

Original Research**Rationale and Design of the Alpha-1 Biomarkers Consortium Study**

Monica P. Goldklang¹ Cheryl Pirozzi² Igor Barjaktarevic³ Surya Bhatt⁴ Sandeep Bodduluri⁴ M. Bradley Drummond⁵ D. Kyle Hogarth⁶ Alison Keaveny⁷ Zhonghua Liu¹ Noel G. McElvaney⁸ Oliver J. McElvaney⁸ Nadine Nuchovich⁷ Sabrina Palumbo¹ Randel Plant⁷ Robert Sandhaus⁹ J. Michael Wells⁴ Andrew Wilson¹⁰ Charlie Strange¹¹ Jeanine M. D'Armiento¹ for the Alpha-1 Biomarker Consortium Study Group*

¹Columbia University, New York, New York, United States

²University of Utah, Salt Lake City, Utah, United States

³University of California, Los Angeles, Los Angeles, California, United States

⁴University of Alabama at Birmingham, Birmingham, Alabama, United States

⁵University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

⁶University of Chicago, Chicago, Illinois, United States

⁷Alpha-1 Foundation, Coral Gables, Florida, United States

⁸Royal College of Surgeons in Ireland, Dublin, Ireland

⁹National Jewish Health, Denver, Colorado, United States

¹⁰Boston University, Boston, Massachusetts, United States

¹¹Medical University of South Carolina, Charleston, South Carolina, United States

**Members of the Alpha-1 Biomarker Consortium Study Group are listed in the Acknowledgements section.*

Address correspondence to:

Jeanine M. D'Armiento
Columbia University
New York, New York
Phone: (212) 305-3745
Email: jmd12@cumc.columbia.edu

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Abbreviations : AATD: Alpha-1 antitrypsin deficiency; COPD: Chronic obstructive pulmonary disease; CT: Computed tomography ; AECOPD: Acute exacerbation of COPD; PBMcs: Peripheral blood mononuclear cells; iPSCs: inducible pluripotent stem cells ; NHLBI: National Heart, Lung, and Blood Institute ; QUANTUM-1: the QUANTitative lung CT UnMasking emphysema progression in AATD; EARCO: European Alpha-1 Research Collaboration; NE: Neutrophil elastase; ECM: Extracellular matrix; Perc15: 15th percentile Hounsfield Unit value; AFD: Airway fractal dimension; FEV₁: Forced expiratory volume in one second; A1BC: Alpha-1 Biomarkers Consortium; QOL: Quality of life; A1F: Alpha-1 Foundation; WCG: Western Institutional Review Board-Copernicus Group; PRO: Patient reported outcome; PHQ-9: Patient Health Questionnaire; SGRQ: St George's Respiratory Questionnaire; CAT: COPD Assessment Test; SOBQ: University of California San Diego, Shortness of Breath Questionnaire; mMRC: Modified Medical Research Council Dyspnea scale; BCSS: Breathlessness, Cough, and Sputum Scale; CLDQ: Chronic Liver Disease Questionnaire; AUDIT-C: Alcohol Use Disorder Identification Test; TLC: Total lung capacity; RV: Residual volume; BMI: Body mass index; UAD: United airway disease; ECLIPSE: Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; AcPGP: Acetylated proline-glycine-proline; MMPs: Matrix metalloproteinases; REDCap: Research Electronic Data Capture; OSMB: Observational Safety Monitoring Board

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ABSTRACT

Rationale: Alpha-1 antitrypsin deficiency (AATD) is the most common genetic cause of chronic obstructive pulmonary disease (COPD), but considerable phenotypic variability exists among affected individuals who share disease-causing variants. Therefore, a multi-center longitudinal cohort study of 270 adult participants with PiZZ AATD will be established with goal of examining how computed tomography (CT) imaging and serum and airway biomarkers can be used to explain differences in phenotypic manifestations and outcomes.

Methods: Study visits at enrollment, 18 months and 36 months will obtain spirometry, patient-reported outcomes and biosampling from blood, nasal mucosa and sputum. Chest CT image acquisition will be utilized for whole lung and lobar estimations of emphysema based on lung density and to test novel measurements of airway remodeling and lung tissue mechanics. Dried blood spot cards will be collected if the participant experiences an acute exacerbation of COPD (AECOPD) during the study. Genetic analysis will be performed with complete SERPINA1 sequencing, and peripheral blood mononuclear cells (PBMCs) will be isolated to generate a repository of inducible pluripotent stem cells (iPSCs).

Results: The cohort will be deeply characterized including imaging, physiology, and symptomatology cross-sectionally and longitudinally over a 3-year follow-up period. A validation cohort from Ireland will independently enroll patients with identical procedures.

Conclusion: This is the first cohort of AATD to incorporate such detailed metrics of disease including quantitative emphysema measures with the overarching goal of improving the understanding of disease heterogeneity in AATD and identifying factors associated with disease severity and progression.

INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD) is the most common genetic cause of chronic obstructive pulmonary disease (COPD). Although AATD is regarded as a classical Mendelian disorder, marked variability in the development and severity of disease-associated phenotypes exists¹⁻³. This variability underscores the need for an improved characterization of the range of clinical outcomes associated with specific disease-causing mutations and the influence of additional modifying genes and environmental exposures on disease presentation. To date, the National Heart, Lung, and Blood Institute (NHLBI) Registry of alpha-1 antitrypsin deficiency^{4,5}, the QUANTitative lung CT UnMasking emphysema progression in AATD (QUANTUM-1)⁶, and the control arms of some comparative studies⁷⁻⁹, are the largest longitudinal studies of patients with AATD. These studies were performed before widespread quantitative computed tomography (CT) scans in the measurement of emphysema, prior to adoption of triple therapy in the management of COPD and reflect historical smoking rates in the United States population. Contemporaneously, the European Alpha-1 Research Collaboration (EARCO) International Registry^{10,11} has recruited a larger numbers of patients with AATD in Europe, with extensive analyses regarding clinical characterization and blood biomarkers, but the study contains only qualitative, not quantitative measures of emphysema, in only a subset of patients. Furthermore, augmentation therapy is not widely available in all countries within the EARCO registry. Therefore, there is still much to be defined regarding the current natural history, treatment strategies for, and the clinical course of AATD for patients in the United States.

Pathophysiology of AATD

Based on population studies^{12,13}, it is estimated that one in 2,800 to one in 5,000 individuals in the United States has a severely deficient genetic variant on both *SERPINA1* sites (ZZ, or rare null alleles), extrapolating to between 67,000 and 117,000 severely deficient patients. Alpha-1 antitrypsin is a serine protease inhibitor that inactivates neutrophil elastase and matrix metalloproteinases to maintain the protease-antiprotease balance in the lung. Individuals with AATD who inherit variant alleles of *SERPINA1* that cause misfolding of the AAT protein within the hepatocyte with resultant low levels of functional plasma AAT^{14,15}. This misfolded protein triggers an endoplasmic reticulum stress response in the hepatocyte with resultant liver inflammation and fibrosis¹⁶. Initial interest in imbalances between proteases and their

endogenous inhibitors stemmed from the observation of an increased incidence of emphysema in smokers with AATD¹⁷⁻¹⁹. Neutrophil elastase (NE) is well known to be a major protease involved in tissue destruction of emphysema^{20,21} and AAT is a key inhibitor of elastase with its functional loss resulting in damage of extracellular matrix (ECM).

CT Imaging in AATD

Unlike the general COPD population that can enroll large numbers of participants to test and validate new therapies based on spirometric measures^{22,23}, the limited patient pool in AATD creates a challenge. Previously, CT-based measures emphysema and lung density have been utilized in AATD clinical trials. The RAPID⁷ and RAPID-OLE²⁴ trials tested the efficacy of weekly alpha-1 antitrypsin augmentation therapy on altering changes in CT lung density (e.g. the 15th percentile Hounsfield Unit value, Perc15) in 180 participants and to date has established Perc15 as the most widely accepted biomarker of lung destruction in AATD. Recent advances in CT image post-processing techniques now facilitate the estimation of lung tissue biomechanics through paired inspiratory and expiratory high resolution chest CT scans. The Jacobian determinant is a measure of local volume change and substantially explains differences between density-based measures of emphysema and the degree of airflow obstruction on spirometry^{25,26}, but has not been evaluated in AATD. Furthermore, complex branching patterns of the airways and subtle variations in these patterns due to disease presence can be quantified using the airway fractal dimension (AFD)²⁷, which has been independently associated with respiratory quality of life, functional capacity, exacerbations, lung function decline, and mortality²⁷, again never evaluated in AATD. This study presents unique opportunities for exploring CT imaging metrics to better understand disease severity and progression. The identification of novel imaging-based biomarkers with clinical and/or pathological relevance has the potential to accelerate the pipeline of therapies available to this vulnerable patient population.

Endpoints in AATD Clinical Trials

General COPD studies have focused on endpoints such as change in forced expiratory volume in one second (FEV₁)²⁸ but require large numbers of patients for statistical significance given variation in FEV₁ over time. Interventional clinical trials require large numbers of patients (in some cases over 1,000) to demonstrate an effect on exacerbation rate^{22,29}. In AATD, this

approach is infeasible due to a limited patient pool. The AATD clinical research community has therefore turned to methods including change in CT lung density as a surrogate marker of lung destruction in clinical trials, including the evaluation of the clinical efficacy of AAT augmentation therapy^{7,24}. The identification of additional blood or imaging biomarkers with clinical or pathological relevance as meaningful intermediate endpoints that may decrease sample size and/or trial length has the potential to improve the pipeline of therapies available to this patient population.

Methods

Study Objectives

The goal of the Alpha-1 Biomarkers Consortium (A1BC) is to better understand the heterogeneity of AATD and associate blood or imaging biomarkers with specific disease phenotypes that might be applied to characterize disease severity or predict clinical outcomes. Biomarkers have been defined by an NIH working group as a “characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”³⁰. In the A1BC, biomarkers are broadly defined to include CT-based measurements of emphysema, serum, plasma and sputum biomarkers of inflammation and lung destruction, alongside outcomes such as spirometry and quality of life (QOL) questionnaires. The study further aims to determine if there are modifying genetic relationships in disease progression of AATD. Sequencing of the *SERPINA1* gene will be performed including in the promoter and enhancer region on all A1BC patients to test the hypothesis that genotype-phenotype correlations exist among PiZZ AATD patients.

Study design

In 2019, the Alpha-1 Foundation (A1F) established a research registry (A1F Registry, NCT04157049) housed within A1F containing over 1,200 discrete data including contact information, extensive patient-reported, and medical record verified clinical data. The first phase of this study was to establish a clinical cohort of Alpha-1 patients with methodology to link A1BC participants to the A1F Research Registry. All self-reported PiZZ AATD participants in the A1F Registry 18 years or older were invited to join the A1BC, with a final enrollment goal of

270 participants. Nine A1F Clinical Resource Centers will be recruiting geographically dispersed United States patients (Table 1 and Figure 1). A separate validation cohort in Ireland has been established. Study design was approved by the Western Institutional Review Board-Copernicus Group (WCG) and both the A1F Registry (NCT04157049) and the A1BC (NCT05297812) are enrolled in clinicaltrials.gov. A1F Registry and A1BC registration numbers will be linked for data sharing. After study completion, patient data and biomaterials will be transferred to the A1F Registry and will be used in accordance with A1F's mission.

Liberal inclusion and exclusion criteria have been selected to ensure that the entire range of AATD pulmonary manifestations in the PiZZ cohort are included (Appendix Table 1). Inclusion criteria are largely based on genotype, adult age (owing to the low incidence of emphysema in children and radiation risk exposure over a lifetime), and a willingness to share data with the A1F. Participants will be included regardless of augmentation therapy status, with data collection regarding dose and interval collected on relevant patients. Crossover of augmentation therapy status during the study will be collected. Patients are excluded if listed for lung or liver transplant. Since CT metrics of emphysema serve as the primary endpoint of this study, patients with diagnoses that could cause alterations in CT imaging were specifically excluded from the cohort; such exclusions have been implemented in other trials utilizing CT densitometry including in COPDGene³¹. Therefore, patients with major thoracic surgeries, lung volume reduction procedures, and automatic internal cardiac defibrillators and similar devices were excluded. Additionally, patients with clinically significant bronchiectasis were excluded³¹. While patients cannot be enrolled in therapeutic clinical trials at the time of A1BC consent and first visit, to not hamper enrollment in the current and upcoming clinical trials across the AATD landscape, the A1BC does not prohibit enrollment in interventional clinical studies after A1BC initial visit. At each 6-month call, patients are queried regarding clinical trial enrollment, and if newly enrolled in additional clinical trials, the clinicaltrials.gov reference number is collected.

In-person visits with blood, spirometry with and without bronchodilators, and CT will occur at baseline, 18 months, and 36 months. Optional induced sputum will be performed on appropriate patients at participating centers (only 3 of 8 centers due to COVID-19 restrictions). Extensive patient reported outcome (PRO) measures will be administered. Participants will be contacted by

telephone every 6 months to update clinical history, obtain current medication lists, and assess for any adverse events. Patients will be invited to complete monthly surveys of exacerbation history. A Whatman 903 blood spot card and fingerstick lancet is provided to all participants at baseline, and participants will be instructed on self-collection of blood on a dried blood spot card at the day of onset, day 3, and day 7 of exacerbations. A schedule of procedures is provided in Appendix Table 2.

Measurements

Questionnaires

This cohort will undergo extensive analysis including clinical characterization of exposure history and health status (Patient Health Questionnaire [PHQ-9], A1BC Exposure Questionnaire). Table 2 lists the questionnaires used to capture clinical manifestations of lung and liver disease associated with AATD; more details are available in the online supplement.

In addition to the above, extensive AATD-related clinical questions are obtained regarding diagnosis, including method of diagnosis, familial index case, and duration between symptom onset and diagnosis. Information regarding childhood or adult asthma, bronchiectasis, and NTM infection is obtained. Specific information about augmentation dosing, intervals, and location of administration will be collected. Other non-pulmonary AATD-related clinical findings are directly queried including neonatal and childhood liver disease, liver fibrosis and cirrhosis, vasculitis, and panniculitis. Clinician-obtained and questionnaire-based information regarding smoking history and alcohol use will be collected.

CT Imaging

All participants enrolled in the study will undergo acquisitions of high-resolution CT scans of the chest at total lung capacity (TLC) and residual volume (RV) at the baseline visit and subsequently at 18 months and 36 months of follow-up. The acquisition protocol will be standardized, and dose adjusted according to body mass index (BMI) using the SPIROMICS chest CT protocol³². Subsequent CT scans will be acquired using the same scanner and the same protocol for each participant at each site, unless there is a significant change in body weight. The

COPDGene phantom will be used to calibrate CT scanners for quality assurance³³. DICOM images will be transferred to the UAB Lung Imaging Laboratory using a HIPAA-compliant secure web portal system³².

Nasal Transcriptome

Based on the united airway disease (UAD) hypothesis which proposes that specific inflammatory process within the respiratory tract manifests in both the upper and lower tracts³⁴, we hypothesized that the nasal transcriptome will be a minimally invasive “window” to view the pathobiology of AATD-related respiratory disease development. Study participants will undergo nasal swab sampling of each nare prior to sputum induction for RNA sequencing and nasal transcriptome analysis. We plan to evaluate the potential of nasal swab-based sampling to reflect the immunological and inflammatory patterns associated with development of lung disease and aim to compare the nasal to peripheral blood transcriptome patterns.

Plasma and Serum Biomarkers

Identification and validation of biomarkers in AATD, especially those present in blood, has remained a challenge. While Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) and other studies have shown differences in lung-derived and liver derived biomarkers between patients with COPD and smoking controls³⁵⁻⁴³ and some of these markers have been associated with disease severity (fibrinogen and CRP)^{44,45}, rate of decline in lung function (CC-16), exacerbation frequency (SP-D and CRP)^{38,44,46-49} and risk of mortality (PARC/CCL-18)^{41,50}, they excluded patients with PiZZ AATD. Furthermore, although sRAGE and SP-D are associated with loss of lung tissue as assessed by CT lung density, these biomarkers are understudied in AATD-associated emphysema. Therefore, these biomarkers need to be assessed in individuals with AATD to clarify whether the associations observed in usual COPD are maintained in this rare condition. Of particular interest will be the ability of the ECLIPSE biomarkers to correlate with clinical outcome measures including rate of loss of CT lung density, as well as their ability to identify patients with frequent exacerbator and/or rapid decliner phenotypes. The optimal technique to measure blood biomarkers that associate with CT density, the hallmark of AAT lung disease, is an unbiased proteomic approach. While the A1BC

may be underpowered for such analyses, an unbiased proteomic analysis will be performed on all A1BC serum samples.

Central to the present investigation is the concept of a loss of balance between pro-inflammatory and anti-inflammatory mediators and between proteases and antiproteases in AATD and multiple potential biomarkers reflective of this derangement will be evaluated. Collagen breakdown by prolyl endopeptidases produce the biologically active extracellular matrix peptide acetylated proline-glycine-proline (AcPGP), a potent chemokine associated with neutrophilic inflammation in cigarette-smoke-mediated emphysema development⁵¹⁻⁵⁵. In addition to neutrophilic inflammation and elastase activity, it is important to note other proteinases have been implicated in COPD pathogenesis; cathepsins and matrix metalloproteinases (MMPs) play important roles in the protease imbalance in human emphysema⁵⁶⁻⁶⁴. Therefore, AcPGPs and levels of MMPs in the circulating blood will be evaluated.

Peripheral Blood Mononuclear Cell Banking and Induced Pluripotent Stem Cells

Patient-derived iPSCs allow disease modeling in differentiated cells that contain the genome of the patient from whom they were derived including any disease-relevant variants. In recent years, iPSC models have been applied to the study of AATD-associated hepatocyte injury and have both recapitulated known disease features and extended understanding of cellular injury mechanisms⁶⁵⁻⁶⁹. PBMCs that can be used to generate iPSCs will be isolated from A1BC study participants and cryopreserved as live cells for banking in an established PBMC/iPSC repository^{70,71}. Specific participant samples with distinct clinical phenotypes or novel genetic variants will be identified for iPSC generation and differentiation to disease-relevant lineages based on phenotypes observed in study data.

Genetic Testing and Detection of Single Nucleotide Polymorphisms (SNPs)

Recent advances in sequencing have led to the description of new mutations in both the exon and intron portions of the SERPINA1 gene⁷². It has been hypothesized that mutations in the promoter region can account for changes in the acute phase response and subsequent increase in AAT during infection. The functional effects of polymorphisms within both the intron and promoter region are unknown, and it is possible that there are mutations within SERPINA1 to account for

the phenotypic differences between patients with the PiZZ allele.

Data Management and Analysis

Columbia University and the A1F have established a Research Electronic Data Capture (REDCap) data entry system. REDCap is a public sourced database, which was a requirement of the NIH study. The REDCap for A1BC has linkage numbers with the A1F Registry to connect current data with historical clinical data.

Statistical Considerations

The goal for powering this study was to assure that longitudinal CT density changes would be seen over 3 years. Two recent studies have used 180 participants over 2 years in a two-arm therapeutic trial (RAPID) and 330 participants over 3 years in a three-arm therapeutic trial (SPARTA). Since our study is not a clinical trial and individuals with normal lung function are invited for participation, the number of 270 participants to follow over 3 years was chosen as a pragmatic goal that was also based on the number of PiZZ patients at participating sites. While the number of patients required to observe clinical and biochemical effects in augmentation naïve patients and/or those stable on augmentation therapy will be higher than the aforementioned AATD proof-of-concept studies, we expect that this number is again covered by the proposed cohort size. In RAPID evaluation of 54 patients from the placebo arm was sufficient to detect a significant change in desmosine/isodesmosine at 24 months^{7,73}. Since large prospective studies correlating inflammatory biomarkers with clinical outcomes and quantitative CT metrics of emphysema in AATD are lacking, this will be the largest prospective biomarker study ever conducted in AATD.

Association of predictors with clinical outcomes

Marginal association analysis will be conducted using linear regression models by regressing the CT measurement of Perc15 on each of the K (K= 28~30 in total) blood biomarkers and lung function parameters (FEV1% predicted using GLI “other” equations and FEV₁/FVC ratio), adjusted for important baseline covariates including ever smoking (100 cigarettes in a lifetime), a categorical variable of 10 pack years of smoking, baseline CRP, age, and sex. We will utilize

different sets of baseline covariates for sensitivity analysis including smoking pack years. To correct for multiple testing, we plan to use both Bonferroni correction to control for family-wise error rate and use q-value to control for false discovery rate. To account for possible correlations among biomarkers, we will use the elastic-net penalized regression models with 5-fold cross-validation to select important biomarkers jointly related to the baseline Perc15 using the R package *glmnet*. Moreover, we will apply the weighted correlation network analysis method using the R package *WGCNA* to discover clusters (modules) of highly correlated biomarkers that are associated with Perc15. For each cluster consisting of multiple biomarkers, we will use kernel machine regression to capture complex nonlinear and interaction effects of multiple biomarkers associated with Perc15. Any missing data will be imputed using the multiple imputation technique implemented in the R package *mice*. We will begin with cross-sectional analysis for data collected at each of the three visits and will use linear mixed models for the repeated measurements of all three visits to assess any longitudinal change over time. Important covariates from repeated measurement analysis including the number and severity of pulmonary exacerbations will be explored.

Findings will be validated by a companion cohort of participants from the National Alpha-1 Registry of Ireland, based at the Irish Centre for Genetic Lung Disease (Beaumont Hospital/Royal College of Surgeons in Ireland, Dublin). Methods for validation will be modeled to explore significant findings from the United States cohort and discrepancies will be probed to determine the differences in covariates between the two cohorts.

Genetic Analyses

With sequencing data of the *SERPINA1* gene, we will first perform standard quality control and data preprocessing. *SERPINA1* sequencing will be performed on the entire gene, with promoter region, exon and introns included. Then, we will perform genetic association analysis using a linear regression model for Perc15 measured at each visit and linear mixed models for the three repeatedly measured Perc15 to identify putative causal genetic variants, adjusted for sex age, and smoking. Gene-environment analysis will be performed with inclusion of an interaction term between genetic variants and important environmental variables (e.g., cigarette smoking). Additionally, we will also perform genetic interaction analysis by including interaction terms

between genetic variants to explore possible epistatic effects.

Study oversight

An observational safety monitoring board (OSMB) has been established to include two pulmonologists and one statistician. The OSMB meets approximately once every 6 months to review patient enrollment, safety related to the study procedures, and adverse events. The open OSMB meeting is attended by NIH and A1F representatives. Adverse events are defined primarily as related to study procedures. Special events of clinical interest will be captured including AECOPD, decompensated liver disease, and enrollment in clinical trials. Furthermore, incidental findings primarily related to lung nodules on CT scans will be collected.

Potential outcomes and conclusions

The above-described study will be the largest cohort of AATD patients with PiZZ genotype prospectively assembled, longitudinally followed and systematically analyzed with regards to biomarkers of lung disease. The characterization of a full complement of biomarkers, including blood biomarkers, CT imaging biomarkers, patient reported outcomes, exposure history, lung function, and genomics in a cohort of individuals with alpha-1 antitrypsin deficiency has never been done at this large scale in a longitudinal fashion. The evaluation of associations between these characteristics and clinical outcomes in cross-sectional analysis as well as over time in the 3-year longitudinal follow-up will improve understanding of the heterogeneity of AATD and identify factors associated with disease severity and progression. The results have the potential to improve care for individuals with AATD by identifying individuals at risk for more severe lung or liver disease manifestations earlier before deterioration and may lead to future interventions that will improve clinical outcomes.

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M.P.G, C.P., I.B., S.Bh., M.B.D., N.G.M, O.J.M., J.M.W, A.W., C.S., and J.M.D. conceived the study and developed the theoretical framework. All authors contributed to and approved the final manuscript.

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Alpha-1 Biomarker Consortium Study Group

Columbia University: Jeanine M. D'Armiento, Monica P. Goldklang, Zhonghua Liu, Laura D. Fonseca, Tina Zelonina, Alexis C. Fisher, Niki Trivedi, Sydney Harris, Sabrina Palumbo, Kiran K. Chada, Devipriya Harinath, Sanjiv Prasad

University of Utah: Cheryl Pirozzi, Cassie E Larsen, Alexis G. Vanwagoner

University of California, Los Angeles: Igor Barjaktarevic, Russell Buhr, John Belperio, Semi Yoon, Vyacheslav Palchevskiy, Lung Wing

University of Alabama at Birmingham: Mike Wells, Surya P. Bhatt, Sandeep Bodduluri, Abhiraj Pudhota

University of North Carolina at Chapel Hill: M. Bradley Drummond, Caleb C. Hemphill, Kelsey B. Haywood, Walker A. Long

University of Chicago: D. Kyle Hogarth, MD, Vanita Patel, MPH

Alpha-1 Foundation: Randel Plant, Alison Keaveny, Nadine Nuchovich, Jennifer Illarramendi, Charles Emala, Jon Hagstrom, Scott Santarella

Royal College of Surgeons in Ireland: Noel G. McElvaney, Oliver J. McElvaney, Suzanne M. Roche, Daniel D. Fraughen

National Jewish Health: Robert A. Sandhaus, Ariana K. Talaie

Boston University: Andrew Wilson, Joseph E. Kaserman, Mark Dodge, Shumin Guan

Medical University of South Carolina: Charlie Strange, Leah Benn, Allison Burton, Gwen Hayden, Chloe Jacobs, Kristen Neff, Melinda M. Talley, Andrea J. Swartz

Declaration of Interest

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TABLES

Table 1. Alpha-1 Clinical Resource Centers with geographic location participating in Alpha-1 Biomarkers Consortium

A1F Clinical Resource Centers	Location
Columbia University Irving Medical Center	New York, New York
Medical University of South Carolina	Charleston, South Carolina
University of Alabama at Birmingham	Birmingham, Alabama
University of North Carolina at Chapel Hill	Chapel Hill, North Carolina
Boston University	Boston, Massachusetts
University of Utah	Salt Lake City, Utah
National Jewish Health	Denver, Colorado
University of California-Los Angeles	Los Angeles, California
University of Chicago	Chicago, Illinois

Table 2. Questionnaires to obtain patient reported outcomes

Questionnaires	Clinical Characterization
Patient Health Questionnaire (PHQ-9), Alpha-1 Antitrypsin Deficiency Cohort Exposure Questionnaire (Appendix Table 3)	Health status
St George's Respiratory Questionnaire (SGRQ) COPD Assessment Test (CAT)	Pulmonary symptomatology
University of California San Diego, Shortness of Breath Questionnaire (SOBQ)	
Modified Medical Research Council Dyspnea scale (mMRC)	
Breathlessness, Cough, and Sputum Scale (BCSS)	
Baseline and Monthly Exacerbation Questionnaires (Appendix Tables 4 and 5)	Exacerbation frequency
University of California San Diego Shortness of Breath Questionnaire (SOBQ)	Dyspnea
Chronic Liver Disease Questionnaire (CLDQ)	Liver health
Alcohol Use Disorder Identification Test (AUDIT-C)	

FIGURES

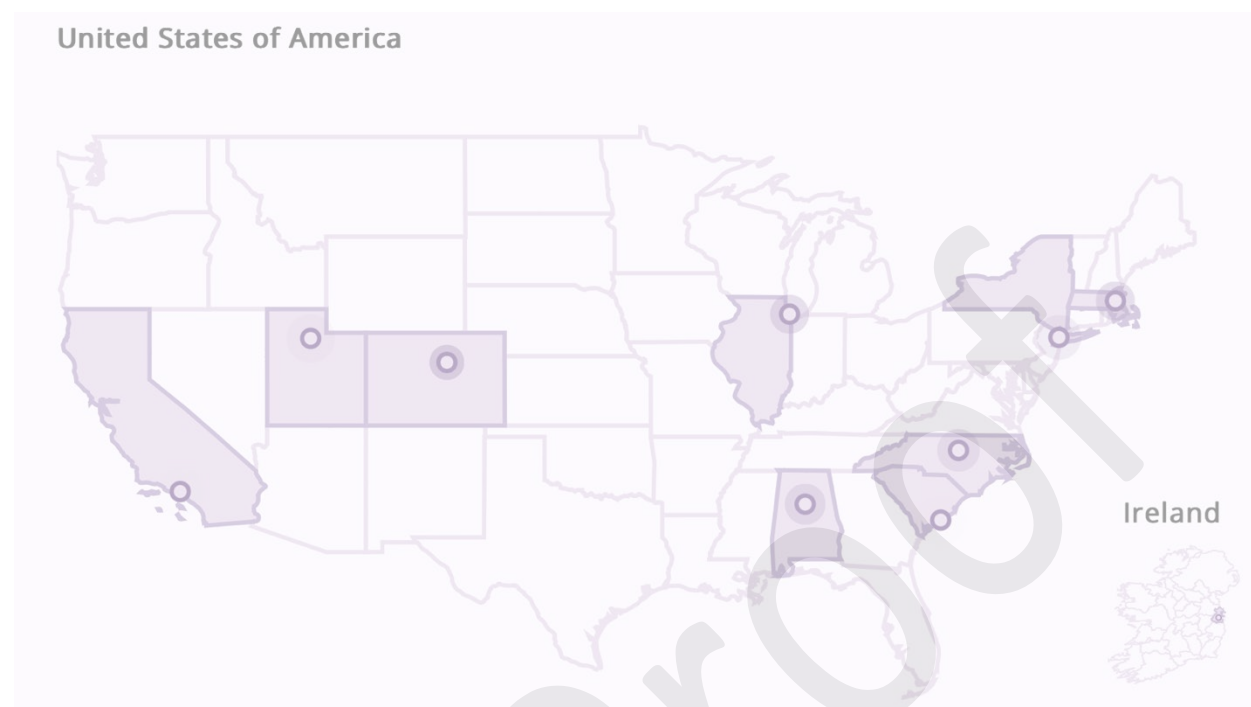


Figure 1. Alpha-1 Clinical Resource Centers participating in the Alpha-1 Biomarkers Consortium are dispersed around the United States, with a validation cohort in Ireland.

APPENDIX**Table 1. Primary inclusion and exclusion criteria for enrollment into the Alpha-1 Longitudinal Biomarker Study**

<i>Inclusion Criteria</i>
1. Males and females, age 18 years or older
2. Understand the study procedures, risks, benefits, purpose
3. Able and willing to comply with the study procedures
4. Have PiZZ alpha-1 antitrypsin deficiency
5. Be an existing member of the Alpha-1 Foundation Clinical Cohort (also known as the Alpha-1 Foundation Research Registry)
6. Agree to have the data collected in this study be shared with the Alpha-1 Foundation Research Registry
<i>Exclusion</i>
1. AATD non-PiZZ status, including heterozygous patients
2. Current lung, hematologic, or solid organ malignancy other than skin or cervical Stage 1 cancers within the past 3 years
3. COPD exacerbation or other pulmonary infection within 6 weeks of baseline visit
4. Pregnancy at the time of the screening visit
5. Inability to lie still in a supine position for 15 minutes during CT acquisition
6. Inability to perform quality-controlled lung function testing
7. Allergy to albuterol
8. Currently receiving intravenous or subcutaneous immunoglobulin for any disease state
9. Past or present major surgery on the lungs including pneumonectomy or lobectomy. Wedge resections, past segmentectomy, and pleurodesis surgeries are allowed
10. Previous lung or liver transplantation or currently on the transplant list
11. Current presence of endobronchial coils or valves in the lung
12. Clinically significant bronchiectasis as defined by the investigator. In general, this would exclude patients with chronic infection of the lungs requiring treatment within the past 6 months including non-tuberculous mycobacterial disease, chronic fungal disease, allergic bronchopulmonary aspergillosis, or known colonization of bronchiectasis with pseudomonas or stenotrophomonas species
13. Participation in the active treatment arm of a therapeutic clinical trial at baseline visit unless using one of the Alpha-1 augmentation therapies in alternative doses
14. Patient with Automatic Implantable Cardioverter-Defibrillator and permanent pacemakers
15. Patient receiving biologic immunomodulators that will affect the assessment of the serum biomarkers (as determined by the site PI)
16. Patient with in-dwelling pleural catheters
17. Any condition that in the opinion of the investigator might adversely influence the study outcome

Pre-proof

Table 2: Study schedule of events

Procedures	Screen	Visit #1 Baseline	PRO AlphaNet	Phone call	PRO AlphaNet	Phone call	PRO AlphaNet	Visit #2	PRO AlphaNet	Phone call	PRO AlphaNet	Phone call	PRO AlphaNet	Visit #3
Months from baseline visit	-1	0	1-5	6 +/- 1	7-11	12 +/- 1	13-17	18 +/- 3	19-23	24 +/- 1	25-29	30 +/- 1	31-35	36 +/- 3
Informed Consent	X (oral)	X (sign)												
Assessment of Eligibility	X													
Review Medical History	X	X		X		X		X		X		X		X
Review Medication List	X	X		X		X		X		X		X		X
Vitals		X						X						X
Blood draw		X						X						X
Dried blood spot card ¹		X						X						X
Urine Pregnancy Test ²		X						X						X
PFT Spirometry Pre & post		X						X						X
CT scan		X						X						X
Induced Sputum* (varies by site)		X						X						X
Nasal Swab*		X						X						
PROs		X	X ³	X ³	X ³	X ³	X ³	X ⁴	X ³	X ³	X ³	X ³	X ³	X ⁴
Assessment of Events of Clinical Significance		X						X						X

Abbreviations: PRO=Patient Reported Outcomes

¹Dried Blood Spot Card = In addition to study visits, collect DBS cards on onset, 3-day and 7-days post a COPD Exacerbation Event.²Urine Pregnancy Test = Complete Radiation Pregnancy Form when applicable.³X= Monthly AlphaNet COPD Exacerbation questionnaire only.⁴X= Excludes Exposure Questionnaire (administered at baseline visit only).

*= Optional procedures.

Table 3. A1BC Exposure Questionnaire

Smoking	
Have you ever smoked cigarettes? No means less than 20 packs of cigarettes, or 12 ounces of tobacco, or less than 1 cigarette a day for one year at any time in your life.	Yes No
2. How old were you when you first started regular cigarette smoking?	
3. Do you smoke cigarettes (as of one month ago)?	Yes No
4. About how many cigarettes do you smoke per day now?	<i>Individual cigarettes, not packs</i>
5. How old were you when you completely stopped smoking?	
6. On average of the entire time you smoked, how many cigarettes did you smoke per day?	
7. Have you ever smoked a pipe regularly? YES means more than 12 oz of tobacco in a lifetime	Yes No
8. How old were you when you first started to smoke a pipe regularly?	
9. Do you smoke a pipe (as of one month ago)?	Yes No
10. How many ounces of pipe tobacco do you smoke per day now?	
11. How old were you when you completely stopped smoking a pipe	
12. On average of the entire time you smoked a pipe, how many ounces of tobacco did you smoke per week?	
13. Have you ever smoked cigars regularly? Yes means more than 1 cigar a week for one year at any time in your life	Yes No
14. How old were you when you first started to smoke cigars regularly?	
15. Do you smoke cigars now (as of one month ago)?	Yes No

16. How many cigars do you smoke per day?	
17. How old were you when you completely stopped smoking cigars?	
18. On average of the entire time you smoked cigars, how many cigars did you smoke per week?	
Vaping	
19. Have you ever used an electronic cigarette or vape product?	Yes No
20. Did your electronic cigarette or vape product contain any of the substances below?	Nicotine Cannabis / marijuana / THC Don't know Other
21. Do you still use e-cigarettes or vape products?	Yes No
22. How often do you use e-cigarettes or vape products?	Everyday Most days 4+ days per week 1-3 days per week Less than once per week Less than once per month
23. How many years in total have you used electronic cigarettes or vape products?	
Second Hand Smoke	
24. Do you currently live in the same household with someone who smokes tobacco products?	Yes No
25. Have you ever lived in the same household with someone who smoked tobacco products?	Yes No
26. Growing up until age 18, were there any adults in your household who smoked at home?	Yes No
27. For how many years in total did you live in the same household with someone else who smoked tobacco products?	
28. Have you been regularly exposed to tobacco smoke in the last 12 months? (Regularly means on most days or nights)	Yes No
29. Do people smoke regularly in the room where you work?	Yes No
Occupational	
30. What is your occupation?	

31. Does your current job expose you to vapors, gas, dust, or fumes?	Yes Don't know No Not applicable
32. In your longest held job, what kind of work did you do. What was your occupation?	
33. Did your longest job expose you to vapors, gas, dust, or fumes?	Yes Don't know No Not applicable
34. Is an air cleaner/filter used in your residence (stand-alone or central)?	Yes No
35. What type of air filter?	Stand-alone / portable Central Don't know
36. Within the last 12 months have you had wet or damp spots on surfaces inside your home other than in the basement (for example on walls, wall paper, ceilings or carpets)?	Yes No I don't know
37. Has there ever been any mold or mildew on any surface, other than food, inside the home?	Yes No I don't know
38. Do you keep a cat inside the house?	Yes No
39. Do you keep a dog inside the house?	Yes No
40. Do you keep any birds inside the house?	Yes No
Cleaning Chemicals	
41. Are you responsible for cleaning or washing in your home?	Yes No
42. Have you ever worked as a cleaner?	Yes No
43. How many days per week did you use cleaning products?	Never Less than one day per week 1-3 days per week 4-7 days per week
44. How many days per week did you use cleaning sprays?	Never Less than one day per week 1-3 days per week 4-7 days per week

Table 4. Baseline Exacerbation Questionnaire

1. Over the past year, how many times have you experienced worsening ("exacerbations" or "flares") of your lung problems?	Every month Every 3 months Every 4 months Every 6 months Once Never
2. Over the past 2 years, have you coughed up sputum/mucus from your lungs on a regular basis for at least three months each year?	Yes No
3. Over the past 12 months, how many times have you experienced the following? Please note these are number of events and not days of hospitalization	
3a. Admitted to the hospital?	0, 1, 2, 3, >3
3b. Admitted to the intensive care unit?	
3c. Seen in the emergency room?	
3d. Seen by a healthcare practitioner for an unscheduled office visit?	
4. If you were hospitalized over the past year, was the primary reason lung related?	Yes No

Table 5. Monthly Exacerbation Questionnaire

1. Did you have a new upper respiratory infection, cold, or flu-like illness in the last month? Please note that you should not repeat events reported one month ago.	Yes No
2. How did you manage any lung problems you had this past month? Check all that apply	I spoke with my regular primary care physician, nurse practitioner, or physician's assistant. I spoke with my pulmonary specialist. I visited a health care practitioner in his/her office. I went to an emergency room or urgent care center. I treated the problem myself. Not Applicable
3. Over the past month, how many times have you been seen for lung disease and experienced the following? Please note these are number of events and not days of hospitalization	
3a. Admitted to the hospital?	0, 1, 2, 3, >3
3b. Admitted to the intensive care unit?	
3c. Seen in the emergency room?	
3d. Seen by a healthcare practitioner for an unscheduled office visit?	
4. Have you experienced any worsening of respiratory symptoms (an "exacerbation" or "flare") in the last month? Please note that you should not repeat events reported 1 month ago.	Yes No
5. Have you had any of these symptoms within the past month? Check all that apply	a) More shortness of breath b) More cough c) Increased sputum amount d) New wheezing e) Worsening of wheezing f) Sputum changed color g) Fever Not Applicable
5.1. Do you have any of these symptoms now? Check all that apply	a) More shortness of breath b) More cough c) Increased sputum amount d) New wheezing e) Worsening of wheezing f) Sputum changed color

	g) Fever
	Not Applicable
6. Did you have chest imaging (chest X-ray or chest CT) in the past month?	Yes No
7. Did you start oxygen or change your oxygen over the past month?	Yes No
If you need to edit a report from one month ago, please report the changes here. Examples of an updated report would include that the event from last month had not ended at the time of last report and you would like to add the total duration of the event or that you began new treatments not reported previously.	