (Month 5):
- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, and IgG.
- Spirometry (post-BD)
- Seventh infusion of study drug
- AE assessment

Day 151:
- Phone assessment by study personnel, including an AE assessment

Day 180 (Month 6):
- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), pregnancy test, if applicable, and IgG.

- Eight and final infusion of study drug
- AE assessment

Day 181:
- Phone assessment by study personnel, including an AE assessment

Day 210 (Month 7):
- Directed physical exam that includes vital signs and blood pressure, interval medication and symptom changes.
- Laboratory evaluations (Table 2) to include CBC, BMP (electrolytes, glucose, BUN, serum creatinine), liver function tests (INR, total bilirubin, ALT, AST), cotinine, pregnancy test, if applicable, and IgG.
- Spirometry (pre/post-BD)
- 6MWD
- Carotid ultrasound
- AE assessment

Day 240 (Month 8):
- Phone assessment by study personnel, including an AE assessment. A variance of 5 days will be allowed for these telephone contacts to account for weekends or holidays.

Day 300 (Month 10):
- Clinic visit and pregnancy test, if applicable (females with childbearing potential). Other subjects will have phone assessment by study personnel, including an AE assessment.

### 2.6.3 Other Variances

A variance of +/-5 days will be allowed for clinic visits and treatments, to account for weekends, holidays or other logistic problems. A variance of 10 days will be allowed for the surveillance visits on day 210, and day 300 (if applicable), for the same reasons.

### 2.6.4 Specimen Collection and Management

Specimen Collection / Documentation: An extra 29 ml of blood for experimental immunologic research studies and endpoint measures will be obtained pretreatment, and on days 30, 90, 150, and 210 after the start of therapy (Table 2). The peripheral blood will be processed to separate mononuclear cells, plasma, and sera, which will be aliquoted, and frozen (-80$^{o}$) until used in batched assays. These assays will be carried out in the laboratories of Dr. Steven Duncan.

Each research sample will be labeled with subject's unique identifier, sample date, and sample collection time. The code number and date on which the specimen is frozen, all other information about the specimen, and subsequent processing will be entered on a specimen processing worksheet.

Covariate information, e.g., concomitant medications, laboratory values, etc., will be obtained at each time point.

Specimen Handling and Labeling (De-Identification)

All research specimens and all records associated with the samples will be labeled only with a unique code that links to the trial data but contains no personal identifiers. The information linking these code numbers to the corresponding subject's identity will be kept in a secure location in the contact PI's office, and will not be available to staff managing samples at the research laboratory.

Specimen Management and Storage: Specimens in excess of immediate assay requirements may be stored indefinitely in a secured freezer under the control of the responsible PI. The coding information linking patient identifiers to the stored samples will be maintained in a locked, secure area that will be accessible only to the study investigator. Subjects may request to have their samples destroyed at any time. These samples will be destroyed immediately upon receipt of the subjects' written request to do so.

Restrictions to Direct Access of Specimens: Specimens will be kept in the responsible study investigators' laboratory indefinitely and will be under the control of the PIs. Investigators or other personnel not involved with the management or operations of the study are not permitted direct access to the specimens.

## 2.7 STUDY ENDPOINTS

### 2.7.1. The Primary End-Point:

The primary endpoint of this trial is a reduction of circulating levels of anti-GRP78 Peptide 25, a clinically relevant surrogate biomarker of "autoimmunity" in these patients. Plasma concentrations of the anti-GRP78 autoantibodies will be measured pre-treatment, and at 1, 3, 5, and 7 months after treatment start.

**General:** Heparinized blood for end-point measures will be obtained from subjects by venous phlebotomy. Gentle centrifugation will separate cells from plasma, which will then be aliquoted and appropriately stored for assays of anti-GRP78 IgG (primary endpoint) and anti-pneumococcal antibodies (a secondary endpoint). A separate unanticoagulated tube (red top) will be used to procure sera. Peripheral blood mononuclear cells (PBMNC) will be isolated from the residual heparinized specimen by resuspension and density gradient centrifugation. PBMNC will be used for B-cell studies (a secondary endpoint), and residual cells will also be stored frozen as dry pellets and in RNase protection media for potential later use (e.g, cloning and sequencing autoreactive B-cell IgG).

Anti-GRP78 IgG levels will be measured in serial specimens by a custom ELISA that has already been developed, validated, and employed (see Figs. 4,5). In brief, biotinylated GRP78 Peptide 25, which was synthesized by Thermo Scientific (Waltham, MA) is diluted to 0.5ug/ml in PBS and added to streptavidin-coated ELISA plate wells and incubated overnight at $4^{o}$ C. After thorough washing and blocking, patient plasma specimens (see Section 2.8.2) are added to the wells at 1:100 dilutions and incubated for 2 hours at room temperature. Following additional washes, alkaline phosphatase-conjugated goat anti-human antibody (1:5000 dilution) is added, incubated, and color developed with a pNPP substrate system (KPL, Gaithers-burg, MD). Optical densities (OD) are determined at 405 nm.

ELISAs against GRP78 and epitopes within this molecule are accurate and reproducible. We used identical aliquots of several specimens in assays performed by multiple different lab operators, separated temporally by up to 1 year. Despite these variables, the ELISA yielded concordant values with $r \geq 0.96$. Similarly, serial anti-GRP78 IgG measures in specimens collected after two-year intervals from a group of COPD patients yielded $r > 0.82$. Most of the differences in OD values in the serial

measures seemed to be inversely concordant with interval changes of patient's clinical status (e.g., anti-GRP78 OD tended to increase as their lung disease severity got worse).

### 2.7.2. Secondary End-Points

**Specific Aim 2 Endpoints**: The secondary endpoints of this trial are: 1.) treatment effects on concentrations of pneumococcal polysaccharide-binding antibodies by ELISA[33,36] 2.) treatment effects on absolute numbers and phenotypes of circulating B-cells by flow cytometry,[8] and 3.) Adverse event comparisons.

Secondary endpoint #1. (anti-pneumococcal antibodies) and #2. (B-cells) will be assessed among blood specimens obtained prior to treatment, and at 1, 3, 5, and 7 months after treatment starts. Secondary endpoint #3 (AE) will be assessed at each clinic visit, telephone encounter and in communications initiated by subjects or study personnel at any other interval.

**a.) Pneumococcal polysaccharide-binding antibody concentrations** in plasma will be measured pre-treatment, and compared to results at the aforementioned intervals after treatment started. The ELISA methodology is straightforward, highly standardized, and fully detailed previously.[36]

**b.) B-cell numbers and phenotypes** will be assessed at the same time-points. Method-ologies are also well established and have been previously detailed.[8] In brief, B-cells will be stained with panels of fluorochrome-conjugated monoclonal antibodies and characterized by flow cytometry. B-lymphocytes will be identified as $CD3^{-}/CD14^{-}/CD16^{-}/CD19^{+}$ cells, and further stratified for comparisons as transitional ($IgD^{+}/CD38^{++}$), $IgM^{+}$ $IgA^{-}$ $IgG^{-}$ switched memory ($IgD^{+}/CD27^{+}$), $IgG^{+}$ switched memory ($IgD^{-}/CD27^{+/-}$), and plasmablasts ($CD19^{lo/+}$, $CD20^{-}$, $CD38^{++}$, $IgD^{-}$, $CD27^{++}$).[8]

**c.) Adverse Events**: The NCI Common Toxicity Criteria Scale will be used to define grades (severity) of adverse events and toxicities. An adverse event is defined as any untoward medical occurrence in a subject, regardless of its relationship to these treatments. Toxicity is an adverse event with a direct relationship to the study drug. All toxicities are adverse events, but not all adverse events are toxicities. Case report forms (CRF) will require responses to all adverse events (and negatives), with a particular focus on allergic reactions, infections (including location and organism), and neuropsychiatric symptoms. Recording adverse events in pre-specified checklists (and free text entries) in all subjects will guard against unintended bias.

**2.7.3 Other Measures that are not a Formal End-Point: Carotid artery ultrasounds (US) will be obtained in participants at baseline evaluations and at the conclusion of formal observations (day 210), in order to determine carotid intimal medial thicknesses (IMT). We do not believe there is a high likelihood there will be a significant treatment effect, given the small number of participants and short duration of therapy in this exploratory trial. Nonetheless, these tests will establish the feasibility of using these assays in subsequent larger and longer trials to examine effects of autoantibody reduction in COPD patients.**

### 2.8 DATA AND POWER ANALYSES

#### 2.8.1 Primary Endpoint Data Analyses:

Baseline demographic and clinical data of participants will be summarized using means (with standard deviations) and medians (with inter-quartile ranges) for continuous variables, and counts and percentages for discrete variables, stratified by treatment group. Differences among groups (to examine the randomization effectiveness), will be assessed by t-tests, or by Fisher's exact test for discrete data, recognizing the limited power of our small trial.

Repeated measures linear mixed models will be used to evaluate differences of immunological parameters (continuous variables) that comprise the primary, and most second endpoints. These models will track the changes over time as well as allow for missing observations, which should be minimal. In general, analyses will follow intention to treat principles, with the last observed outcome over all time points being used as the primary measure for a given variable.

The sample size of 18 subjects for this clinical trial is based on exposing a minimum number of patients to potential risks of the experimental therapy, while having the potential to obtain useful information. Given that this trial is unprecedented, it is impossible to use preliminary data to definitively derive power analyses. Indeed, an important purpose of this study is to generate credible and relevant preliminary data in this population to power subsequent larger trials. Trials to detect clinical efficacy (e.g., change in pulmonary function tests, reductions in acute exacerbations, etc.) will almost certainly require treatment of many subjects over prolonged periods.

With these limitations in mind, we can assume belimumab effects in COPD patients are similar to those among patients with SLE. Thus, we can expect to see reductions of anti-GRP78 autoantibody concentrations by ~30%, in addition to ~50% reductions of several circulating B-cell subpopulations, by or after approximately 6 months of therapy. With respect to the primary endpoint (reduction of anti-GRP78 IgG): If the true difference in the experimental and control means is 30%, with standard deviations that are two–thirds of this (20%), we will need to study 12 experimental subjects and 6 control subjects to reject the null hypothesis that the population means of the experimental and control groups are equal, with probability (power) 0.8. The Type I error probability associated with this test of the null hypothesis is 0.05.

The 2:1 experimental arm: control enrollment will increase the amount of data regarding effects and safety of this drug in COPD patients, and only increases the necessary subject number to reach 80% power by two, compared to 1:1 enrollment.

**2.8.2 Secondary Endpoint Data Analyses:** Analyses will again follow intention to treat principles, with the last observed outcome over all time points being used as the primary measure for a given variable. Multiple imputation will be used based on subject characteristics to assess the obvious issues with last observations carried forward in these analyses. Trajectories over time, for a given patient and outcome measure, will be displayed via line graphs, and assessments conducted using linear mixed models on the repeated time points. Comparisons between the treatment arms of the polysaccharide binding assay concentrations and B-cell parameters at various time points will be made using repeated measures techniques. Transformations of these tests may be needed, such as velocities, if many individuals are unable to complete the entire treatment protocol. The proportion of subjects with adverse events and the count of adverse events per subject will be compared by treatment using Fisher's exact test and the exact Poisson tests, respectively.

**2.8.3 Interim Analysis**: Since the primary endpoint of this trial is a laboratory test and surrogate biomarker, rather than a clinical outcome parameter, and the subject number is small, no interim analysis will be conducted for efficacy.

## 2.9 ANTICIPATED RESULTS, ALTERNATIVES, AND PITFALLS

### 2.9.1 Specific Aim 1 (Primary Endpoint).

**Specific Aim 1 Anticipated Results:** Based on the known action of belimumab in other disease populations,[33,34] we anticipate anti-GRP78 reductions will be greatest in experimental arm subjects.

**Specific Aim 1 Alternative Experimental Therapies:** In comparisons to other agents used for treatment of autoantibody diseases, belimumab is almost certainly the safest, particularly with respect to risks for later infections.[23,33,34,37] We conservatively assume all subjects will have lower airway microbial colonization.[20] The potential that autoantibody reduction treatment could promote infections is a singular consideration in this subject population. Nonetheless, at least among SLE patients, belimumab reduces pathogenic autoantibodies, but leaves protective host defense antibody titers largely preserved.[33] Confirming this happens too in COPD patients is an important secondary endpoint. Experts with use of belimumab in SLE do not rigorously evaluate their patients for infections prior to initiating therapy (e.g., pre-treatment bronchoscopy is not indicated), nor reduce doses with infections other than those that are serious (W. Chatham, personal communication).

In contrast, other potential agents, including cyclophosphamide, alemtuzumab and bortezomib, experimental anti-CD19 monoclonal antibodies, other anti-T-cell agents, and rituximab are more toxic and far more likely to promote infections (including opportunistic infections). Moreover, with the exception of rituximab, the efficacy of these other, riskier agents for treatment of autoantibody-mediated autoimmune disorders has not been established.

**Specific Aim 1 Potential Pitfalls:** Even if the experimental regimen decreases autoantibodies and is safe, it is unlikely to reverse the lung destruction, and vascular and other tissue injuries that already afflicted these patients prior to the start of therapy. Nonetheless, slowing the rate of disease progression, particularly for otherwise untreatable morbid or lethal disorders, is still a worthwhile goal.

Our subject numbers are small, and this will limit our power to detect small intergroup differences, especially if there are biological differences of drug actions within our demographically and ethnically heterogeneous study population. This problem(s) will be confounded if there are several subject drop-outs due to the development of acute exacerbations, or if they lose interest and quit participating.

We have attempted to minimize the potential number of acute exacerbation drop-outs by excluding those patients with the single greatest risk for these complications (i.e., those who have a history of same). And the UAB LHC has a very good record for retaining trial subjects (approaching 100% in several studies). Furthermore, our data analysis methods should still enable us to make meaningful intergroup comparisons if there are subject drop-outs (see **Data Analyses**).

The alternative would be to propose a much bigger and more expensive initial trial, in the face of having absolutely no prior experience in COPD patients with this particular agent, or even this general approach. However, this would expose many more subjects to potential risks of the experimental intervention, prior to having any indication of effectiveness or safety.

The effectiveness of belimumab in autoimmune COPD patients is currently unknown, and it is conceivable that the autoimmune responses in this lung disease population are actually more fulminant, or otherwise more refractory, than those of SLE patients. Of course, a rationale for the

proposed study is to begin to explore this possibility. If, for whatever reason(s) COPD autoimmunity is resistant or partially resistant, it will further hinder our ability to find a treatment effect. Based on results of clinical trial results in other disease populations, and general biological considerations,[23,33,34,37] we are confident that belimumab will, in fact, detectibly reduce anti-GRP78 IgG in the treatment arm, and will likely alter circulating B-cell populations as well, even given the inherent limitations of this trial.

There are almost certainly other processes, and other autoantibodies, not-yet-discovered, that may also be involved in the development and/or progression of COPD and its comorbidities. **The concurrent presence of diverse autoantibodies is a feature of autoimmune syndromes**.[38-41] As one of many examples, SLE and scleroderma are notable for the presence of particular autoantibodies, but >100 other autoantibodies are also present in these patients, and many of these immunoglobulins are highly related to particular disease phenotypes.[40,41] Even if other autoantibody specificities are also important, as we suspect, our data show that GRP78 autoreactivity ***per se*** is highly associated with the presence and severity of COPD and many of its associated comorbidities (Figs.2-5). It is implausible to postulate that belimumab will have differential effects on concentrations of anti-GRP78 IgG compared to other (if undiscovered) pathogenic autoantibodies. Hence, even if other important and yet-to-be-recognized autoantibodies are present, values of our already developed and reproducible anti-GRP78 ELISA should change in parallel, and thus these results will be a useful generalizable measure of the autoantibody changes that occurred during this trial.

Admittedly this trial is a high risk endeavor, which is a characteristic of pioneering studies. Nonetheless, we believe that voluminous data support the central premise here. Furthermore, the enormous individual and public heath burdens that are attributable to these diseases, and their refractoriness to currently available medications, mean that the potential rewards of this novel therapeutic approach are also very high.

### 2.9.2 Specific Aim 2 (Secondary Endpoints) Anticipated Results and Pitfalls

**Specific Aim 2 Anticipated Results:**

a.) **Pneumococcal polysaccharide-binding antibody concentrations**: Based on results of analogous studies that examined belimumab effects in other populations (primarily SLE patients), we anticipate the experimental treatment will have only modest effects on concentrations of these important host defense immunoglobulins. Proving these antibodies are also minimally, if at all, affected in COPD patients (a novel trial population) is an important element of this pilot study. Dr. Duncan is an expert with these types of assays and no technical problems are anticipated.

**b.) B-cell Effects:** Again, based on analogous literature in SLE populations, we anticipate a gradual shift in circulating B-cell phenotypes among the experimental treatment subjects, particularly those at the higher doses. In particular, we anticipate increases in proportions of less differentiated lymphocytes and corresponding decrements of more differentiated phenotypes. Dr. Duncan is also experienced with these assays and no technical problems are anticipated.

**c.) Adverse Events:** Based on multiple studies involving thousands of patients treated with analogous regimens, we anticipate high-grade adverse events will not be significantly more frequent among experimental arm subjects, and the experimental regimen will have an acceptable risk:benefit ratio.[33,34,37] Examining the safety of belimumab in this potentially high-risk population is a critical aspect of our small and conservatively biased pilot trial. It is conceivable this study will show that even carefully selected and closely observed COPD patients cannot tolerate any immune

suppression, even relatively benign belimumab. **Even if so, these negative results (albeit disappointing) will be highly valuable, given the increasing interest (and seemingly increasing amount of supportive data and rationale) for therapeutic targeting of the autoimmune responses in these diseases**.[9,10]

### 2.10 STUDY TIMELINES AND MILESTONES

**Timelines and Milestones**: Infrastructure and regulatory requirements should be completed by ≤6 months after funding is awarded (Table 3). The personnel of this project have considerable clinical trial experience. Our potential subject population is so many times greater than the necessary study numbers that we anticipate being able to meet our target goal enrollment before the end of the project duration.

| | YEAR 1 | | | | YEAR 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | **Q1** | **Q2** | **Q3** | **Q4** | **Q1** | **Q2** | **Q3** | **Q4** |
| **Regulatory/Admin Issues** | **x** | **x** | | | | | | |
| **Subject Recruitments (#)** | | | **3** | **4** | **5** | **4** | **2** | |
| **Data Analysis and Reporting** | | | | | | | | |

**Table 3.** Anticipated enrollment and key study milestones.

## 3 HUMAN SUBJECTS

### 3.1 SUBJECT POPULATION

Human Subject Characteristics:
The study population will be eighteen (18) adult patients with COPD and autoantibodies against GRP78. The subjects will be recruited from the clinics or registries of the Lung Health Center (LHC) at the University of Alabama at Birmingham (UAB). The trial subjects must provide written informed consent prior to participation. Surrogate consent will not be allowed. No special vulnerable populations will be studied.

The demographics of the study population will reflect the characteristics of patients who suffer from COPDand are evaluated at at the University of Alabama at Birmingham (UAB) clinics and LHC.. These will be older patients (COPD is predominantly a disease of mature and elderly individuals). The ethnicities of the subjects will similarly reflect the demographics of our clinic populations that are afflicted with this disease. We anticipate that our recruited population will be 50% female, 75% Caucasian and 25% African American)

Recruitment goals (~9 subjects/year) are easily feasible, based on the large number of potential participants available here at UAB.. This pulmonary clinics at this academic medical center

routinely sees hundreds of new patients annually, and the majority of these have COPD. The UAB LHC has a long history of success in the recruitment and retention of participants in various studies, and has routinely been among the top recruitment sites in several large, NIH funded, multicenter clinical trials. A full time recruiter is employed at the LHC, who relies on in-clinic contact with potential participants, as well as print, television, and media advertising, as needed. There are currently over 1000 patients with COPD in the LHC databases and several hundred in the active research registries of the Center.

### 3.1.1 Inclusion of Women and Minorities

Women who meet the inclusion criteria, and have none of the exclusion criteria, will be enrolled without restriction as dictated by the study protocols. Because of the use of a study medication, woman of child bearing potential must meet specialized inclusion/exclusion criteria to minimize this risk. We will make efforts to enroll participants in this research in a distribution that mirrors the clinic population at our center (50% female, 75% Caucasian and 25% African American)..

### 3.1.2 Inclusion of Children

Children under 18 years of age will not be enrolled because they do not develop this disease.

## 3.2 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria have been selected to study a patient population with COPD and autoimmunity. The exclusion criteria are selected to exclude patients with increased risk for the experimental intervention.

**Inclusion Criteria**

1.) A history of past tobacco smoking (≥10 pack years), but quit for ≥6 months at the time of enrollment. Smoking cessation will be confirmed by serum cotinine assays.

2.) Clinical diagnosis of at least moderate COPD as defined by the Global Initiative for Obstructive Lung Disease (GOLD) criteria:

a. Post bronchodilator $FEV_1/FVC < 70\%$,
b. Post bronchodilator $FEV_1$ between 25% and 80% predicted, with or without chronic symptoms (i.e., cough, sputum production).

COPD

3.) Ability and willingness to give informed consent.

4.) Levels of plasma autoantibodies that have specifities against a 15–mer peptide sequence within the GRP78 molecule (aa. 246-260) that are in the upper three quartiles of values present in COPD patients. We have discovered that circulating levels of IgG autoantibodies with avidity against this peptide (GRP78 Peptide 25) are associated with the magnitude of carotid artery intimal medial thickness (IMT) among COPD patients (Figure 5). Limiting enrollments to subjects with readily detectible autoantibodies (e.g., those with levels in the $25^{th}$ percentile or above) will easily enable us to ascertain if the experimental treatment (belimumab) results in a significant reduction of these autoantibodies.

5.) Age 40-75 y.o. COPD is a disease of older individuals.

**Exclusion Criteria**

1.) History of prior acute COPD exacerbations or no more than one moderate exacerbation in the last year and no exacerbations four months prior to enrollment. A past history of an acute exacerbation is the single biggest risk for recurrence.[36] Exclusion of these higher-risk subjects will minimize drop-outs.

2.) Oral steroids or cellular immunosuppressant use (e.g., cyclophosphamide) within 6 months.

3.) History or clinical or laboratory evidence of other autoimmune syndromes.

4.) Inability or unwillingness to complete the treatment and surveillance protocols.

5.) Eligible for lung transplant at time of enrollment. This exclusion will mitigate any potential, however slight, that a patient could be rejected for transplantation due to surgeon concerns about this novel therapy (and will also obviate early drop-outs due to transplantation).

6.) History of malignant neoplasm within the last 5 years.

7.) Evidence of serious suicide risk including any history of suicidal behavior in the last 6 months and/or any suicidal ideation in the last 2 months or those, in the investigator's judgment, pose a significant suicide risk.

8.) History of a primary immunodeficiency.

9.) Significant IgG deficiency (IgG level < 400 mg/dL).

10.) Have an IgA deficiency (IgA level < 10 mg/dL).

11.) Currently on any suppressive therapy for a chronic infection (such as tuberculosis, pneumocystis, cytomegalovirus, herpes simplex virus, herpes zoster or atypical mycobacteria).

12.) Hospitalization for treatment of infection within 60 days of screening.

13.) Use of parenteral (IV or IM) antibiotics (antibacterials, antivirals, anti-fungals, or anti-parasitic agents) within 60 days of screening.

14.) Current drug or alcohol abuse or dependence, or a history of drug or alcohol abuse or dependence within 365 days prior to screening.

15.) History of a positive HIV test or positive screening test for HIV.

16.) Serologic evidence of current or past Hepatitis B (HB) or Hepatitis C (HC) infection based on positive tests for HBsAg or HBcAb, or HCAb.

17.) History of an anaphylactic reaction to parenteral administration of contrast agents, human or murine proteins or monoclonal antibodies.

18.) Any other clinically significant abnormal laboratory value in the opinion of the investigator.

19.) Any intercurrent significant medical or psychiatric illness that the investigator considers would make the candidate unsuitable for the study.

20.) Women of Child Bearing Potential (WCBP) must have a negative urine pregnancy test at screening, and agree to 1 of the following:

Complete abstinence from intercourse from 2 weeks prior to administration of the 1st dose of study agent until 16 weeks after the last dose of study agent (Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception)

OR

Consistent and correct use of 1 of the following acceptable methods of birth control for 1 month prior to the start of the study agent, during the study, and 16 weeks after the last dose of study agent:

- Oral contraceptive, either combined or progestogen alone
- Injectable progestogen
- Implants of levonorgestrel or etonogestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches
- Intrauterine device (IUD) or intrauterine system (IUS) with <1% failure rate as stated in the product label
- Male partner sterilization (vasectomy with documentation of azoospermia) prior to the female subject's entry into the study, and this male is the sole partner for that subject. For this definition, “documented” refers to the outcome of the investigator's/designee’s medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject’s medical records
- Double barrier method: condom and occlusive cap (diaphragm or cervical/vault caps) plus spermicidal agent (foam/gel/film/cream/suppository)

21.) Use of Excluded Medications:

- Anti-B-cell therapy:
  - Wash out of 5 therapeutic half lives after prior B-cell therapy, or until pharmacodynamic effect would be minimal (e.g., 1 year following rituximab)
- 365 days Prior to Belimumab:
  - Any biologic investigational agent (e.g., abetimus sodium, anti CD40L antibody, BG9588/ IDEC 131)
- Investigational agent applies to any drug not approved for sale in the country in which it is being used

- 30 Days Prior to Belimumab (or 5 half lives, whichever is greater)
    - Any non-biologic investigational agent
- Investigational agent applies to any drug not approved for sale in the country in which it is being use
- Live vaccines within 30 days prior to baseline or concurrently with belimumab

# 4 RECRUITMENT AND INFORMED CONSENT PROCEDURES

## 4.1 RECRUITMENT METHODS

Subjects will be recruited from the outpatient population followed at The Kirklin Clinic and UAB LHC. The primary care physician/clinical care team, who may be study investigators or colleagues of the study investigators, will first identify potential research subjects. The clinician investigators will discuss the research project with the potential subjects.

## 4.2 INFORMED CONSENT PROCEDURES

The consent process will begin via one of two possible pathways:

1) Referral of the prospective participant to the investigators/research coordinator by a physician who has knowledge of the proposed research, and obtains patient consent for the research team to approach the patient/surrogate.

2) Individuals who have provided signed IRB-approved HIPPA compliant consent for participation in clinical trial research registries.

Subjects must provide informed consent prior to performing any of the study procedures. The information about this study will be given to subjects in language understandable to subjects. Only physician investigators will present the study to the potential subjects. The physician investigator will verbally present a general outline of the research plan, including inclusion and exclusion criteria, to the prospective participant. The consent form, outlining the design of the study, will include the risks and benefits of participating, and will be reviewed and the investigator will answer any questions. Prospective participants may take as much time as required to make an informed decision. Written informed consent will be obtained from each participant prior to performing any research study procedures.

In addition, older potential study participants whose competency to consent is in question will be tested for sufficient comprehension and recall of the information presented. Prospective subjects who do not remember the important facts about participation in the research study after repeated testing will not be included in the study. The investigators will also assess whether a participant understands experimental procedures over time, including assessment throughout the full duration of participation in the study.

# 5 POTENTIAL RISKS AND BENEFITS

### 5.1.1 General Risks of Study Protocol and Procedures

The potential subject risks specifically related to the study protocol procedures could include loss of confidentiality or actual physical harm due to experimental intervention as outlined in 5.1.2.

### 5.1.2 Potential Risks of Experimental Intervention

The potential subject risks specifically related to the study protocol procedures could include:

Venipuncture:
Common Risks: temporary, minor discomfort, bruising.

Other Risks: infection, bleeding and phlebitis.

Belimumab: Common risks are temporary and self-limited or treatable with over the counter (OTC) medication (e.g., Benadryl, Tylenol). The common adverse effects reported with belimumab include nausea, diarrhea, and fever, as well as hypersensitivity and infusion-site reactions. However, there were few differences between treatment and placebo patients in the monitored clinical trials. Infusion reactions were reported in 17% (251/1458) and 15% (99/675) of patients receiving belimumab and placebo, respectively. Serious infusion reactions (excluding hypersensitivity reactions) were reported in 0.5% of belimumab patients and 0.4% of placebo patients.

Serious infection rates were comparable among drug and placebo cohorts (6.0% and 5.2%, respectively). Progressive multifocal leukoencephalopathy (PML) resulting in neurological deficits, including fatal cases, has been reported in SLE patients receiving immunosuppressant pharmacotherapy, including belimumab.

There were 14 deaths during the controlled period of three clinical trials: 3/675 in placebo, 5/673 in belimumab 1 mg/kg; 0/111 in belimumab 4 mg/kg, and 6/674 in belimumab 10 mg/kg. Causes of death included infection, cardiovascular disease, and suicide.

There are no known drug interactions, and the only absolute contraindication is a history of anaphylaxis to belimumab.

## 5.2 ALTERNATIVE TREATMENTS

The alternative treatments for the subjects participating in this investigation are to continue their usual medical care under the direction of their attending physician.

## 5.3 POTENTIAL BENEFITS

Participation in the proposed research may or may not provide a direct benefit to the subjects. Potential benefits from the participation in this protocol include enhanced survival, improved respiratory symptoms, and decreased exacerbation frequency and/or severity. Identification of the mechanism(s) mediating these outcomes will facilitate risk-stratification for these adverse outcomes, and development of targeted treatment strategies for the future.

Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits. The research presents a balance of risks and expected direct benefits similar to that available in the clinical setting.

Importance of the Knowledge to be Gained: The preliminary data in this application outline a hypothesis for the development and/or progression of COPD and associated comorbidities. The protocol specifically seeks to address that hypothesis. If the study intervention is found to be both safe and effective in the study population, the treatment of these patients would be altered significantly, and ultimately could lead to a change in the disease natural history. Completion of these protocols will begin to address important questions related to COPD and associated diseases.

## 5.4 RISK MANAGEMENT PROCEDURES

### 5.4.1 Protection Against Loss of Confidentiality

All research interventions/activities will be conducted in private patient care areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected.

To avoid any violation of subject confidentiality, all data will be stored in a password-protected database as well as Case Report Forms (CRFs), identified only by study ID number. A confidential database linking patient identifying information with study ID number will be maintained by the PIs.

All demographic and clinical information about the subject will be stored in a secured study database under the supervision of the PIs for this protocol. All staff will sign confidentiality statements.

Specimens will be stripped of subject identifiers and stored according to a similar coding protocol as described above. These specimens will be stored safely in the custody of the PIs responsible for the individual assays. These Investigators will limit future access to any remaining sample to only those investigators with prior IRB approval for their studies.

The PIs will retain the data for the entire period of this study. The investigators may continue to use and disclose subjects de-identified information for the purpose of this study for a minimum of seven years after final reporting or publication of the study. If the subject and/or legal representative decide to withdraw or be withdrawn from study participation, they may request that the study data and samples be destroyed.

All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety. All principal and co-investigators, coordinators, and other Key Personnel are required to participate in courses and be certified as mandated by local IRBs regarding human subjects' protections.

### 5.4.2 Protection Against Risks of Experimental Procedures

Despite the documented safety profile of the study medication in other human disorders of abnormal immune regulation, the study has been designed with a focus on protecting patients against risk including:

Selection of a target patient population with a high risk of morbidity and mortality due to the absence of definitive medical treatment for the disorder

Involvement by trained staff/investigators with experience in the administration of belimumab

Prior human experience with the study medication in other autoimmune disorders, including (SLE).

Selection of a study population that is not taking other immunosuppressive agents will lessen risks of infections.

Continuous monitoring by an independent Data Safety Monitoring Board (DSMB).

Use of low rates of infusions (≥1 hour), with continuous monitoring and observations, during study drug administrations. Subjects will be visited and observed by study personnel during each of their treatments. Subjects will also be contacted by phone on the day following their study drug administrations. Assessments for adverse events will occur at each of those contacts.

Infusion reactions occurred more frequently on the first two infusion days and tended to decrease with subsequent infusions. Delay in the onset of acute hypersensitivity reactions has been observed and recurrence of clinically significant reactions after initial resolution of symptoms following appropriate treatment, have been observed. Therefore, patients will be monitored during and for an appropriate period of time after administration of belimumab. Subjects will remain under clinical supervision for 3 hours after completion of the first 2 infusions. Should symptoms of acute hypersensitivity occur, an extended period of monitoring may be appropriate, based on clinical judgement. This may include, but is not limited to, monitoring vital signs and observing any untoward reactions. Beyond the first 2 infusions, subjects will be monitored during and for an appropriate period of time after infusion according to the study sites' guidelines or standard operating procedure for IV infusions. Subjects will be made aware of the potential risk, the signs and symptoms of such reactions, and the importance of immediately seeking medical attention. Delayed-type, non-acute hypersensitivity reactions have also been observed and included symptoms such as rash, nausea, fatigue, myalgia, headache, and facial edema.

Subjects will be made aware of the potential risk, the signs and symptoms of allergic reactions, and the importance of immediately seeking medical attention. In addition, other physicians who have responsible for various aspects of these subjects medical care will have continuous access to study investigators and will be encouraged to immediately report suspected adverse events. Subjects and family members will similarly be encouraged to contact investigators for suspected adverse events. Subjects will be given a wallet-size card explaining that they are in a research study and that they are assigned to receive either belimumab or a matched placebo. The card will include telephone numbers of the responsible investigator. Subjects will be instructed to carry the card at all times.

Some autoimmune diseases have an increased risk of suicidal behavior and/or ideation. For this reason in studies of patients with autoimmune disease, patients will be clinically assessed for suicidal ideation and/or behavior at each visit by experienced medical personnel. It is not known if the increased risk of suicidality with some autoimmune diseases is also present in COPD patients.

PML will be considered in any subject presenting with new-onset or deteriorating neurological signs and symptoms. The subject will be referred to a neurologist or other appropriate specialist for evaluation. If PML is confirmed, the study agent will be discontinued, and the event **will be immediately reported to GSK.**

Medical treatment for conditions that arise as a result of study participation will not be treated by study personnel. The subject will be informed of the problems identified, and this information will be transmitted to the responsible attending physician or physician designated for the subject. In the event of physical injury resulting from the research procedures, medical treatment will be available but not offered free of charge. In addition, financial compensation is not available for wages lost because of injury related to the research protocol. This will be emphasized at the time of consent.

## 5.5 DATA SAFETY MONITORING PLAN

### 5.5.1 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB), independent of the study investigators, will monitor the clinical trial outlined in this proposal for the duration of this proposal. The DSMB will be selected by study investigators, in concert and with approval of the sponsor (GSK), from among experts in the field. The Food and Drug Administration has already provided an exemption from IND requirements for this protocol.

The DSMB will be expected to meet as needed, but not less than every six months, to review the progression of the study including patient enrollment, protocol compliance, and adverse event reports. The DSMB will conduct interim monitoring of accumulating data from research activities to assure the continue safety of human subjects, relevance and appropriateness of the study, and the integrity of research data. An emergency meeting of the DSMB may be called at any time by the DSMB Chair should participant safety questions or other unanticipated problems arise.

The UAB study team will assume the following general responsibilities:

- Maintain copies of FWA and Institutional Review Board (IRB) approvals.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the sponsor and DSMB.
- Maintain documentation of Serious Adverse Event reports and submit these to the sponsor and DSMB for timely review.
- Post on ClinicalTrials.gov

The study PIs will establish a data collection protocol and mechanism. Data will be collected on paper Case Report forms. Numerous data quality checks, such as duplicate entry checks, double-entry verification, and real-time data checking (e.g., ID verification, range checks) will be implemented to ensure that the data are accurate, complete and secure. Data entry personnel will be trained and certified in the correct use of the system.

## 5.6. ADVERSE EVENTS:

### 5.6.1. Definition of an Adverse Event:

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Note: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE) unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae

"Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the definition of an AE or SAE

Events that **do not** meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition

### 5.6.2 Definition of a Serious Adverse Event

A serious adverse event is any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

   NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires hospitalization or prolongation of existing hospitalization

   NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE

d. Results in disability/incapacity, or

   NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption

e. Is a congenital anomaly/birth defect

f. Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse

g. All events of possible drug-induced liver injury (see Section 5.6.5) with hyperbilirubinaemia defined as ALT $\geq$ 3xULN **and** bilirubin $\geq$ 2xULN (>35% direct) (or ALT $\geq$ 3xULN and INR>1.5, termed 'Hy's Law' events (the INR threshold value stated will not apply to patients receiving anticoagulants)

***Laboratory and Other Safety Assessment Abnormalities Reported as AEs and SAEs***

Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator are to be recorded as AEs or SAEs. However, any clinically significant safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition, are **not** to be reported as AEs or SAEs.

*Pregnancy*

Any pregnancy that occurs during study participation must be reported to GSK. To ensure subject safety, each pregnancy must be reported within 2 weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy, brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the study treatment, must be reported to GSK within 24 hours of learning of its occurrence.

The National Cancer Institute Common Toxicity Criteria Scale will be used to define grades (severity) of adverse events and toxicities. An adverse event is any untoward medical occurrence in a participant who received study drug, regardless of its relationship to the study drug. Toxicity is an adverse event with a direct relationship to the study drug. All toxicities are adverse events, but not all adverse events are toxicities. This is a determination made by the study investigator. The study investigators will classify adverse events as "definitely or most likely," "possibly," or "very unlikely" due to the study drug. Toxicity will be defined as an adverse event that is definitely, most likely, or possibly caused by the study drug.

The severity of adverse changes in physical signs or symptoms will be classified as follows:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention not indicated.

- Grade 2 (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appreciate instrumental ADL (Activities of Daily Living).

- Grade 3 (Severe): medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

- Grade 4 (Life-threatening): consequences; urgent intervention indicated.

- Grade 5 (Death): event is a direct cause of death.

Assuring patient safety is an essential component of this protocol. The contact PI has primary responsibility for the oversight of the data and safety monitoring. The study investigators will evaluate all adverse events. All subjects who have adverse events, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome.

All untoward medical occurrences observed in subjects will be recorded on the participants' adverse event CRF by the study coordinator under the supervision of a PI, with a particular focus on infections (including location and organism), metabolic or hemodynamic perturbations (e.g., hyperglycemia, hypotension, etc.), liver function test abnormalities, and other, less frequent complications (e.g., neurologic and psychiatric symptoms), as well as having provision for free text entries. Recording all adverse events in pre-specified checklists (and free text entries), will guard against unintended bias. The CRFs will then be reviewed for completeness and internal

consistency.

The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the PI considers it medically justifiable to terminate follow-up.

The PIs will work with the coordinator(s) to prepare a detailed written summary of serious, unexpected, and treatment related adverse events, and will compare, and contrast the event with prior events. The detailed written summary will be provided to the DSMB, IRB, and sponsor.

In addition, the DSMB Report addressing the following information will be submitted to the IRB at the time of continuing review annually or more often as required.

### 5.6.3 Adverse Events Reporting Timeline

The investigators or site staff are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE. AEs will be collected from the start of study treatment and until the follow up contact. SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed **as related** to study participation (e.g., study treatment, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK concomitant medication, will be recorded from the time a subject consents to participate in the study up to and including any follow up contact.

The investigators will report life-threatening or fatal unexpected adverse events associated with the use of the study drug or procedures to the DSMB, and the IRB within 24 hours of discovery of the incident with subsequent follow-up submission of a detailed written report.

All SAE will be reported via email notification **within 24 hours of awareness** to the following sponsor (GSK) contacts including:

1) ISS Chairperson
2) ISS Managing Director
3) GMAL or designee
4) LOC Physician
5) LOC Safety Contact
6) LOC Study Accountable Person
7) GCSP Safety Physician
8) GCSP Safety Scientist
9) US.NAPS@gsk.com

Serious (but not fatal or life-threatening) and unexpected adverse events associated with the use of the study drugs or procedures must be reported to the DSMB and the IRB within 5 working days with subsequent follow-up submission of a detailed written report.

***Adverse Events of Special Interest:***

The following adverse events of special interest will be assessed for frequency in the data analyses and final report.

- Serious Hypersensitivity or Infusion Reactions
- Serious infections, including herpes zoster and opportunistic infections
- Malignancy
- Suicidal thought, intent or behaviour

**5.6.4 Stopping Rules**:

**Individual subject-specific stopping rules:** A study participant will be discontinued from further study drug treatment/Intervention(s) administration if any of the following occur:

Any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

An unexpected fatal or life-threatening adverse event, which requires discontinuation of study treatment.

Patients who develop acute exacerbations that require an addition to their lung medications, or a hospitalization, will be terminated from the study. Any subject who develops new conditions or statuses that would have been an exclusion criterion, will be terminated from the trial.

Request by the subject to withdraw from the study.

**Specific Liver Chemistry Stopping Criteria:**

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

- ALT Absolute: ALT ≥ 8x upper limit of normal (ULN)
- ALT Increase:
  - ALT ≥ 5xULN but <8xULN persists for ≥2 weeks
  - ALT ≥ 3xULN but <5xULN persists for ≥4 weeks
- Bilirubin: ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin)

All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants.

- INR: ALT ≥ 3xULN and INR>1.5, if INR measured
- Cannot Monitor:
  - ALT ≥ 5xULN but <8xULN and labs cannot be monitored weekly for ≥2 weeks

- ALT ≥ 3xULN but <5xULN and labs cannot be monitored weekly for ≥4 weeks

- *Symptomatic: ALT ≥ 3xULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity*
  - New or worsening symptoms believed to be related to liver injury (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia)

**Required Actions, Monitoring, and Follow up Assessments following ANY Liver Stopping Event Actions:**

The study treatment will be Immediately discontinued

The event will be reported to GSK within 24 hours

Liver event follow up assessments will be performed (as stated below)

The subject will be monitored until liver chemistries resolve, stabilize, or return to within baseline (see MONITORING below)

Treatment will not be restarted or rechallenged in the subject

Monitoring:

For bilirubin or INR criteria:

Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs

Monitor subjects twice weekly until liver chemistries resolve, stabilize or return to within baseline

A specialist or hepatology consultation is recommended

For All other criteria:

Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24-72 hrs

Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline

Follow Up Assessments:

Viral hepatitis serology (Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody)

Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).

Fractionate bilirubin, if total bilirubin≥2xULN

Obtain complete blood count with differential to assess eosinophilia

Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form

Record use of concomitant medications including acetaminophen, herbal remedies, other over the counter medications

Record alcohol use

For bilirubin or INR criteria:

Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins)

Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009])

Liver imaging (ultrasound, magnetic resonance, or computerized tomography) and /or liver biopsy to evaluate liver disease

*Increased Monitoring Criteria with Continued Therapy*

**Criteria**

- ***If ALT ≥5xULN and <8xULN and bilirubin <2xULN without symptoms believed to be related to liver injury or hypersensitivity, and who can be monitored weekly for 2 weeks***

  ***OR***
- ***ALT ≥3xULN and <5xULN and bilirubin <2xULN without symptoms believed to be related to liver injury or hypersensitivity, and who can be monitored weekly for 4 weeks***

**Required Actions**

- ***Notify GSK within 24 hours of learning of the abnormality to discuss subject safety***
- ***Subject can continue study treatment***
- ***Subject must return weekly for repeat liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) until they resolve, stabilise or return to within baseline***
- ***If at any time a subject meets the liver chemistry stopping criteria, proceed as described above for Required Actions and Follow up Assessments following ANY Liver Stopping Event***
- ***If ALT decreases from ALT ≥5xULN and <8xULN to ≥3xULN but <5xULN, continue to monitor liver chemistries weekly***

***If, after 4 weeks of monitoring, ALT <3xULN and bilirubin <2xULN, monitor subjects twice monthly until liver chemistries normalize or return to within baseline.***

**Study-wide stopping rules:** A study-wide stopping rule will be based on the comparison of serious adverse events rates between the two treatment groups. This safety endpoint will be

used instead of the primary endpoint since the primary endpoint is not a clinical marker of efficacy. The DSMB and sponsor (GSK) will have responsibility for invoking a study-wide stopping rule based on their evaluations of the data.

### 5.6.5 Parameters to be Monitored

The following progress will be monitored throughout the course of the research to ensure the safety of subjects as well as the integrity and confidentiality of their data.

- An evaluation of the progress of the research study, including subject recruitment and retention, and an assessment of the timeliness and quality of the data.
- A review of collected data (including adverse events, unanticipated problems, and subject withdrawals) to determine whether there is a change to the anticipated benefit-to-risk assessment of study participation and whether the study should continue as originally designed, should be changed, or should be terminated.
- An assessment of external factors or relevant information (eg. Pertinent scientific literature reports or therapeutic development, results of related studies) that may have an impact on the safety and study participants or the ethics of the research study.
- A review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data.

### 5.6.6 Frequency of Monitoring

The PIs will review subject safety data as it generated. The PIs and research staff will meet or converse bi-monthly when the study is being initiated, and then thereafter at least at monthly intervals, or more frequently if necessary, to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints, and confidentiality of subjects. There will be an evaluation of the progress of the research study, including assessments of data quality, time lines, participant recruitment, accrual, and retention. The PIs will also review the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and whether the study should continue as originally designed or should it be re-evaluated and changed.

# 6. STUDY ADMINSTRATION

## 6.1 REGULATORY AND ETHICAL CONSIDERATIONS

The clinical study will be conducted in accordance with the IRB-approved clinical protocol; International Conference of Harmonization (ICH) guidelines on Good Clinical Practice, and relevant policies, requirements, and regulations of the IRBs, and applicable federal regulations, including those required under an IND exemption.

The clinical trial will be registered with ClinicalTrials.gov to comply with Section 801 of Public Law 110-85.

The investigators will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigators will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

## 6.2 PROTOCOL DEVELOPMENT

### 6.2.1 Activation of a protocol

The PI and the Co-PI are responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting serious adverse events, violations and deviations per IRB guidelines.

To meet these requirements, the PI and the Co-PI will be responsible for the following minimum standards:

- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the PIs.
- Ensure that there is only one version of the protocol.
- Oversee the development of data collection forms (case report forms).

## 6.3 PROTOCOL MANAGEMENT

### 6.3.1 Protocol distribution

The Contact PI will distribute the final approved protocol and any subsequent amended protocols to all study staff.

## 6.4 INFORMED CONSENT REQUIREMENTS

All study participants in this study will be provided a consent form describing the study and providing sufficient information for participants to make informed decisions about their participation in this study. The study participant MUST be consented with the IRB approved consent form before the participant is subjected to any study procedures. The approved consent form MUST be signed and dated by the study participant and the investigator or other qualified study staff member obtaining the consent.

## 6.5 IRB DOCUMENTATION

### 6.5.1 IRB Renewal Approval

Annual IRB renewal approval is required in order to continue research and recruit participants onto a protocol. There is no grace period for continuing approvals.

## 6.6 QUALITY CONTROL AND QUALITY ASSURANCE

To ensure the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet GCP and, when appropriate, regulatory guidelines, the DSMB and/or sponsor may conduct a quality assurance audit (site monitoring) of the site records at any time during or after completion of the study. Audits of a random selection of data, and all protocol consents will also be reviewed.

Monitoring will be scheduled periodically throughout the conduct of the study to assure compliance with the approved protocol, and to verify the completeness and accuracy of study data. Monitoring also aids in identifying any research-related problems for the investigator to correct. A brief written report on each audit or site visit will be prepared by the auditor(s) and sent to the sponsor, DSMB, and PIs after each site visit.

## 6.7 DATA HANDLING AND RECORD-KEEPING

### 6.7.1 Data recording/Case Report Forms

Case report forms (CRFs) are the primary data collection instruments for the study. All data requested on the CRFs must be recorded, and any missing data must be explained. If a space is left blank because the procedure was not done or the question was not asked, “N/D” must be noted. If the item is not applicable to the individual case “N/A” must be noted. All entries must be printed legibly in black ink on the paper case report forms. In the event of any entry errors, corrections must be made by drawing a single straight line through the incorrect entry, writing the initials of the person making the correction, recording the date when the correction is being made, and entering the correct data above the strike through.

Data elements that are extracted from the medical record (such as participant history or official clinical interpretations of images, pathology, or surgery results) and recorded on the case report forms (CRFs) will be audited against the appropriate component of the medical record. The investigator will review, approve and sign/date each completed CRF; the investigator’s signature serving as attestation of the investigator’s responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

*Source Data* are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. Information recorded on the CRF must be consistent with the *Source Data* recorded on the *Source Documents* or discrepancies must be explained.

Source data are found in all information, original records of findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents represent the first recording of any observations made or data generated about a study participant while he or she is enrolled in a clinical trial. Source documents for each study participant substantiate the data that are submitted in the CRF.

If data is abstracted from medical charts that are not filed at the investigative sites (e.g. hospital charts), copies of these records should be filed in the research chart. However, every attempt must be made to obtain all records/charts that were used to abstract any study data for this

protocol at the time of the audit visit. This will prevent any discrepancies and the inability to verify the document and the data reported.

The CRFs must be kept current to reflect subject status at each phase during the course of the trial. In all cases, subjects must not be identified on the CRF by name. Appropriate coded identifications (i.e. Subject ID number) will be used. Every effort will be made to collect complete data for each study visit. Causes of *missing data* will be fully documented. With respect to safety evaluation, it is not planned to impute missing data.

### 6.7.1 Record maintenance and retention

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be easily accessible when needed (e.g., for audits or regulatory inspections) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of the records may be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution must be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original. In addition, they must meet accessibility and retrieval standards, including regeneration of a hard copy, if required. The investigator must also ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for creating the reproductions.

The minimum retention time will meet the strictest standard applicable, as dictated by local laws/regulations, and/or institutional requirements.

# 7 COSTS AND PAYMENTS

## 7.1 COSTS

Staff (Coordinator and laboratory technician) salaries will be borne the sponsor. The sponsor will also pay for study-specific assays, tests, procedures, and therapeutics.

## 7.2 PAYMENTS

Participation in this protocol is completely voluntary. Subjects will be compensated a maximum of $1425 for their participation in this research study. Parking will be provided at no cost while at the research clinic.

# 8 QUALIFICATIONS AND SOURCES OF SUPPORT

## 8.1 QUALIFICATIONS OF THE PRINCIPLE INVESTIGATORS

**Steven R. Duncan, M.D., Principal Investigator, Professor of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham School of Medicine**. Dr. Duncan is the Director of the Program in Immunology of

Chronic Lung Disease in PACCM and contact PI of this proposal. He has considerable clinical trial and translational immunology experience. He will be responsible for supervision, financial administration, obtaining necessary regulatory approvals including IRB approval, final protocol development, and coordinating research activities in conjunction with the other study investigators. Dr. Duncan will also assist Dr. Dransfield in the identification, enrollment, provision of care, and monitoring of the study subjects. In addition, he will supervise GRP78-binding antibody ELISAs and other immunological assays, coordinate and facilitate collections of research specimens, and participate in data analyses and manuscript preparation.

**Mark T. Dransfield, M.D., Principal Investigator, Professor of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham (UAB) School of Medicine**. Dr. Dransfield is the Director of the Lung Health Center at UAB, and an attending physician on the pulmonary and critical care services at UAB and the Birmingham Veterans Hospital. He will have primary responsibility for the identification, enrollment, provision of care, and monitoring of the study subjects. In addition, he will assist with coordination and collection of research specimens, and participate in data analyses and manuscript preparation.

**Kaiyu Yuan, M.D., Research Technician, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham School of Medicine**. Dr. Yuan has been the lab manager for Dr. Duncan since the latter relocated to UAB. Dr. Yuan will continue to work under the direct supervision of Dr. Duncan. His primary responsibilities will include isolation and/or production (transfection expression) of GRP78 protein to be used in assays, and subsequent collection and transport of clinical specimens, performing the ELISA for GRP78-binding antibodies, appropriate storage and archiving specimens and collation and safe storage of data. These anti-GRP78 IgG ELISA will need to be performed promptly and throughout Years 1 and 2 to determine potential subject eligibility.

### 8.2 SOURCE OF SUPPORT

GlaxoSmithKline

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