Protocol Title: **Long Term Nitric Oxide Bioavailability on Vascular Health in Chronic Obstructive Pulmonary Disease (COPD-LT)**

Principal Investigator: *Ryan A. Harris, PhD, CES, FACSM*

1. Objectives

Researchers have found a link between chronic obstructive pulmonary disease (COPD) and heart disease; however, a link is all they have found. In a previously funded grant, using a double blind, randomized experimental design, we explored the effect of an acute dose of Kuvan or an antioxidant cocktail (1000mg of vitamin C, 600IU of vitamin E, and 600mg alpha-lipoic acid) on vascular health in patients with COPD. Consequently, we found in separate experiments, that a single dose of both antioxidants and Kuvan transiently improves vascular health in patients with COPD. The current project is an attempt to expand on our previous findings and explore the effects of sub-chronic use of antioxidants and Kuvan on sustaining the improvements in vascular health in COPD.

2. Background

Chronic obstructive pulmonary disease (COPD) affects up to 14 million people and is among the top five leading causes of death worldwide. Although COPD is a disease of the lungs, recent evidence indicates that COPD is associated with multiple systemic consequences including vascular endothelial dysfunction. Recently, it has been suggested that more patients with COPD die from cardiovascular disease and coronary heart disease than of direct pulmonary complications. Examination of the mechanisms that contribute to a reduction nitric oxide (NO) bioavailability resulting in vascular endothelial dysfunction in patients with COPD are important as endothelial dysfunction has been indicated to be an independent predictor of future atherosclerotic cardiovascular disease and events.

NO Bioavailability and Vascular Function. Evidence indicates that a reduction in concentrations of NO, a hallmark of proatherogenic states, facilitates atherosclerosis. The flow-mediated dilation (FMD) technique, which represents a fundamental assay of NO bioavailability, has provided means to evaluate vascular endothelial function noninvasively and has been shown to correlate with invasive testing of coronary artery endothelial function.

Vascular Endothelial Function in COPD. The NO dependent mechanism for vasodilation is considered the most vital endothelial mediated function because of its antiatherogenic property. Moreover, endothelial dysfunction has been proposed as the earliest identifiable event in the process of atherosclerosis. In fact, recent evidence indicates that endothelial dysfunction is associated with an increased risk for CVD in several different clinical populations and can even be considered a predictor of COPD. To our knowledge, there is limited data describing in vivo vascular endothelial function in patients with COPD. With respective limitations, each study reports an impaired FMD in patients with COPD which appears to be associated with the degree of airway obstruction; however, examining the role of NO bioavailability in contributing to endothelial dysfunction in patients with COPD and the molecular mechanisms involved have yet to be determined.

Tetrahydrobiopterin (BH4) in COPD. Tetrahydrobiopterin(BH4) is an essential cofactor required for NO production through the "coupling" with nitric oxide synthase (NOS). Accordingly, the uncoupling eNOS has been indicated to contribute to vascular oxidative stress and endothelial dysfunction in animal models. In fact, an improvement in FMD has been observed in current smokers following BH4 supplementation. Additionally, recent evidence indicates the essential role of BH4 in regulating pulmonary vascular homeostasis. Moreover, a recent review addressed the role of BH4 in regulating vascular endothelial function and potential therapeutic implications of BH4 in various clinical populations, which included chronic smokers; however, no studies have investigated the contributing role of BH4 in "recoupling" eNOS and increasing NO bioavailability in patients with COPD. The proposed novel investigation aims to identify if decreased BH4 levels are contributing to the reduction in NO bioavailability and vascular endothelial dysfunction in COPD. If so, these findings may provide support for the use of BH4 supplementation to provide a salutary effect on vascular endothelial function in COPD.

Oxidative Stress and COPD. A shift in the antioxidant/free radical balance in favour of free radicals is termed oxidative stress. Superoxide anion (O_2^-) and other free radicals are highly energized molecular species, structurally distinct in that they contain one or more unpaired electrons in their atomic orbital [33]. Although low concentrations of free radicals appear to have important roles in cell signaling, an excess of free radicals can cause a wide spectrum of cellular damage, including lipid peroxidation, alteration of

intracellular redox state, inactivation of enzymes, and damage to DNA. Unsurprisingly, elevated oxidative stress has been observed in smokers[34-36] and in COPD[24, 37]. Since the initial observation that O_2^- and other free radicals inactivate nitric oxide[38], which plays a fundamental role in regulating endothelial function, it seems logical that the elevated oxidative stress observed in COPD would contribute to endothelial dysfunction. One method to reduce oxidative stress is through a reduction in superoxide anion (O_2^-), which would limit the degradation of NO, thus increasing its bioavailability. Antioxidant supplementation has been utilized as a means to reduce oxygen free radicals and increase NO bioavailability in several clinical populations[39-41].

3. Inclusion and Exclusion Criteria

List the inclusion/exclusion criteria:

- **Inclusion**
  - 24 patients with medically diagnosed COPD
  - 24 apparently healthy controls
- **Exclusion**
  - FEV1/FVC >0.7 (normal lung function in patients only)
  - Clinical diagnosis of heart disease or diabetes
  - Vasoactive medications (i.e. nitrates, beta-blockers, Viagra etc.)
  - uncontrolled high blood pressure
  - high blood pressure in your lungs
  - thyroid problems
  - Fluid in the lungs
  - Sleep apnea
  - Anemia
  - Raynaud's Phenomenon
  - GI bleeding
  - Gangrene of the digits
  - History of low platelets or coagulopathies
  - Phenylketonuria (PKU)
  - Pregnant
  - Lactation
  - Any allergy to Kuvan

4. Number of Subjects/Records/Samples Collected

A maximum of 48 volunteers will be needed for this entire research study. For patients, there are 2 protocols which each consist of 6 visits each. We would like to recruit 12 patients with COPD that will be asked to complete the Kuvan protocol and an additional 12 patients that will be asked to complete the Antioxidant protocol. Apparently healthy controls (n=24) will be asked to only complete visit one.

5. Recruitment Methods

Patients with COPD will be recruited from the Pulmonary and Critical care medicine unit, family medicine, internal medicine, and local outreach clinics associated with Georgia Regents University. We will also use I2B2 to pull up inclusion and or exclusion criteria for potential participants as well as power chart to gather contact information; for this purpose a Waiver of Consent, Waiver of HIPAA Authorization along with the telephone screening forms that will be used to contact potential participants will be approved by the designated IRB before patient recruitment takes place. The main source of control subject recruitment will be the parents of the subjects enrolled in other studies here at the Georgia Prevention Institute (GPI). The GPI sees approximately 10,000 subject visits per year (both children and adults).

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6. Procedures Involved

Body Composition. The subjects body fat, bone, and lean tissue will be measured at the beginning of the study with a low level x-ray machine called dual-energy x-ray absorptiometry (DXA) for short. These tests give the subjects body about 4 extra days worth of the type of background radiation that everyone receives every year from the environment. The subject will be asked to lie still on a table with their eyes closed while a mechanical arm moves above their body measuring their body composition. Body Mass Index (BMI) will also be calculated using measurements of the subject's height and weight. Waist to Hip Ratio (WHR) will be measured using a vinyl tape measuring around their waist and hips.

Pulmonary Function Test. All volunteers will perform a lung function test to measure how well their lungs work. This involves taking a deep breath and then blowing into a tube as hard as they can, until they cannot blow any further. We will also measure how well the air crosses through the lungs by having each participant breathe a mixture of gasses (10% He, 21% O2, 0.3% CO, and balance N2) and having them hold their breath for 10 seconds. Following the baseline lung function test, it is possible that the volunteer will be excluded from the study if their lung function does not meet the criteria above.

Blood Samples. Blood samples will be taken from a vein in your arm by a certified technician before and after each protocol. For each experimental visit, a total of about 40 ml of blood (~ 2-3 tablespoons) will be taken. This amount is less than 1/10 of the amount that is withdrawn during a normal blood donation (500 ml or 15 fluid ounces). We will use this blood to test for blood fat (cholesterol) and the concentration of certain hormones, and to determine the level of harmful chemicals found in the blood. You may only drink water after dinner the night before this test.

Muscle Function Test. For this test, the subject will lie on their back. We will place a cuff around their upper thigh and place an oxygen measuring device and four electrodes on the middle of their thigh muscle. These electrodes will be used to stimulate their thigh using a commercial electrical stimulation unit. The stimulation will be set so that the muscles contract strongly, but we will adjust the stimulation to a level that the subject can tolerate. We will perform two measurement trials, each consisting of a short (15 second) electrical stimulation period followed by a series of 15 short cuff inflations to block the blood flow to the leg. There will be a 2-5 minute rest period between trials.

Arterial Stiffness Evaluation (PWV). A device will be used to measure how fast the participant's blood travels. This test is called pulse wave velocity or PWV. How fast the subject's blood travels is also a measure of how stiff their arteries are. Stiff blood vessels/arteries are linked to heart disease. To start the

test, participants will be asked to lie still in a resting position for about 20 minutes. The research assistant will place white patches called electrode sensors on their body. A pen-like device is gently applied on the carotid artery (neck) and then the femoral artery (groin) and the radial artery (wrist) to record how fast their blood flows between each of the points.

Arterial Function (FMD). This test looks at how artery expands with changes in blood flow and is used to characterize the health of the subject's arteries. The subject will lie on their back for 20 minutes to rest. We will then measure the function of the artery by scanning an artery in the arm. Following baseline measurements, a blood pressure cuff placed around the subjects forearm will be inflated to 250 mm Hg to stop blood flow for 5 minutes. Blood flow and velocity and arterial diameters will be recorded for 2 minutes following the release of the cuff.

Ankle Brachial Index (ABI). This test is done by measuring the blood pressure in both the arm and the lower leg (ankle) while the subject is lying down. We will use this test to screen for peripheral vascular disease.

Six-Minute Walk Test (6MWT). This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes. A line, 30 m in length, will be drawn out in tape with cones at each end of the line to mark the turnaround points.

Skin Blood Flow. The laser imaging camera is a special camera that shines a low energy laser light on the surface of the skin to measure blood flow. The FLPI makes graphs, photos, and movies of skin blood flow. There are no known risks associated with the laser imaging camera. The small skin probes can hurt the eye if you stare into the light for a long time; however, we do not turn on the laser until the probes are taped to a surface of the skin.

Iontophoresis. Acetylcholine, sodium nitropruside and L-NAME are vasoactive substances that will be used to increase and decrease skin blood flow. The amount of drug delivered is based on the amount of current being delivered. Using this technique with such a low level current, there should be no drug that enters the body. Although the current being used is very small (less than 200mA), participants may feel mild skin irritation on the hand or the forearm where the chambers are placed.

Local Heating. We measure the temperature of the skin under the ring holders. The skin will feel very warm but should not hurt. The heating of the water will make the skin of the arm red under the holders like when the subjects takes a hot bath. The redness will not last more than a few hours. Some people may be more sensitive to the heating than others and therefore if the arm gets too hot, we will reduce or stop the heating.

In this study there will be 5 experimental visits following the preliminary visit each being four weeks apart. For the Kuvan (BH4) protocol, depending on the randomized group the subject is in they will receive either 5 mg, 10 mg, or 20 mg per kg of treatment.

Tentative Schedule of Events:

*Note subject may or may not be asked to perform all procedures during each testing day depending on

time and availability. Also, some preliminary day measures may not be completed on the preliminary day and may be completed some other time during the course of the study.*

Preliminary Day

- Consent
- ABI
- IMT
- DXA Scan
- Pulmonary Function Test
- 6 min Walk Test

Experimental Testing Days

- Continued consent
- PWV
- FMD
- Skin Blood Flow/Local Heating
- Blood Draw
- Pulmonary Function Test

Risk Assessment

All procedures in this study have been routinely performed in research studies in the past and are of low risk to participants.

Venous Blood Draw. Potential risks related with taking a blood sample are few but include slight bruising, pain, a temporary feeling of faintness, and phlebitis. Rarely, there may be a small blood clot or infection at the site of the needle puncture. All blood draws will be performed by a research team member or a nurse who is certified in drawing blood; in either case sterile techniques will be used.

Pulmonary Function Test. There are no known risks associated with this test.

Muscle Function Test. Electrical stimulation of the muscles may be uncomfortable and could be painful. If the muscle stimulation is too painful, we will adjust the stimulation to a level that the subject can tolerate. The study does not require the stimulation to be painful for the study to be successful. Because the stimulator "exercises" the muscle, the muscle may be sore for a few days following the protocol.

Body Composition. The DXA will expose the subject to a minimal amount of radiation. The DXA scan will expose the participants body to about 3 mrem per scan. Everyone receives a small amount of unavoidable background radiation from the natural environment each year. The radiation dose from the DXA scan is equal to less than three to four extra days' worth of natural background radiation. Radiation at significantly higher levels is known to cause cancer; however, the amount of radiation in this study is very low and is not expected to cause bodily injury during the subject's lifetime. The radiation dose discussed

here is what the participant will receive from this DXA study only. Radiation dose from multiple exposures is cumulative. Our radiation devices are monitored regularly for continued performance and approved for testing. The subjects will be asked if they are receiving additional x-ray exposure from other sources during the consenting process.

Pregnancy. Radiation at large doses is known to have an effect on a developing fetus. Although radiation doses from these studies are extremely small, if the subject is a premenopausal female a urine pregnancy test will be conducted to rule out pregnancy.

Arterial Health and Stiffness. The risks associated with the flow-mediated dilation, pulse-wave velocity, and intima media thickness tests are minimal, if not none. Potential risks include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with the lubricant gel are skin irritation and possible break out of rash.

Kuvan. The potential risks associated with Kuvan are headache, diarrhea, abdominal pain, vomiting, upper respiratory tract infection, and nausea. These risks are the most commonly reported in approximately 4% of patients chronically treated with Kuvan.

Antioxidant Cocktail. (1000mg of vitamin C, 600IU of vitamin E, and 600mg alpha-lipoic acid) There are no known risks associated with taking the antioxidant cocktail.

Skin Blood Flow: The laser imaging camera is a special camera that shines a low energy laser light on the surface of the skin to measure blood flow. The FLPI makes graphs, photos, and movies of skin blood flow. The subject will be able to see a harmless red light on their skin. There are no known risks associated with the laser imaging camera. The small skin probes can hurt the eyes if the subjects stare into the light for a long time. We do not turn on the laser until the probes are taped to a surface of the arm.

Iontophoresis: Acetylcholine, sodium nitropruside and L-NAME are vasoactive substances that will be used to increase and decrease skin blood flow. The amount of drug delivered is based on the amount of current being delivered. Using this technique with such a low level current, there should be no drug that enters the body. Although the current being used is very small (less than 200mA), the subject may feel mild skin irritation on the hand or the forearm where the chambers are placed.

Six-Minute Walk Test: Potential risk related to this test are few but include chest pain, shortness of breath, leg cramps, staggering, sweating, and pale skin color in the face. Every effort will be made to minimize these risks. A staff member who is certified in basic life support will be present at all times. We will have a chair available throughout the test if the patient needs to take a break. Oxygen will also be readily available.

Local Heating: We measure the temperature of the subject's skin under the ring holders. The skin will feel very warm but should not hurt. The heating of the water will make the skin of the subjects arm under the holders red like when taking a hot bath. The redness will not last more than a few hours. Some people may be more sensitive to the heating than others. If the subjects arm feels too hot, the subject has been instructed to tell us, and we will reduce or stop the heating.

Visits Required:

There are six visits required for all COPD subjects for this study, a preliminary visit, and 5 experimental day visits (baseline, 4 weeks, 8 weeks, 12 weeks, and a follow up visit on week 16). Control subjects will need to only complete one experimental day visit.

Subjects may participate in both protocols but they do not have to. If they do wish to participate in both protocols, they must wait at least 4 weeks in between protocols.

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7. Data and Specimen Management

a. Describe the data analysis plan, including any statistical procedures: The proposed investigation is an open-label dose response investigation. More importantly, this investigation is a pilot project that is based on the transient findings of our previous funded American Heart Association grant. Nonetheless, for the Kuvan protocol we will employ a 3 x 5 (dose x time) mixed factorial ANOVA with the repeated factor being time. For the Antioxidant protocol we will employ a Repeated measures ANOVA. For both protocols we will run planned comparisons of the treatment response to the control data to determine the effects of the treatment relative to the baseline value of the control. In an attempt to reduce the family wise error rate, planned comparisons will also be made for the interaction between baseline and 12 weeks for each dose with comparisons made with the withdraw period.	☐ N/A
b. When applicable, provide a power analysis: The power and sample sizes could not be determined from the acute intervention study.	☐ N/A
c. Describe how data and specimens will be handled:	☐ N/A

i. What information will be included in that data or associated with the specimens?

All specimens will be collected with deidentified information. Specifically, the ID, date, specimen type (i.e. serum or plasma), and analyte to be measured will be included on the tube label.

ii. Where and how data and/or specimens will be stored?

Data are stored in subject folders, specimens are stored de-identified in -80 freezers

iii. How long will the data and/or specimens be stored?

Until analyzed

iv. *Who will have access to the data or specimens?* All investigators who are on the protocol
v. *Who is responsible for receipt or transmission of the data and/or specimens?* Dr Harris
vi. *How will data and/or specimens be transported?* *A*ccording to biosafety procedures

8. Provisions to Monitor the Data to Ensure the Safety of Subjects

This study involves no more than minimal risk study and this section is not required. ☐ **N/A**

The plan might include establishing a data monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.

a. Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe. Subjects will be monitored the entire time they are in the LIVEP lab. The PI and study team will evaluate the safety for each participant by changes in BP and HR before, during and after treatment.
b. Describe what data are reviewed, including safety data, untoward events, and efficacy data. During consenting we ask what drugs they are currently taking and we also ask on every visit if there have been any changes to the drugs. We will frequently monitor BP, and HR to determine how the treatment is affecting them.
c. Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants). Data is consistently collected during their visits which will monitor how their body is reacting to the drug or cocktail treatment.
d. Describe the frequency of data collection, including when safety data collection starts. This done with every visit, subjects BP, and HR are consistently monitored during the duration of their visit
e. Describe who will review the data. The PI will review the data on a daily basis. Because Biomarin is the manufacture of Kuvan and is providing the drug for this study, the researchers may also disclose information to BioMarin Pharmaceutical Inc.
f. Describe the frequency or periodicity of review of cumulative data. This will be evaluated during each visit.

g. Describe any conditions that trigger an immediate suspension of the research.

If the PI thinks that the study is causing harm to the participant they will withdraw that subject from the study.

9. Withdrawal of Subjects ☐ N/A

In the event that participating in the study might be harmful to the subject's health and well-being, we will end their study participation. In addition, if we feel that the participants are not adhering to the treatment we have the right to end their study participation. At any time, the subjects also have the right to stop participating in the study.	☐ N/A
For any reason the subjects ask to withdraw from the study they will be paid an hourly rate of $10.00 an hour.	☐ N/A
	☐ N/A

10.Risks to Subjects

All procedures in this study have been routinely performed in research studies in the past and are of low risk to participants.

Venous Blood Draw. Potential risks related with taking a blood sample are few but include slight bruising, pain, a temporary feeling of faintness, and phlebitis. Rarely, there may be a small blood clot or infection at the site of the needle puncture. All blood draws will be performed by a research team member or a nurse who is certified in drawing blood; in either case sterile techniques will be used.

Pulmonary Function Test. There are no known risks associated with this test.

Muscle Function Test. Electrical stimulation of the muscles may be uncomfortable and could be painful. If the muscle stimulation is too painful, we will adjust the stimulation to a level that the subject can tolerate. The study does not require the stimulation to be painful for the study to be successful. Because the stimulator "exercises" the muscle, the muscle may be sore for a few days following the protocol.

Body Composition. The DXA will expose the subject to a minimal amount of radiation. The 1 DXA scan will expose the subjects' body to about 1.5 mrem radiation dose. Everyone receives a small amount of unavoidable background radiation from the natural environment each year. The radiation dose from the DXA scan is equal to less than 2 extra days' worth of natural background radiation. Radiation at significantly higher levels is known to cause cancer; however, the amount of radiation in this study is very

low and is not expected to cause bodily injury during your lifetime. The radiation dose discussed here is what you will receive from this DXA study only. Radiation dose from multiple exposures is cumulative. Our radiation devices are monitored regularly for continued performance and approved for testing. The subjects will be asked if they are receiving additional x-ray exposure from other sources during the consenting process.

Pregnancy. Radiation at large doses is known to have an effect on a developing fetus. Although radiation doses from these studies are extremely small, if the subject is a premenopausal female a urine pregnancy test will be conducted to rule out pregnancy.

Arterial Health and Stiffness. The risks associated with the flow-mediated dilation, pulse-wave velocity, and intima media thickness tests are minimal, if not none. Potential risks include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with the lubricant gel are skin irritation and possible break out of rash.

Kuvan. Kuvan is FDA approved for the use in phenylketonuria (PKU). Although Kuvan has not been approved for the use in COPD, it is generally well tolerated. The potential reported risks associated with Kuvan use in PKU are headache, diarrhea, abdominal pain, vomiting, upper respiratory tract infection, and nausea. These risks are the most commonly reported in at least 4% of patients chronically treated with Kuvan.

Antioxidant Cocktail. There are no known risks associated with taking the antioxidant cocktail.

Skin Blood Flow: The laser imaging camera is a special camera that shines a low energy laser light on the surface of the skin to measure blood flow. The FLPI makes graphs, photos, and movies of skin blood flow. They subject be able to see a harmless red light on their skin. There are no known risks associated with the laser imaging camera. The small skin probes can hurt the subject's eye if they stare into the light for a long time. We do not turn on the laser until the probes are taped to a surface of the arm.

Iontophoresis: Acetylcholine, sodium nitropruside and L-NAME are vasoactive substances that will be used to increase and decrease skin blood flow. The amount of drug delivered is based on the amount of current being delivered. Using this technique with such a low level current, there should be no drug that enters the body. Although the current being used is very small (less than 200mA), you may feel mild skin irritation on the hand or the forearm where the chambers are placed.

Local Heating: We measure the temperature of the subject's skin under the ring holders. The skin will feel very warm but should not hurt. The heating of the water will make the skin of the subjects arm under the holders red like when taking a hot bath. The redness will not last more than a few hours. Some people may be more sensitive to the heating than others. If the subjects arm feels too hot, the subject has been instructed to tell us, and we will reduce or stop the heating.

Adverse Events

According to the ICH definition, an adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an

abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the investigational product.

This definition includes intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions. Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

Adverse event information will be collected in an ongoing fashion through patient reporting AEs to their physician or health care provider. Seriousness and relatedness will be assessed by the treating physician, with appropriate reporting.

A designated primary contact person based at the treatment center will be responsible for the collection and reporting of AEs for patients participating in the program.

Serious Adverse Events

A serious adverse event (SAE) is defined as any AE that:

- Results in death
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, an AE that occurs in the child or fetus of a subject exposed to the product prior to conception or during pregnancy
- Important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above.

More than one of the above criteria may apply to any specific event.

Pregnancy

Pregnancy in a subject being treated with the product should be reported immediately (within 24 hours of becoming aware of the pregnancy) to BioMarin Pharmacovigilance by using the FDA 3500A (MedWatch Form). Every effort should be made to follow the patient through resolution of the pregnancy (termination or delivery) and report the resolution of the FDA 3500A (MedWatch Form) to BioMarin Pharmacovigilance.

SAE and Pregnancy Reporting

In Investigator IND studies,

KUVAN™ serious, related, unlabelled, (unexpected) adverse events will be reported to the FDA as required by 21 CFR 312.32 by the Investigator/Sponsor. These reports may be filed utilizing the Form FDA 3500A (MedWatch form). The Investigator/Sponsor will provide BioMarin Pharmacovigilance, with a copy of this report.

All serious adverse events (SAEs) and pregnancy reports whether or not considered drug-related should be reported to BioMarin Pharmacovigilance (contact information below) within 24 hours of receipt by the investigator/sponsor by using the FDA 3500A (MedWatch Form).

For Comparator Drugs / Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer.

For Comparator Drugs / Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer.

Clinicians should not wait to collect additional information that fully documents the event before notifying BioMarin's Pharmacovigilance Department of an SAE or pregnancy. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore it is important that clinicians submit additional information requested by BioMarin Pharmacovigilance as soon as it becomes available.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements.

Contact information for BioMarin's Pharmacovigilance Department is as follows:

BioMarin Pharmaceutical Inc.
105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
Email: drugsafety@bmrn.com

Product Complaints Reporting

A product complaint ("Complaint") is any direct, written, electronic, or oral communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, labeling, purity, stability, appearance, effectiveness, safety, and/or design of a drug product after it is released for distribution.

Complaints that simultaneously fall under Adverse Event definitions under this Protocol need only be reported via the Adverse Event reporting procedure set forth in this Protocol.

Investigator or designee (Reporter) shall capture the following Complaint information as relates to Kuvan used under this Protocol:

- Date complaint received
- Product Name and Lot Number
- Indicate if the product is available for return to BioMarin for investigation
- Quantity Affected
- Detailed Description of complaint
- Study Protocol Number
- Investigator Name

• Site Contact • Site Number • Subject Number • Name and contact information of the person who is reporting the complaint as well as name and contact information of the complainant. The reporter will be contacted by BioMarin Product Complaint Quality Assurance Department. Investigator or designee (Reporter) will use his or her best efforts to report Complaints to BioMarin within five days of learning of the Complaint. Investigator or designee will submit the complaint information by email at productcomplaint@bmrn.com or by fax at 415-523-1457.	
There are no costs for participating in the study; however, the subjects will be responsible for the costs of transportation to the Georgia Prevention Institute for clinic visits. There is no cost for the medications/supplements used during the study or for any of the procedures and tests required for this study. The cost of the lab draws during the study will be covered by the study investigators	☐ N/A
a. If applicable, describe risks to others who are not subjects.	X N/A

11. Potential Benefits to Subjects

COPD is the 3rd leading cause of death in the US. Participation in this study will advance our knowledge and understanding of the link between heart and lung disease and contribute to the treatment and prevention of COPD. Although there is no direct benefit to the participant, access to the results and information on 1) cholesterol values, 2) skeletal muscle function test, and 3) functional and structural measurements of the health of the participants arteries will be available. They may also choose whether they receive the results of these assessments; however, if the participant choose to receive the results, Dr. Harris or another qualified research member will explain the results to the participant in detail.

12. Confidentiality

To minimize the risk of the subject confidentiality, no testing material will be linked to the name, SSN, medical record number, or any other identifiable markers. Instead, each participant will be assigned a random identification number (ID). Those unique ID numbers will be used in the data file. A list of participants' names and their ID numbers is kept in a separate file and only the Principal Investigator and collaborating investigators named on the informed consent document will have access to this file. E-mail addresses and phone numbers are not shared with outside parties.

13. Consent Process

On the preliminary visit when subjects first arrive they will be undergo the informed consent process. The RA will go over everything that will be involved in the study in detail. If the subject understands they will be asked to initial each page after the RA has explained that page of the document to them. The RA will answer any questions the subject may have during the consent process. In addition, when finished going through the ICD, the RA will ask the subject again if he/she has any questions and will get confirmation that the subject still wants to participate in the study. If the subject agrees they will sign the document on the last page followed by the RA's signature indicating the subject understands everything. Dr Harris will confirm that the subjects understand the study and are still willing to participate. Dr Harris will also answer any questions that the subjects might have. All subjects will be aware that they have the right to stop the test at any time.

14. Compensation for Research-Related Injury

This section is not required when research involves no more than Minimal Risk to subjects. ☐ **N/A**

Because of Georgia Regents University policy, the institution is not able to offer financial compensation should the subject be injured as a result of participating in this research. However, the subject is not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research, including the institution. Each risk described has been considered and all safety precautions have been put in place to protect the subject. The subjects are aware if they have any questions or concerns during the study they will need contact us immediately.

15. Resources Available ☐ N/A

The study doctor, Dr. Caralee Forseen, will be on hand to work with patients if a medical consequence occurs as a result of the research. Patients will also be directed to the GRU clinics or ER if necessary. If the issue is psychosocial, then patients will be directed to the GRU Psychology department to talk to one of the licensed and trained psychologists on staff.
All research staff are required to sign by their duties associated with each study that they understand and know how to carry out their duties in any event. These documents can be found in the HAC binders in the office of the PI.

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