Title of the Study: CCRC: Randomized trial of L-arginine in severe asthma patients grouped by exhaled nitric oxide levels (NCT #01841281)

Principal Investigator: Nicholas Kenyon, MD, MAS

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PROTOCOL

PURPOSE AND PROCEDURES:

1. Describe the study format (pilot, phase I, phase II, or III) and whether it is single or multi-center; industry-sponsored or investigator initiated; and the funding source.

This is an investigator-initiated, early phase II study funded by NHLBI.

2. Describe the specific aims of the study, research methods and procedures.

We hypothesize that a subset of adult severe asthma patients will respond to supplemental L-arginine and derive clinical benefit from the addition of this therapy to standard-of-care medications. We hypothesize that these patients will have lower exhaled NO concentrations (<20 ppb) than -non-responders. The aim is:

1. To test the hypothesis that adult severe asthma subjects with exhaled breath NO concentrations <20 ppb will have fewer American Thoracic Society (ATS)-defined asthma exacerbations over 3 months when treated with L-arginine compared to subjects with FeNO > 25.

The major impact of this study will be to identify the adult severe asthma cohort that will benefit from supplemental L-arginine therapy to define the underlying mechanisms of arginine benefit in asthma. This follows our initial 20 subject trial of L-arginine in asthma subjects (Kenyon et al., Pharmaceuticals 2011) that was designed to determine how L-arginine was metabolized (by testing serum markers) and whether certain participants had clinical benefit.

To do this, we will recruit a total of 50 ATS-defined asthmatic subjects with $\geq$2 asthma exacerbations in past two months and enroll them in a randomized, blinded, placebo-controlled, cross-over designed trial of L-arginine and placebo. We will compare 25 subjects with -low‖ FeNO <20 with 25 subjects that have -high‖ FeNO > 25 ppb.

Study Design:

Clinical Trial Study Design-- Aim #1*:* The proposed study design is a randomized, double-blind, placebo-controlled, cross-over Phase II trial. We will perform a 30 week (12 wk. treatment A – 4 to 6 wk. washout – 12 wk. treatment B) blinded, cross-over design clinical trial, during which we treat 25 -high FeNO‖ and 25 -low FeNO‖ severe asthmatics with L-arginine at a dose of 0.05 g/kg IBW (ideal body weight) twice a day (e.g. 6-8 g/day) and placebo. The study will be performed at the CTSC Clinical Research Center. All patients will be on standard controller therapy including appropriate doses of inhaled corticosteroids and long-acting bronchodilators. The randomization process and disbursement of medication is done at the UC Davis Investigational Drug Service.

2. If applicable, address how the study will involve the use of drugs, devices, biologics, or radioactive materials (both FDA approved or investigational).*

L-arginine (Jarrow Formulas; Used in prior UC Davis IRB-approved Pilot study; clinicaltrials.gov # NCT00280683) : Commercially available amino acid supplement available in numerous formulations. We will purchase L-arginine tablets containing 1 g of elemental L-arginine (1204 mg of L-arginine HCL) developed by Jarrow Formulas in Los Angeles. They are easy to swallow despite its large size, and there is no taste. In our last L-arginine trial, Jarrow Formulas agreed to make placebo for us which will look identical to the arginine tabs. (Jarrow Formulas: 1824 S. Robertson Blvd Los Angeles, CA 90035)

50 study subjects will take L-arginine orally, at 0.05 g/kg twice a day (e.g. 6-10 g/day). The 50 subjects will take a placebo capsule twice a day, to be formulated by the UC Davis Medical Center pharmacy. Subjects will be asked to discontinue use of any nutritional supplements prior to the start of the study. L-arginine is a safe,

naturally occurring amino acid that is available as a supplement at grocery stores and health food stores without a prescription. Since it is a readily available nutritional supplement, it is not regulated by the Food and Drug Administration and is readily available for public consumption.

3. Address if therapeutically removed tissue will be collected, what types, and for what purposes.

Blood samples will be drawn (15-20 mls/1-2tbsps) at each study visit to analyze for antibodies to standard environmental and occupational allergens.

4. Specify the nature, frequency and duration of tests, if any.

Screening visit:

After signing the informed consent, severe asthma subjects will be enrolled for screening sample collections—exhaled breath samples for exhaled nitric oxide measurements. All subjects will have their medical records and past studies for characterization of their severe asthma as described above. All subjects will breathe into collection devices to collect both exhaled gases.

Study visits 1-4

After reviewing the signed informed consent and answering questions regarding the protocol, severe asthma subjects will be enrolled for sample collections—blood and breath. All subjects will breathe into collection devices to collect both exhaled gas and exhaled breath condensate. The EBC collection device is approved for the measurement of pH and is supplied by a commercial supplier. The subjects will not receive any study medications, biologics, or radioactive materials at this time. The study drug and placebo will be mailed to the subject by the Investigational Drug Service, housed in the UC Davis hospital. This is recharge based service. The visit 1-4 procedures are:

- *Exhaled breath nitric oxide collection:* Exhaled breath samples will be collected by the investigators from each participant at the time of the study visit. Samples will be collected in Mylar bags using the off-line exhaled breath technique, as described in the 2005 ATS/ERS standardized measurement recommendations document. Two breath samples will be collected in individual bags at a flow rate of 250 L/sec, and measurements will be recorded within 12 hours. A pressure gauge at the collection apparatus (standard on the Sievers NO analyzer offline collection apparatus) will allow for the participant and interviewer to control exhaled breath flow rates. All samples from Sacramento participants will be analyzed on a Sievers 280i NO Analyzer (Boulder, CO) in Dr. Kenyon's laboratory.

- *Exhaled Breath Condensate (EBC) Collection:* Collections of EBC from each participant will occur immediately after collection of the exhaled breath samples. Participants will breathe into a chilled collection tube for 10 minutes as recommended in the 2005 ATS/ERS statement. Collection tubes will be purchased from Respiratory Research (Charlottesville, VA), a commercial entity that has received FDA approval for measuring EBC pH in subjects with cough. Immediately after collection, the tubes will be frozen on dry ice for transport to Dr. Davis' lab. Total time of sample collection should be 10 minutes. Breath samples will undergo chemical analysis at Dr. Davis' laboratory. Volatiles in breath samples will be concentrated using Solid Phase Microextraction (SPME). Analyte compounds will then be loaded on a chromatographic column for a nominal pre-separation and will be detected with a differential mobility spectrometer, according to protocols previously developed in Dr. Davis' lab.

- *Blood draw* 16 cc of blood will be drawn at each visit. Serum will be collected for cytokine profiling and NOx measurements and L-arginine metabolites. Whole blood will be processed for genotyping.

- *Office Spirometry will* be performed with portable office spirometer (Spriolab II).

- *Physical examination and symptom questionnaire*

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Brief Study outline: 1 screening visit and 4 study visits

Screening Visit	– Explanation of protocol – Informed consent – History and physical examination -- Two exhaled NO measurements (to determine if subjects > or < 25 ppb)
Study Day 1	– History and physical examination (brief) to verify stable clinical status – Blood collection #1 – Spirometry #1 – Exhaled NO measurement #1 – Sputum collection #1
	– Respiratory symptom questionnaire #1 – Start symptom and peak flow diary – Start oral L-arginine or placebo
Study Day 80	– Updated clinical history – Blood collection #2 – Spirometry #2 – Exhaled NO measurement #2 – Sputum collection #2 – Respiratory symptom questionnaire #2
Study Day 110	– Updated clinical history – Blood collection #3 – Spirometry #3 – Exhaled NO measurement #3 – Sputum collection #3 – Respiratory symptom questionnaire #3
Study Day 190	– History and full physical examination – Blood collection #4 – Spirometry #4 – Exhaled NO measurement #4 – Sputum collection #4 – Respiratory symptom questionnaire #4 – Stop oral L-arginine or placebo

5. If blood samples will be collected, identify in what manner:

   Xx venipuncture_____venous catheter ___arterial puncture ___arterial catheter ___cutaneous

Blood sample (15-20 mls/1-2 tbsps.) will be drawn at each study visit by PI and Co-Investigators.

6. Any additional procedures (noninvasive) involved in this study activity must be described.

No other procedures

7. If the study involves incomplete disclosure, provide the rationale.

Not applicable

8. If this activity will be utilizing existing data, specify the source and how the data will be retrieved, reviewed, coded and stored.

Not applicable

9. Address the location and duration of the study.

The expected duration of the study is 5 years. Accrual to the study is expected to be completed in 4 years. For each subject, the duration of the study will be 30-32 weeks.

Dr. Kenyon Laboratory: Dr. Kenyon presently shares an open 1050 ft2 lab with Dr. R. Wu, Dr. J. Last, and Dr. R. Harper in the Genomics and Biomedical Sciences Facility (GBSF) building. The room has 22 benches and is designed for biochemical and molecular biology work as well as *in vivo* mouse work.

CCRC: 10 highly skilled RNs who provide 24/7 care to subjects enrolled in clinical research studies, whether they be admitted to the CCRC. The RNs are specially trained in Human Subjects Protection, Good Clinical Practices, and Research Protocol Adherence; all are Chemotherapy, Conscious Sedation, and ACLS certified. We will apply for the Nurse Practitioner who provides expanded services to investigators (i.e., clinical procedures, exams)

10. Clarify how you plan to monitor data to ensure subject safety (i.e., labs monitored for abnormalities, questionnaires monitored for suicidality).

We will have a Data Safety Monitoring Board (below) to review subject safety data, including bronchoscopy visits. We will not be monitoring blood tests. The PI and Co-Investigators will review study exhaled NO values and spirometry data to ensure subjects are not experiencing an asthma flare. Subjects will be seen by asthma care experts (PI, Co-Is) if this is the case and UCAN subjects will be referred to UCAN.

11. Address whether you have the appropriate resources (study personnel and facilities) to conduct this study.

We have ample facilities and personnel to conduct this study. All study visits will take place in the UC Davis clinics.

12. Describe the role of each key member of your study personnel

SUBJECT SELECTION:

1. Identify the subject population.

We plan to enroll subjects with Severe Asthma (*Severe Asthma—American Thoracic Society Statement (51)).* Subjects will be recruited primarily from the PI's subspecialty UCAN™ asthma clinic. Severe asthma subjects must have at least 1 major and 2 minor criteria. Major criteria are 1) Requirement for treatment with high-dose ICS (>800mcg fluticasone or equivalent) and 2) Treatment with oral steroids >50% of year. Minor criteria are 1) Requirement for additional daily treatment with other controller medication (LABA, theophylline, LTRA, e.g.), 2) Asthma symptoms requiring SABA on daily basis, 3) Persistent airway obstruction (FEV1<80%, PEFR>20%), 3) 1 or more urgent care visits/year, 4) 3 or more oral steroid bursts/year, 5) Near-fatal asthma event in past.

2. Address how subjects will be recruited: XXX direct person to person solicitation,____by telephone,____letter, ___advertisement,____press release, notices,____other. Provide the text.

Study locations and Subject Characteristics:
The Sacramento community has a large degree of ethnic diversity; approximately 10% are of African American ancestry, 10% Asian and 20% Hispanic. This is reflected in our UC Davis Pulmonary clinics and our Severe Asthma/ UCAN™ clinic. Our community has always been very responsive to our recruitments. The study locations will be the existing UC Davis Pulmonary clinics that house the UCAN clinics, the UC Davis CTSC Clinical Research Center that is also located in the VA Mather Hospital, The UCAN clinic is affiliated with the University of California, Davis and is one of two specialized, chronic disease management clinics in the university. Two respiratory therapists (1 Certified Clinical Research Coordinator) have 100% appointments in this clinic to assist in the treatment of referred patients with severe asthma.

3. State from where subjects will be recruited, when and how many.

Study locations and Subject Characteristics:

The study locations will be the existing UC Davis Pulmonary clinics that house the UCAN clinics, the UC Davis CTSC Clinical Research Center that is also located in the VA Mather Hospital, the locations are: UC Davis Pulmonary clinics (including the UC Davis Asthma Network clinic) at 2825 J. Street, Suite 400, Sacramento, CA. One primary source of patients will be the UC Davis Asthma Network clinics (UCAN), where Dr. Kenyon serves as Co-Director. Dr. Kenyon and colleagues see many subjects with diagnosed severe asthma and many of these subjects will fit the criteria for enrollment in this study. We maintain a database of over 700 subjects treated by us in the past 10 years. The UCAN asthma clinic saw approximately 720 referred patients from 1999-2007. 77% of the referrals were women with a mean age of 46.3±15.3 (mean± SD) years. (Kivler et al JACI 2002, 109:S314, Kivler et al. AJRCCM 2003, 167:A209).

4. Specify the age of the research subjects.

Asthmatic patients aged 18-80 will be enrolled.

5. List all criteria for including and excluding subjects.

Inclusion and Exclusion Criteria: Inclusion a) Adult patients >18 years or older with ATS definition of severe asthma, b) History of recent exacerbations ≥ 2 in past 2 months) or Asthma Control Test score < 20/25. Subjects will meet this definition based on the use of high dose ICS and LABA use (Table 3) plus an even distribution among minor criteria 2-5. E*xclusion:* a) Less than 18 years of age; b) baseline FEV1 <30% predicted, c) pregnant or nursing women, e) current smokers or subject with >15 pack year history, f) any history of intolerance of, or reaction to, L-arginine.

Controller Medications for Study: Upon enrollment, all subjects will be treated with high dose ICS and LABAs throughout the 8-month period. The overwhelming majority of the UCAN™ patients are on one of these two ICS. In addition, all subjects will be on scheduled LABAs, and will be aware of the FDA Black Box warning on these medications. Additional controller medication including leukotriene inhibitors will not be stopped, and it is expected that the use of these other medications will be roughly equal between the two groups.

6. If women and minorities are excluded, provide rationale for such exclusion.

Women and minorities will not be excluded. Many enrolled subjects will be women as severe asthma in adults is approximately 60-70% women.

SPECIAL/VULNERABLE POPULATON (if applicable)*:*

Surrogate consent for participation in a research study should be employed only to the extent that it is consistent with the intent of the Common Rule (45 CFR 46, Subpart A) and all other federal and state laws and regulations pertaining to protecting human subjects participating in research. Carefully review the IRB Policy on *Surrogate Consent for Research* for compliance with all applicable laws, regulations, and conditions of this policy. Investigators are reminded that use of surrogate consent shall apply on a case-by-case basis within the protocol.

1. Identify the vulnerable population:____children,____mentally handicapped,____pregnant women,____fetuses, ___prisoners,____cognitive impairment,____life-threatening disease, or____social or____economically disadvantaged. Address what additional safeguards you will put into place to protect the rights and welfare of this population.

No vulnerable populations of workers are anticipated.

2. If you are seeking IRB approval for use of surrogate consent, justify the appropriateness of such use and describe your specific plan for the assessment of the decision-making capacity of the subject(s).

No surrogate consents.

RISKS:

1. Address whether there is a possibility of physical, psychological, social or legal injury from participation in this study and assess the likelihood and seriousness of those risks.

There is a very low probability of physical injury from study participation. The potential risks of each procedure are described in detail below. There are no anticipated psychological, social, or legal risks involved in this study. All procedures used have been used for many years, without identified excessive risks.

L-arginine: Risks of ingesting L-arginine are minimal. There is a remote possibility of an allergic reaction to the supplement. It may cause abdominal cramping and bloating. L-arginine is a naturally occurring amino acid which is available over the counter at health food stores and in snack bars. Oral supplementation with 6-21 g per day has been studied and is safe and well tolerated (2). Recent clinical trials have used oral L-arginine in the same dosage as we plan to use (0.1 g/kg), without any untoward effects (3).

Venipuncture: Risks from phlebotomy include pain, possible bruising or bleeding at the puncture site, and infection. It may rarely cause fainting.

Urine Test: No known risks

Spirometry: No known risks. There is a potential for breathlessness, fatigue, or lightheadedness.

Exhaled NO Measurement: No known risks. It may cause transient breathlessness, fatigue, or lightheadedness.

2. If the methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

This is discussed in preceding two paragraphs.

3. Identify your plan for protecting subject privacy and confidentiality.

For this study, the material and data will be collected specifically for the purposes of the research project. Identifying information is kept by the hospital supplying the material but this is not available to the research team apart from Dr. Kenyon's team. Full clinical and physiological characterization is undertaken, and the information recorded. Study subjects are coded and not identifiable to the non-clinical research team. The link to the original subjects will be kept by the PI, and research coordinator involved in recruitment. For subjects in this study the following data will be collected from their records: Age, sex, ethnicity, clinical history, asthma diary card, spirometry, PC_{20} methacholine, CT scan reports, 6-minute walk test results, skin tests, total IgE. The PI (Kenyon), Co-I, clinical fellows, and the research RRTs will have access to individually identifiable private information about the human subjects.

4. Explain your plan for reporting adverse and serious adverse events to the IRB.

Medical information obtained during this study will be kept confidential and accessible only to the principal investigators and assistants unless the law requires disclosure. The information will be used in a manner that does not publicly disclose identity. The greatest risk is the release of information from health records. We will protect health records so that the name, address, and phone number will be kept private.
Absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena. The name, address, phone number, and any other identifying information will be taken off anything associated with the specimens before it is given to the researcher. This would make it very difficult for any research results to be linked to the subject. Also, people outside the research process will not have access to results about any one person, which will help to protect subject privacy. Subjects' clinical records are stored on the UC Davis Electronic Medical Record system and backed up on the university Citrix server. A written clinical record form containing unidentifiable data is kept in a locked cabinet in Dr. Kenyon's research office. A recruitment log linking patient to study number is kept in this locked research office. A database will be developed by the CTSC informatics staff and backed up on the password protected Citrix server.

Description of how AEs related to tests will be are graded
AEs will be graded using a standard grading scale, if the need arises: 0=none, 1=mild, 2=moderate, 3=severe, 4=life-threatening. The expectation of the AE will be graded as: A=unexpected. The attribution scale is 1=definitely not related, 2=possibly related, 3=probably related, 4=definitely related to the study drug and/or intervention.

Plan for AE Reporting

Research subjects are encouraged to report any adverse effects to the PI, Co-PI or study personnel, who will suggest ways to minimize the problems. The PI and C0-PI will review all adverse events. The review may lead to the recommendation that the subject be withdrawn from the study. When appropriate, the subject will be referred to their primary physician. In accordance with NIH regulations, reportable adverse effects arising from any of the procedures will be submitted promptly to the UC Davis Office of Human Research Protection and the PI will be notified of any subsequent reporting by the UCD OHRP to the NIH. Although they are not anticipated to occur, Unanticipated (non-serious) or Serious Adverse Events will be reported to the IRB within 5 working days of awareness of the event. Copies of reports should be received by the IRB office no later than 15 calendar days after the event. In addition, adverse events in all categories will be included in the PIs annual reports to the IRB as required by the federal regulations. All subjects will be seen by the PI and co-investigators. Research

subjects are followed by their regular physicians, so they will continue to receive usual care. Dr. Kenyon and co-investigators will review safety parameters prior to enrollment. The PI reviews and reports on the progress of the study on a semi-annual basis. The PI will monitor study progress, outcomes and participant safety, and may make recommendations on changes to the study protocol. The PI will review safety on an ongoing basis. All reportable adverse events will be compiled, and reported in summary form, on an annual basis to the IRB, and at the conclusion of the study. Unanticipated adverse events and serious adverse events will be reported to the IRB. The protocol is reviewed annually by the IRB in conjunction with adverse event information.

ClinicalTrials.gov Requirements: Aims 1 will be reported on the clinicaltrials.gov website. We will register with ClinicalTrials.gov within 21 days after enrollment of the first patient. Summary result information will be reported within one year of completion of the trial.

5. If the study involves the use of placebo, justify why this is appropriate.

While this study includes a placebo, it is an -add-on‖ placebo. Subjects will be on their standard asthma medication. This is part of the inclusion criteria for the study. This will be monitored at study visits.

BENEFITS*:*

1. Address if there is a benefit to individual subjects or to the group or class.

There is no benefit to the participant in this study. If we find that L-arginine improves asthma exacerbation rates in severe asthma patients, this will be a significant advance. Also, if we find that the exhaled breath condensate (EBC) method confirms established NO profiles for asthmatics, we can again use the EBC method for further research and this non-invasive method would be an advance. Overall, we must state that there is no known direct benefit to the current study population.

2. Address if there is no direct benefit to the subject.

There is no benefit to the subjects/participants in the study.

RISK-BENEFIT RATIO:

1. Address whether the risks to subjects are reasonable in relation to the benefits.

This study is a minimal risk for Aim I. No serious adverse events are anticipated. The only interventions will be a physical exam, questionnaire, routine spirometry (in healthy controls), blood draw, and exhaled gas collection. There is unlikely to be much direct benefit to individual participants in the short term, although it is apparent that some patients recruited from the community need modification of their treatment which can be advised to their general practitioner. It is hoped that the results from the study will lead to the development of better treatments for severe asthma in the future, and so in the long term, all participants with severe asthma may benefit.

COSTS/COMPENSATION TO SUBJECTS*:*

1. If the study involves the possibility of added expenses to the subject or to a third party, such as an insurer (e.g., longer hospitalization, extra laboratory tests, travel) address the magnitude of those expenses and how this is justified.

The study will not include planned added expenses to the participant.

2. Describe the amount and type of compensation that will be paid to subjects and how that compensation will be staged/pro-rated.

Aim #1 Clinical Trial: Subjects will be compensated $40/study visit or $160/total at time of completion of L-arginine trial. Reimbursement is for time and travel.

DISCLOSURE OF PERSONAL AND FINANCIAL INTEREST:

1. Disclose any personal and financial interest in the research as well as the extent of personal and financial interest in the sponsor.

The investigators have no financial interest in the research. It is funded by NIH.

INVESTIGATIONAL DRUG (if applicable):

L-arginine supplementation: L-arginine, an essential amino acid, is a readily available amino acid supplement that already has clinical uses. An L-arginine trial should be considered minimal risk since toxicities are well documented in humans. To date, the most promising therapeutic use for arginine is in sickle cell disease patients with acute chest syndrome. A dose of 0.1g/kg/day of L-arginine in divided doses is planned for the proposed study. Oral supplementation with 6-21 g per day has been studied and is safe (42), and an important clinical trial in pulmonary hypertension adopted the same dosage (0.1 g/kg), without any untoward effects (40).

High level doses of L-arginine may lead to excessively high levels of asymmetric dimethyl arginine (ADMA), a NOS inhibitor, therefore, in our proposed study we choose an L-arginine dose in the low -therapeutic‖ range.

L-arginine is classified as a supplement and is subject to the Self-determination of IND exemption based on 21 CFR 312.2. Specifically,

1. The investigational drug (L-arginine) is lawfully marketed in the United States
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product. (Instead, this would lead to publication and a multi-center Phase III study).
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product
4. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

1. Provide available toxicity data on the drug(s).

Oral supplementation with 6-21 g per day has been studied and is safe (42), and an important clinical trial in pulmonary hypertension adopted the same dosage (0.1 g/kg), without any untoward effects (40). High level doses of L-arginine may lead to excessively high levels of asymmetric dimethyl arginine (ADMA), a NOS inhibitor, therefore, in our pilot study and in the proposed study we choose an L-arginine dose in the low -therapeutic‖ range.

2. Describe previous studies on humans.

We have published our finding of L-arginine pilot study at this dose in asthmatics in 2011 (Kenyon et al. Pharmaceuticals 2011; full reference immediately below). It is safe with minimal side effects. Furthermore, there

is a 15-year history of human studies with L-arginine.

3. Address whether this study will have a Data Safety Monitoring Board in place.

Data and Safety Monitoring Plan (DSMB)
As this study will be considered moderate risk because of the research bronchoscopy associated with a Phase II clinical trial, a biannual review of all study visits clinical data, bronchoscopy reports, and any adverse events will be performed by an independent Data Safety Monitoring Board. As required by NIH supported clinical centers, the UC Davis CTSC has the mechanism to establish and maintains DSMBs for diverse clinical trials throughout the institution. We will install a DSMB with expertise in clinical trials, regulatory support, interventional bronchoscopy and statistics. This board will be submitted to the UC Davis IRB for approval. Names of individuals who will serve on the data safety monitoring board:

Statistical Analysis Methods

Study design and participants

We used a randomized, triple-blinded, placebo-controlled, cross-over design trial to evaluate the efficacy of oral L-arginine as an add-on therapy in adults with severe asthma. We performed a two-group study stratifying participants by fractional exhaled nitric oxide (FeNO) levels, ”low” FeNO <20 ppb versus “high” FeNO > 25 ppb. The patients were recruited from the University of California-Davis Asthma Network (UCAN™) clinic.

Outcomes

The primary endpoint was the total number of “moderate” exacerbations during the 12-week interventional period, as defined by recent the ATS/ERS 2009 statement *[2]*. A moderate asthma exacerbation is defined as any of the following: 1) a drop in am PEF>30% from baseline on two consecutive days, 2) need for initiation of oral steroids or am increased dose of inhale corticoid steroid on any two consecutive days, 3) doubling of short-acting β-agonist use (e.g. number of puffs of albuterol)/ day for 2 consecutive days [3-5]. The secondary clinical endpoints were recorded at each of the six study visits, these included: 1) asthma control test score, 2) FEV1 (liters), 3) FVC (liters), 4) FEV1/FVC (%), and 5) Weight. The secondary biological endpoints including FeNO and untargeted metabolites.

Metabolites measurement

Longitudinal plasma metabolite levels were measured by hydrophilic interaction liquid chromatography (HILIC) in both positive and negative modes and gas chromatography (GC) mode. The peak intensity was measured for the compounds and were annotated to specific known metabolites. Relative standard deviation (RSD) for each compound found in more than one mode in quality control samples were calculated and the intensities of compound with lowest RSD was chosen for the downstream analysis.

Clinical trial analysis

For the sample size calculation, we used a conservative power estimate based on t-test analysis of 2x2 cross-over design [6]. For a sample size of 50 people, we would have 95% power to detect a 0.6 standard deviation change in the mean lung function at alpha = 0.025 and accounting for 10% attrition rate. Improvements of this magnitude are consistent with those reported in recent crossover trials in asthma with either FEV1 [7], symptoms scores [8], or AM PEFR [9] as outcomes. Statistical analyses for the clinical trials were performed using SAS University Edition (SAS Institute Inc., Cary, NC, USA.) and R (public access). Data were analyzed on an intention-to-treat basis and the significant level was defined by p value less than 0.05. A generalized linear mixed model was used for analysis accounting for correlated repeated measurements which enable us to account for expected missing data points [10]. This model considered differences in baseline levels of function, by allowing for person-specific random effects (random intercepts). The distribution of exacerbation events was assumed to be a Poisson distribution. For

the primary hypothesis, we included an indicator in the model that allows for the subjects to be on active treatment. We first examined the carryover and period effect then tested for the treatment effect and examined the interaction term between treatment and FeNO. For secondary clinical outcomes, we performed the same analysis except we used linear mixed effect for the continues endpoints.

Identify baseline metabolites as predictors for treatment response

We further defined the treatment response by reducing exacerbation events by 33% during L-arginine treatment. The individual event reduction was calculated by (events during treatment period minus events during placebo period)/events during placebo period. Subjects with zero event at baseline or did not complete both treatment and placebo phase were excluded. The baseline metabolomic profiling (first visit) was used for the downstream analysis. Partial Lease Square-Discriminant analysis (PLS-DA) and hierarchical cluster analysis (using Euclidean as similarity measure and Ward's linkage as clustering algorithm) were performed using MetaboAnalyst 4.0 [11]. to identify the predictive metabolites. Clinical characteristics were compared between treatment response versus non-response groups using t-test (continuous characteristics) or fisher's exact test (categorical characteristics with small sample sizes).

Longitudinal metabolomic profiling analysis

A linear mixed model was used to test the association between the longitudinal metabolite intensities and the clinical outcomes including FeNO at each visit. Metabolites pattern change between two biological conditions including 1) treatment vs. placebo, 2) high vs. low FeNO groups during treatment period, 3) treatment response vs. non-response groups during treatment period were tested using the method of multivariate empirical Bayes (MEBA) time-series analysis [12] built in the MetaboAnalyst 4.0. Hotelling-T2 statistics were used to rank the metabolites with different temporal profiles between two biological conditions under the study.

References

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