Study Protocol for

Targeting Pulmonary Perfusion in Alpha-1 Antitrypsin Deficiency

NCT03008915

IRB# AAAP9855
Version 1.6
April 11, 2017

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Funding Source: Alpha-1 Foundation

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1. Study Purpose and Rationale

The purpose of the Alpha-1 Foundation-funded study "Targeting Pulmonary Perfusion in Alpha-1 Antitrypsin Deficiency" is to determine whether pulmonary vascular changes in alpha-1 antitrypsin (AAT) associated chronic obstructive pulmonary disease (COPD) are modified by either aspirin use or AAT replacement therapy.

COPD and emphysema cause significant morbidity and mortality in patients with AAT deficiency (AATD).[1,2] Emphysema is defined as airspace enlargement and alveolar wall destruction,[3] whereas COPD is defined by spirometric airflow limitation. AAT has been associated with lower lobe emphysema, which often occurs before airflow limitation. Emphysema on CT has been associated with impaired cardiac function,[4] increased dyspnea,[5] and increased all-cause mortality in those with and without COPD[6,7] AAT augmentation is the only medication to target emphysema, and it is a weekly intravenous therapy that is costly and burdensome to the patient. AAT replacement was FDA-approved based on its ability to re-establish normal levels of AAT in the lung, and the only clinical endpoint with high quality positive results was a reduction in emphysema progression.[8-11]

Platelets are involved in many vascular diseases and when activated have been shown to cause microvascular dysfunction.[12-14] Platelets are activated by von Willebrand factor (vWF)[15] and in chronic vascular inflammation.[16] As pulmonary capillaries are integral to alveolar structure, platelets may also be involved in the pathogenesis of emphysema. In fact, human genome-wide[17] and DNA methylation association studies[18] of COPD have found associations for platelet-related genes including serotonin receptor 4 (*HTR4*), vWF (*VWF)*, and its platelet receptor glycoprotein 1bα (*GP1BA*). Moreover, the serine proteases neutrophil elastase and cathepsin G, both inhibited by AAT, increase platelet activation.[19-22] In animal studies activated platelets form aggregates in the pulmonary microvasculature[23,24] and cause vasoconstriction.[25,26] Finally, the addition of platelet factor 4, which is released by activated platelets, increased emphysema in an animal model of neutrophil elastase-induced emphysema.[27]

Human studies also suggest a relationship between platelet activation and COPD. Two small studies found that platelet activation was associated with COPD and hypoxemia.[28,29] A larger study of COPD hospitalizations found that thrombocytosis was associated with greater 1-year mortality, which was reduced in those on aspirin.[30] In the Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study (AAAE9235) we found that progression of percent emphysema on computed tomography (CT) was more rapid in those with higher vWF (P=0.05) and slower among participants taking aspirin compared to those not (P=0.008). In summary, platelet activation has been associated with COPD in humans, and aspirin may reduce mortality in COPD and predict slower progression of emphysema; however, whether aspirin improves pulmonary microvascular blood flow in COPD or AATD is unknown.

Endothelial cell perturbation and reduced pulmonary blood flow may be relevant to the pathogenesis of emphysema[31,32] and important in AATD. AAT has been shown to attenuate endothelial apoptosis in animal models of emphysema.[33,34] We examined the endothelial hypothesis of emphysema in the MESA COPD Study (AAAD6395), a case-control study of COPD, in which we found pulmonary microvascular blood flow on contrast-enhanced magnetic resonance imaging (MRI) to be inversely associated with CT percent emphysema and COPD stage, and associated with $CD31^+$ endothelial microparticles (EMPs), which reflect endothelial apoptosis.[35,37] In the Alpha-1 Foundation-funded study "Pulmonary Vascular Damage in Alpha-1 Antitrypsin Deficiency", which followed the MESA COPD protocol, we found greater $CD31^+$ EMPs in AATD compared to MESA COPD cases and controls, and a non-significant reduction in pulmonary microvascular blood flow in AATD compared to controls. Importantly, those with

AATD on AAT augmentation had lower $CD31^+$ EMPs and a trend toward greater pulmonary microvascular blood flow compared to those not on AAT therapy. These findings demonstrate increased endothelial damage in AATD compared to non-AATD COPD and suggest amelioration of endothelial damage by AAT augmentation; however whether AAT augmentation reduces $CD31^+$ EMPs and improves pulmonary microvascular blood flow is unknown.

Therefore, we propose to accrue 15 subjects with PiZZ AATD-associated lung disease who are on AAT augmentation therapy into a study protocol that includes: 1) a Phase IIa cross-over randomized controlled trial (RCT) of aspirin *vs.* placebo and 2) a withdrawal study with a follow-up visit off AAT augmentation therapy in order to test the following hypotheses:

1) Randomization to aspirin 81mg daily increases pulmonary microvascular blood flow on MRI and improves secondary endpoints compared to placebo in participants with AATD-associated lung disease receiving standard care (including AAT augmentation therapy).
2) $CD31^+$ EMPs will be lower and secondary endpoints will be improved when participants are on AAT augmentation therapy compared to off AAT augmentation therapy, while receiving other standard medical treatment.

Successful completion of these aims would demonstrate that aspirin and AAT augmentation therapy improve pulmonary microvascular blood flow and reduce CD31+ EMPs in AATD-related lung disease, would support future studies evaluating aspirin in emphysema in AAT, demonstrate additional benefits of AAT augmentation, and support the use of pulmonary MRI and EMPs as outcomes for clinical trials in COPD.

2. Study Design

a. The proposed study is a single-center Phase IIa randomized cross-over study and withdrawal study.

b. The study will accrue 15 subjects and enroll up to 20.

c. Inclusion criteria:
   i. Alpha-1 antitrypsin deficiency (PiZZ genotype)
   ii. 40 years of age or older
   iii. Evidence of emphysema on CT scan as read by a Radiologist
   iv. On intravenous alpha-1 antitrypsin replacement therapy

d. Exclusion criteria:
   i. Platelet count < 150,000/dL, history of intracranial hemorrhage or severe GI bleed, use of systemic anticoagulant, physician prescribed use of antiplatelet drug (including aspirin and P2Y12 receptor inhibitors), or known severe liver disease;
   ii. Immunosuppression by use of medications (including oral prednisone) or medical condition (organ transplantation or actively-treated malignancy), including autoimmune conditions that require immunomodulatory treatment (e.g. anti-TNF agents);
   iii. Known atrial fibrillation or left ventricular (LV) systolic heart failure;
   iv. Contraindication to MRI, including pregnancy, weight > 300 lbs (due to weight limits of the machine), those with pacemakers, aneurysm clips, cochlear implants or other implanted electronic devices, or severe claustrophobia;

v. Chronic renal insufficiency (estimated GFR < 45 L/min/1.73 m^2 or self report) due to slightly increased risk of nephrogenic systemic fibrosis from gadolinium administration and aspirin-related renal insufficiency. GFR will be calculated using the MDRD equation based on the most recent laboratory sample available (within the last 30 days required). If one is not available at the time of MRI a point-of-care Creatinine will be run and this value will be used instead of the laboratory sample;

vi. Frequent exacerbations of respiratory symptoms (two or more in the last year); and

vii. Exacerbation of respiratory symptoms within the previous 6 weeks, such as that requiring hospitalization, oral prednisone or antibiotics to control symptoms.

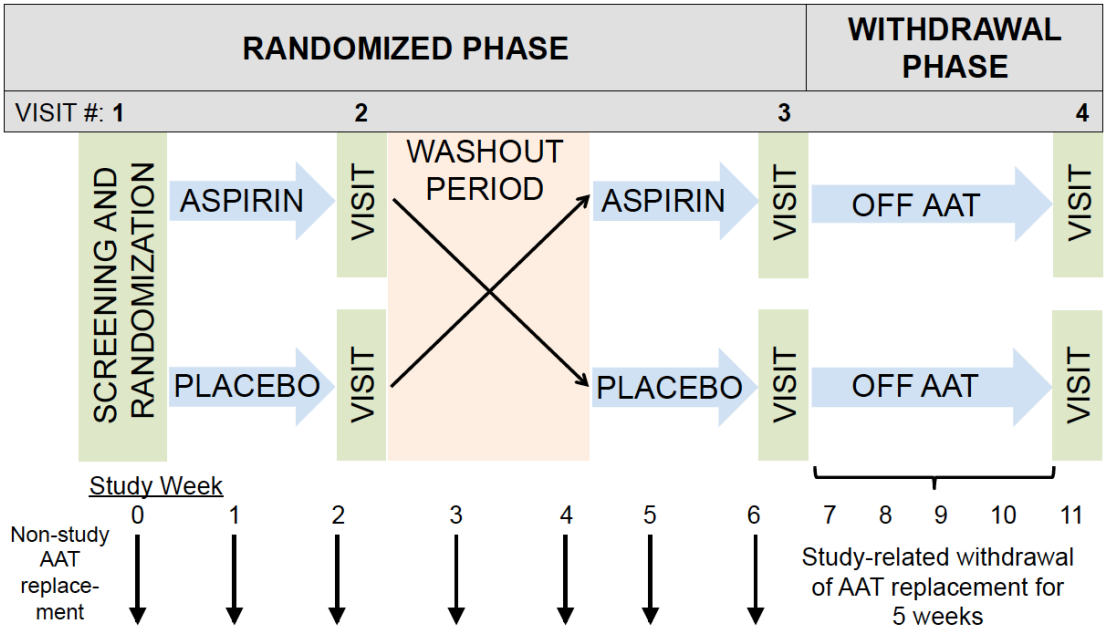

Figure 1. Study Design

Table 1: Study Components

Visit #:	1	2	3	4
Exam component	Screening visit	After treatment 1	After treatment 2	Off AAT augmentation
Anthropometry	X	X	X	X
Blood pressure		X	X	X
Questionnaires	X	X	X	X
Phlebotomy				
Endothelial microparticles		X	X	X
Creatinine, CBC	X	X	X	X
Platelet activation		X	X	X
Alpha-1 antitrypsin level		X	X	X
Urine specimen		X	X	X
Spirometry, pre- and post-bronchodilator	X	X	X	X
Diffusing capacity	X	X	X	X
Pulse oximetry	X	X	X	X
Six minute walk test		X	X	X
Non-contrast chest CT	X			
Cardiopulmonary MRI				
Pulmonary microvascular blood flow		X	X	X
RV, LV, cardiac output		X	X	X

e. Endpoints
   i. Aspirin RCT
      (1) Primary endpoint: pulmonary microvascular blood flow
      (2) Secondary endpoints:
         a. Pulmonary perfusion: pulmonary microvascular blood volume, pulmonary microvascular transit time, and above measures of pulmonary perfusion in areas of emphysema on chest CT
         b. CD31+ endothelial microparticles (reflecting endothelial apoptosis), CD62+ endothelial microparticles (reflecting endothelial activation)
         c. Spirometry: post-bronchodilator FEV_1 and FEV_1/FVC
         d. Pulse oximetry
         e. Diffusing capacity
         f. Six minute walk test
         g. Alpha-1 antitrypsin level
         h. Cardiac MRI: LV EDV, RV EDV, LV SV, cardiac output
         i. Questionnaire: MMRC dyspnea scale, COPD Assessment Test

   ii. Alpha1 Augmentation Withdrawal Study
      (1) Primary endpoint: CD31+ endothelial microparticles (reflecting endothelial apoptosis)
      (2) Secondary endpoints:
         a. CD62+ endothelial microparticles (reflecting endothelial activation), platelet microparticles (reflecting platelet activation)
         b. Pulmonary perfusion: pulmonary microvascular blood flow, pulmonary microvascular blood volume, pulmonary microvascular transit time, and above measures of pulmonary perfusion in areas of emphysema on chest CT
         c. Spirometry: post-bronchodilator FEV_1 and FEV_1/FVC
         d. Pulse oximetry
         e. Diffusing capacity
         f. Six minute walk test
         g. Markers of platelet activation: beta-thromboglobulin, platelet factor 4, thromboxane B2, von Willebrand factor
         h. Cardiac MRI: LV EDV, RV EDV, LV SV, cardiac output
         i. Questionnaire: MMRC dyspnea scale, COPD Assessment Test

f. Subjects will undergo telephone pre-screening before Visit 1. At Visit 1 they will be consented, after which a platelet count and creatinine will be drawn to confirm eligibility. Participants will then complete questionnaires, perform spirometry, diffusing capacity and pulse oximetry. Eligible patients will then be randomized as to the order of intervention vs. placebo and will undergo non-contrast chest CT. Randomization will be stratified by smoking status with full concealment of allocation; subjects and the investigative team will be blinded as to treatment assignment. The intervention will be 81mg aspirin and a matched placebo, each taken once per day in the morning. Each treatment will last approximately 2 weeks (Figure 1, previous page) with Visits 2 and 3 occurring at the end of treatment 1 and 2, respectively. Each treatment may be continued for up to 18 days, if needed, to coordinate scheduling of the participant visit and procedures. There will be a 2-week washout period between the treatments, and the total length of the randomized protocol will be approximately 6 weeks. The CU Research

Pharmacy will dispense study medications. During the study period, subjects will be asked to continue the same medication regimen and avoid using over-the-counter aspirin and other non-steroidal anti-inflammatory drugs.

g. Timing with respect to AAT infusions: We will recruit subjects who are on AAT augmentation therapy, and the timing of study visits will be coordinated with their weekly dosing of AAT to minimize differences between study visits other than the intervention. We will schedule patient visits on days 1-3 post-infusion in order to study the effects of peak AAT levels.

h. Withdrawal of AAT augmentation therapy:
Subjects will be asked to stop AAT augmentation therapy for approximately 5 weeks and return for Visit 4. Withdrawal of AAT for 5 weeks (a maximum of 5.5 weeks) will allow AAT levels to drop briefly to those seen in the absence of AAT augmentation.

3. Statistical Procedures

a. Data analysis: Data analysis will be performed primarily by the PI at Columbia University. The primary analysis will use paired t-tests to compare pulmonary microvascular blood flow on aspirin compared to placebo, and $CD31^+$ EMPs on AAT augmentation therapy compared to off augmentation following the intention-to-treat principle. Similar analyses will be performed for secondary endpoints. Exploratory analyses will also evaluate for different effects in subgroups by smoking status, COPD severity, use of supplemental oxygen, and by severity of reduction in DLCO and will evaluate for period and carryover effects. Approaches to missing data will include: 1) minimization through short study duration and efforts to retain 100% of participants for 3 months and 2) sensitivity analyses using a variety of imputed values (worst-case scenario, baseline observation carried forward, last observation carried forward).

b. Statistical power: Power was estimated for a paired t-test to compare an individual's pulmonary microvascular blood flow before and after the intervention. For 15 subjects with power of 80% and α=0.05, we will be able to detect a minimally significant difference of 0.30 log $CD31^+$ EMPs (1.2 SDs of the difference in log $CD31^+$ EMPs for those on replacement and not), and a minimally significant difference of 48.4 mL blood/min*100mL lung (1.6 times the SD of the difference in pulmonary microvascular blood flow [PMBF] for those on replacement and not). Power will be adequate for $CD31^+$ EMPs and moderate for PMBF. As power was derived from a cross-sectional analysis, this is a conservative estimate and we expect that power will be improved when comparing changes within an individual.

4. Recruitment and Consent

a. Recruitment: Participants will be recruited from multiple sources. First, we will recruit among the 30 participants who previously enrolled in the Alpha-1 extension of the MESA COPD Study over the telephone (AAAD6395). All of these participants agreed to be contacted for future research purposes on their consent forms. Second, we will email,

call, meet in person with, or mail flyers to pulmonologists within the recruitment region who see patients with alpha-1 antitrypsin deficiency to ask them to refer any patients who might be interested. The flyer may also be posted on the pulmonary and lung transplant floors of Presbyterian Hospital at Columbia University. Third, we will distribute study information through AlphaNet, a network for Alpha-1 patients that is affiliated with the Alpha-1 Foundation (see attached recruitment materials). Fourth, we will utilize the Alpha1 Research Registry (run out of the Medical University of South Carolina), who maintains a database of approximately 5,000 persons with alpha-1 antitrypsin deficiency and can send study information to a subset who meet our basic eligibility criteria and live in the geographic region. Fifth, we will contact leaders of support groups for alpha-1 antitrypsin deficiency in the recruitment region to consider presenting material about our study at their meetings, allowing us to present the material, or distributing recruitment materials to their list-serve via email. The recruitment region includes NY, NJ, CT, PA, DE and MA.

Interested persons will be contacted directly by telephone to discuss the study (following the attached script). The interviewer will then administer a Screening Questionnaire to check inclusion and exclusion criteria, and determine willingness to participate.

For persons who are screened over the telephone but found to be ineligible for the study, we will keep record of their name, telephone number and Screening Questionnaire for the duration of the study in a locked cabinet in the study coordinator's office. The rationale for this is 1) to ensure that we do not contact the same person twice, and 2) in the event that we change eligibility criteria, we would be able to contact the appropriate persons for potential inclusion in the study. We will send an information sheet to those who are screened but ineligible to inform them that they were contacted for this study.

b. Informed Consent: Each participant will go through the process of informed consent (and signature documentation of this) with the PI, Dr. Aaron. Informed consent includes consent to be randomized to take the study drug and placebo in either order, holding their home augmentation therapy for approximately 5 weeks, and return for three study visits. Prior to initiation of the study, the consent form will be approved by the IRB. The alternative to participating in the study is not participating in the study, or participating in part of the study as it is entirely voluntary. If subjects wish to withdraw from the study, we will request to perform measurements at the time of drop out if the subject is amenable.

5. Study Procedures

The study will consist of the following components, as outlined in Table 1 (next page):

1. Anthropometric measurements. Height and weight will be measured to the nearest 0.1cm and 0.5kg with a scale and stadiometer at visits 1-4.
2. Blood pressure. Resting blood pressure is measured at visits 1-4 in the right arm after 5 minutes in the seated position with the automated Dinamap Monitor Pro 100. Three readings are taken and the 2nd and 3rd readings are averaged for use in analyses.
3. Questionnaires. Questionnaires will be administered to all study participants at visits 1-4 including respiratory symptoms, smoking habits, medication use, occupation, and health information.

4. Phlebotomy. A blood sample will be collected at visit 1 to confirm eligibility. Fasting blood samples will be collected at visits 2-4 to measure EMPs, creatinine, complete blood count, measures of platelet activation, and alpha-1 antitrypsin level. The total amount to be drawn will be no more than 45mL. A 10mL syringe will be transported immediately for EMP analysis. A second blood sample may be collected during visits 2, 3 and 4 for a point-of-care Creatinine, if the laboratory measurement is not resulted at the time of MRI.
5. Urine specimen. Approximately 1 cup of urine will be collected at visits 2-4, this is for storage for potential future measurement of platelet activation.
6. Spirometry. At visits 1-4 pre and post-bronchodilator spirometry will be performed according to American Thoracic Society (ATS) guidelines. Testing will be performed before and 15 minutes after administration of 2 puffs of albuterol (180 mcg) from a metered dose inhaler.
7. Diffusing capacity. This non-invasive test is similar to spirometry, but involves less effort. All participants will undergo single breath DLCO at visits 1-4.
8. Pulse oximetry, or oxygen saturation. Resting oxygen saturation (off supplemental oxygen for 5 minutes, if used) will be measured non-invasively with a pulse-oximeter at visits 1-4.
9. Six minute walk test. The 6MWT will be conducted on an established course according to ATS standards by a certified and trained technician with continuous non-invasive monitoring (e.g. oxygen saturation).
10. CT scan. The CT scan will be performed using a GE 64 multi-detector computed tomography (MDCT) scanner at visit 1. The protocol comprises a full scan of the chest following instructions for a very deep breath. The purpose of this test is to measure the full lung and lung segments and regions, and the total pulmonary vasculature volume. At the end of the study, participants will be asked to breathe out and hold it for a few seconds to be able to take an expiratory phase scan. The estimated effective radiation exposure for the inspiratory and expiratory scans is 6.5 mSv total. Women age 40-55 years will have a urine pregnancy test performed prior to CT and MRI scanning.
11. Magnetic Resonance Scanning on MRI scanners. The MRI exam will be performed at visits 2-4. Before having the MRI exam, participants will be asked questions about their medical history and any contraindications to MRI. The MRI will be a pulmonary angiography with the administration of gadolinium to visualize the pulmonary vascular bed. For this exam, the participant will lie on a table inside the MRI for about 30 minutes and will wear a facemask delivering supplemental oxygen at 8 L/min to reverse pulmonary hypoxic vasoconstriction. Gadolinium contrast will be injected into the antecubital vein through an 18-20 gauge IV. The type of gadolinium will be gadoterate meglumine (a macrocyclic agent, Dotarem), and the dose per scan will be 0.03 mmol/kg (0.06 ml/kg). The scanning will be at Functional Residual Capacity (FRC), which is the volume of air left in the lungs after a normal, passive exhalation. Participants will be asked to breathe out to a resting phase, and will be asked to practice achieving and holding their breath during the expiration phase.
12. Storage of Blood and urine. Blood and urine from visits 2-4 will be stored at Columbia and at University of Vermont, identified only by study ID and following standard procedures. It will be accessible only to study investigators and, if the participant approves, outside investigators with IRB approval. Blood and urine will be kept indefinitely.

6. Results Reporting

a. Clinically relevant results (spirometry, CT scan report, LV mass and ejection fraction and Class A and B incidental findings and, if highly abnormal, blood pressure and blood test

results) will be provided to participants and if they allow it, to their physicians.

b. Incidental Findings. Images will be reviewed within 2 weeks as follows: all CT scans are acquired on clinical scanners and enter into the clinical workflow and are read by a credentialed Radiologist as part of clinical work and a clinical report is produced; all cardiac MRI and pulmonary perfusion sequences will be reviewed by a credentialed Radiologist and a safety read will be produced for incidental findings. When medically significant incidental findings occur the Reporting of Incidental Findings in Research Form will be submitted to the IRB.

c. Typical incidental findings include nodules and signs of low-grade infection, rarely cancer, LV hypertrophy and low ejection fraction. Clinical reports are sent out and, additionally, the PI calls participants in the case of incidental findings in a timeframe consistent with the severity of the incidental finding.

7. Potential Risks

a. Interventions

i. Aspirin 81 mg daily x 2 weeks. Aspirin is a commonly used over-the-counter medication that is generally regarded as safe. The most common adverse effects of aspirin are minor bleeding and gastrointestinal upset. Serious adverse effects are rare, but include intracranial and gastrointestinal hemorrhage. The increased attributable risk of intracranial hemorrhage for daily aspirin use (most at 81 mg, some at 325 mg dose) was 0.4 events per 1000 person-years, and attributable risk of gastrointestinal hemorrhage due to peptic ulcer disease was 2 events per 1000 persons-years.[36] Renal function can be transiently reduced with aspirin, as with any nonsteroidal anti-inflammatory drug, but this is rare in the absence of chronic kidney disease.[37] In AATD there is no documented increase in adverse effects of aspirin. However, those with cirrhosis or liver failure should avoid aspirin due to increased risk of variceal bleed[38] and acute kidney injury.[37] Aspirin will be administered for at least 2 weeks, and may be continued for up to 18 days if needed to coordinate scheduling for the participant and Visit 2 or 3 procedures.

ii. Placebo daily x 2 weeks. There are no risks of the placebo medication. Participants will be asked not to take aspirin and other nonsteroidal anti-inflammatory drugs during the study period, but are able to take Tylenol if needed. We plan to exclude subjects who take aspirin prescribed by a physician, minimizing the potential risk of withholding a participant's usual medication. Placebo will be administered for at least 2 weeks, and may be continued for up to 18 days if needed to coordinate scheduling for the participant and Visit 2 or 3 procedures.

iii. Stopping alpha-1 antitrypsin replacement therapy. Participants will be asked to stop AAT replacement therapy for 5 weeks, so that AAT levels drop to levels seen in the absence of AAT replacement. AAT replacement therapy may be withheld for up to 40 days if needed to coordinate scheduling for the participant and Visit 4 procedures. AAT replacement therapy is the standard of care for AATD in the U.S. and was FDA-approved because it was shown to restore AAT in the lung to normal levels.[39] Further evidence for its use is based mostly on observational data: an alpha-1 registry study compiling 5 years of data found a lower mortality in those on augmentation compared to those not, and a slower decline in FEV_1 in those with poor lung function

on augmentation therapy compared to those not on augmentation therapy;[40] a meta-analysis of randomized and non-randomized studies found a slower rate of FEV_1 decline in those with initial FEV_1 between 30-65% predicted who were on augmentation therapy compared to those not on therapy;[41] and a retrospective study found lower exacerbation rates and less severe exacerbations in subjects when they were on augmentation therapy compared to when they were not on augmentation therapy.[42] A few RCTs have also found a borderline reduction in the progression of CT emphysema in those on AAT augmentation compared to those not,[8-10] with one meeting statistical significance.[11] One of these RCTs evaluated exacerbations in 77 participants over 2 years, they found the overall exacerbation rate to be similar among the two groups, however a post-hoc analysis found a reduction in severe exacerbations (those requiring hospitalization) in those on augmentation therapy.[9] In summary, there is no data to suggest that short term withdrawal of AAT augmentation will lead to a clinical change or exacerbation. The participants in this study will miss approximately 4 doses of AAT augmentation over approximately 5 weeks (5.5 weeks maximum), this is 1/24 of the 2-year duration of the RCT mentioned above and there would be little change in emphysema or exacerbation rates expected to occur during this interval. However, we will mitigate the potential risk of increased exacerbations during this short withdrawal period by excluding participants who have experienced frequent exacerbations (2 or more exacerbations in the last year).

b. Study Procedures

i. Anthropometry, blood pressure and pulse oximetry. There are no direct risks or discomforts from participation in the anthropometry, blood pressure or pulse oximetry to measure oxygen saturation.

ii. Questionnaires. There is minimal risk related to questionnaires, this involves the social-psychological risk from the inadvertent disclosure of confidential medical information. We guard against this by maintaining study information identified by study number only in a filing system separate from the name and address files.

iii. Phlebotomy. The risks associated with blood collection are minimal and include the potential to feel a little pain or get a bruise at the place where the blood is drawn. It is possible, but not likely, that there may be swelling or bleeding at the site. A second blood collection (for point-of-care Creatinine in MRI) will be avoided if possible by collecting a blood sample at the time of IV placement.

iv. Urine specimen. Minimal risk.

v. Spirometry. Spirometry is non-invasive and involves minimal risks. Spirometry can induce shortness of breath, coughing or chest tightness, and may cause the participant to feel lightheaded. Albuterol will be given as part of the pulmonary function testing. Albuterol is a commercially available, FDA-approved drug. The common side effects associated with albuterol are transient mild tachycardia, tremulousness and nervousness.

vi. Six minute walking test (6MWT). The 6MWT may cause a participant to experience shortness of breath or chest tightness while doing the test. Treatment will be available if this occurs. There is a very small risk of abnormally high or low blood pressure, fainting, abnormally slow, fast or irregular heartbeat, and heart attack. We will minimize this risk by excluding higher risk participants (those with resting heart rate

below 50 or above 110 beats per minute, chest pain within the last 4 weeks, or new/worsening chest pain, shortness of breath or fainting within the last 8 weeks).

vii. CT Scan. MDCT involves exposure to low doses of x-ray radiation. The estimated effective radiation dose that an average sized participant will receive from the MDCT will be approximately 6.5 mSv, which is the radiation dose that everyone receives in about 2.1 years of background radiation in the greater New York City area.

viii. MRI Scanning. The MRI procedures do not expose participants to ionizing radiation. All subjects will be screened to exclude those with contraindications to MR, including those with pacemakers, aneurysm clips, cochlear implants or other implanted electronic devices. Subjects with a history of occupational exposure to small metallic projectiles will be further evaluated with orbital radiographs as needed. There is also a psychological risk if an individual becomes claustrophobic while inside the MRI machine. Claustrophobic episodes may be traumatic to the patient, however it resolves after removal from the scanner and usually does not have any known lasting effects. We minimize this risk by excluding subjects with a known history of severe claustrophobia. Subjects will be in direct verbal contact with the MRI operator at all times and MRI examinations will be terminated at any time upon the request of the subject. The MRI scanner areas at the sites are equipped with standard emergency equipment for a hospital-based MRI center.

ix. Gadolinium (contrast agent). There is a risk of allergic reaction after the gadolinium injection; with less than one in 300,000 chances that this will severe. Metallic taste in the mouth, tingling in the arm, nausea, or headache occurs in less than 1% (less than 1 in 100) people. Insertion of the needle may also cause minor pain, bruising and/or infection at the site. Participants with chronic renal insufficiency (estimated glomerular filtration rate [eGFR] < 45 mL/min/1.73 m2) will be excluded to eliminate the risk of nephrogenic systemic fibrosis. Gadolinium has recently been reported to persist in the brain; the significance of this finding is unknown and there is no reported harm. Persistance of a gadolinium signal in the brain has been reported to be associated with renal failure, linear gadolinium agents compared to macrocyclic agents, and cumulative dose.[43,44] We will guard against this potential harm by using a macrocyclic agent (gadoterate meglumine, Dotarem). The macrocyclic agents are not reported to persist in the brain,[45,46] and we use a very low dose so that our cumulative study dose of 0.09 mmol/kg is less than the clinical dose for 1 scan of 0.1 mmol/kg. We will also exclude those who have received gadolinium agents within the past 6 months.

x. Supplemental Oxygen During MRI. There is a risk that supplemental oxygen via non-rebreather mask (at 8 L/min, with an FiO2 of 50-60%) will lead to retention of carbon dioxide, as it reverses hypoxic pulmonary vasoconstriction. If this occurs it could cause somnolence, confusion and decreased respiratory rate. We will mitigate this risk in five ways: 1) participants with current use of supplemental oxygen, history of intubation for COPD exacerbation, history of being a "retainer" (high carbon dioxide levels in the blood), or history of becoming drowsy or confused when given too much oxygen will not receive supplemental oxygen; instead they will undergo MRI using the rate of oxygen used at home, if prescribed; 2) respiratory rate and pattern will be monitored by the MRI technician during the MRI; 3) verbal communication will be maintained with the participant while they are in the MRI scanner; 4) a physician will be present during oxygen administration for the first MRI, and for subsequent MRIs a registered nurse (certified in advanced cardiac life support) will be in the vicinity

during oxygen administration; and 5) emergency equipment will be immediately available in case the participant's respiratory rate drops. If there is any change in mental status or respiratory rate, supplemental oxygen will be discontinued immediately, and not used for the subsequent visits.

xi. Findings on Imaging. The imaging may reveal incidental findings that require further testing or interventions that may be harmful, costly or cause distress. Please note that all CT and MRI scans are read by board-certified radiologists; the former get a clinical read, the latter, a safety read. In the event of incidental findings, the PI will contact the participant and their physician, if desired by the participant. If the participant does not have a physician, we will refer then to an appropriate clinic for follow-up. At the time an incidental finding of clinical significance is reported to the PI, the PI will provide the IRB with image numbers, study subject numbers, type of scan, date of scan, description of the incidental finding of clinical significance, date of communication with the patient and outcome, if it is known.

8. Potential Benefits

a. Benefits to society
The information learned from this study may benefit the alpha-1 community, as it will increase scientific knowledge about changes in the pulmonary vasculature in lung disease related to AATD. Benefits also include that it may further our understanding of how emphysema occurs in lung disease related to alpha-1 antitrypsin deficiency, potentially leading to new treatments, and will explore intermediate endpoints for the only specific alpha-1 targeted therapy, AAT augmentation. As emphysema is also prominent in some subtypes of COPD, there would also be potential benefit to the larger population of individuals with, or at risk for, COPD.

b. Benefits to the individual
The imaging may reveal subclinical findings that may lead to interventions that are beneficial to some participants. In particular, results of the National Lung Cancer Screening Study suggest that screening current and former smokers with CT scanning reduces both lung cancer-specific and all-cause mortality

9. Data Safety and Monitoring Plan - please see separate document.

10. Privacy and Data Security

All data will be treated confidentially. Discussions of informed consent and all study-related matters will take place in a private room. Personal, identifiable information such as name, date of birth, consent form and contact information will be kept in a locked file cabinet located in the study coordinator's locked office, along with the code linking to study ID. Data analysis will occur at Columbia University only by the PI and co-investigators. The analysis datasets will consist of coded data, which can only be linked back to the participant by the code in the study coordinator's office, will be kept on our institution's clinical trials server which is password protected, encrypted and accessible only to those involved in the study, and on endpoint devices which will be encrypted and password protected. Questionnaires and study records will be identified by study ID only.

Data that will be transmitted include:

- Spirometry data - to the Spirometry Reading Center, John Hankinson
- CT images - to University of Iowa, the MESA COPD CT Reading Center
- Pulmonary perfusion on MRI - to Hannover Medical School, Hannover, Germany

Transmitted data will always be secure. Data transmitted to and from the Spirometry Reading Center will be identified only by study ID, acrostic and date of test; it will not be personally identifiable. Data will be sent to Iowa and Hannover using the secure data sharing portal DatAnywhere (file size up to 2 GB) or a SFTP (files over 2 GB). Although these are secure means of sharing data, we will safeguard the data by using only acrostic and study ID to identify study subjects and the data will not include any of the 18 HIPAA identifiers. Data will not be transmitted to these sites until IRB/ethics approval is obtained.

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