

## Patterns of Neurocircuitry Activation In Severe Asthma (PANISA)

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## **Background**

The control of asthma is a complex process and is influenced by many factors. Efforts to identify and establish the regulation of airway inflammation have largely focused on immune cells, their products of inflammation, and the response of resident cells and tissues of the lung to these products. It is also known that the response to inflammation is heterogeneous both in type, intensity and consequences, i.e. as they relate to disease severity and treatment responsiveness. While research on mechanisms of asthma has primarily been interested in processes which are confined and related to the airway and lung, and their function, it is also known that environmental factors are important to the initiation of these responses and the resulting inflammation, altered lung function and disease severity. In addition to traditional environmental factors, such as allergens and respiratory infections, mental-related response, such as anxiety and depression, can contribute as well to asthma. Thus, extra-pulmonary factors, like the brain, can affect underlying asthma **and** asthma can influence the function of non-pulmonary organs. Little is known about these relationships both in terms of mechanisms and consequences.

As noted, in contrast to an extensive understanding of how the lung in asthma responds to respiratory viral infections or airborne allergens, available information is far more limited on the influence of non-pulmonary factors, such as the brain, on asthma and, conversely, asthma on brain function and subsequent behavior such as anxiety, depression or stress. A number of observations have begun to support the importance of “asthma/brain” interactions, as well as “brain/asthma” influences. This is the focus of this pilot proposal.

In previous and ongoing work, we have shown that allergen activation of airway inflammation in asthma is associated with an activation of specific neurocircuits, the intensity of which predicts airway inflammation, as measured by increases in sputum eosinophils to the inhaled allergen, and lung function as reflected on a late-phase fall in pulmonary functions. Epidemiological evidence also points to strong associations between mental depression and the severity of underlying asthma. What is not clear from these associations is whether the presence of mental depression arises as a consequence of disability from asthma, or the presence of mental depression enhances asthma, or, most likely, if these interactions are the result of a vicious cycle in which mental depression and asthma create an ongoing “loop” of activity to further the intensity of both conditions. Because associated mental depression is a major risk factor for poor outcomes in asthma, efforts to establish insights into this relationship promise to have significant clinical importance and benefit.

The overall purpose of this study is to compare the neurocircuitry activation patterns of severe asthmatics to mild to moderate asthmatics and healthy controls. To begin to further address possible relationships of asthma and brain function, we propose the following hypothesis, “patients with defined characteristics of severe asthma will have *distinct patterns of persistent neurocircuitry activation*. We further propose that the *detection* of ongoing neurocircuitry activation occurs because of persistent and active airway inflammation in severe asthma. Finally, we propose that the *intensity* of specific neurocircuitry activation will relate to the severity of underlying asthma.”

## **Approach**

**Study population.** The Severe Asthma Research Program (SARP) was established in 2002 by the NIH to determine and define the characteristics and causes of severe asthma, as this phenotype of asthma has greatest disease morbidity and associated disease burdens – costs,

limited quality of life, and associated adverse effects from both disease and necessary higher doses of medications particularly corticosteroids – osteoporosis, hypertension, diabetes, and depression.

The UW has been part of the SARP since its origin. Since SARP III started in 2012 (IRB #2012-0571), UW recruited 107 subjects into its program with 45 adult subjects meeting criteria for severe asthma.

Subjects with severe asthma were defined by the ATS-ERS definition that involves three stages of assessment (2012):

- I. **Stage 1: Confirm an asthma diagnosis** and differentiate from difficult-to-treat asthma through evaluations by an asthma specialist for at least 3 months
- II. **Stage 2: Differentiate severe asthma from milder asthma.** When a diagnosis of asthma is confirmed and comorbidities are addressed, severe asthma is defined as “asthma which requires treatment with high-dose inhaled corticosteroids\* plus a second controller or systemic corticosteroids, with or without a second controller, to prevent it from becoming uncontrolled or which remains uncontrolled despite this therapy.”

\*Note: thresholds for high-dose inhaled corticosteroids are defined as  $\geq 440$  mcg fluticasone equivalent/day for children 6-11 years and the highest marketed LABA/ICS combination for subjects 12 years and older. If the second controller is not a LABA, then  $\geq 880$  mcg fluticasone equivalent/day, plus a second controller is required for subjects 12 years and older. High-dose inhaled corticosteroids or systemic corticosteroids must be taken for the 3 months prior to enrollment and for at least 6 of the past 12 months.

- III. **Stage 3: Assess for uncontrolled asthma** by any one of the following four criteria:
  - a. Poor symptom control evidenced by either an Asthma Control Questionnaire score consistently  $> 1.5$ , an Asthma Control Test Score  $\leq 20$ , or “not well controlled” by NAEPP or GINA asthma treatment guidelines, or
  - b. Frequent severe exacerbations as reflected by  $\geq 2$  bursts of systemic corticosteroids ( $> 3$  days each) in the previous 12 months, or
  - c. Serious exacerbations reflected by at least one hospitalization, ICU stay or mechanical ventilation in the previous 12 months, or
  - d. Presence of airflow limitation evidenced by  $FEV_1 < 80\%$  predicted (in the face of reduced  $FEV_1/FVC$ ).

Evidence for any one of these four criteria either on current high dose inhaled corticosteroid therapy or with tapering of that therapy identifies the patient as having “severe asthma”. Fulfillment of this definition predicts a high degree of future risk both from the disease itself (exacerbations and loss of lung function), as well as from side effects of the medications.

Because of the longstanding nature of their asthma and its severity, the consequences of their disease will be more apparent and more likely to reflect a greater risk for systemic effects of this

disease including the possibility of persistent neurocircuitry activation, what might include chronic anxiety, or loss of concentration. SARP subjects will be the focus of this study.

Identified adult, severe asthma SARP subjects from the UW cohort will be approached for participation in this study. SARP subjects will be called via telephone if they had previously agreed to be contacted for future studies during their SARP consenting process. Since no medication withhold is being sought, potential subjects will be scheduled for visit 1 (V1) over the phone and consent will occur at the research center on V1. Before being scheduled for V1, the subjects will undergo a preliminary phone screen to determine basic eligibility.

### Inclusion Criteria

An asthma subject is eligible for participation in the study if all of the following criteria apply:

- Current enrollment in the SARP 3 (2012-0571) study,
- Severe asthma,
- Male or female with no health concerns that might affect the outcome of the study,
- Women of child-bearing potential (WCBP) must have a negative urine pregnancy test (urine HCG) ,
- In the opinion of the investigator, capable and willing to grant written informed consent and cooperate with study procedures and requirements,.
- Ability to tolerate a simulated fMRI brain scanning session, and
- Ability to give valid informed consent to participate by signing and dating a written consent form.

### Exclusion Criteria

A subject is not eligible to participate in this study if any of the following criteria apply:

- Psychotropic medication use that might affect function of neurocircuitry implicated in our hypotheses (at the discretion of a study physician or Co-Investigator),
- Contraindication for MRI,
- Needle phobia or claustrophobia,
- Unable to distinguish specific colors used in Stroop task,
- History of a diagnosed Bipolar Disorder, Schizophrenia, or Schizoaffective Disorder,
- Pregnant or lactating females,
- Upper or lower respiratory infection within 1 month of the visit,
- Unstable asthma as indicated by self-report of increased symptoms or increased beta-agonist use over the 2 weeks preceding the visit,
- Current smokers (defined as smoked within the last year) or a former smoker with a history of >5 pack years,
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- Any condition which, in the opinion of the investigator, might interfere with participation in the study,
- Inability or unwillingness to perform required study procedures

Subjects having (1) an upper or lower respiratory infection within 4 weeks and/or (2) unstable asthma within 2 weeks prior to of the date of the phone screen: may be contacted and re-screened at a later date if meeting all other criteria reviewed during the initial phone screen.

## **Procedures**

Completing the study activities will take approximately four hours. The study team's goal is for the majority of subjects to complete all study activities in one visit. However, subjects will have the option to complete the informed consent process and MRI Simulation at their first visit, and return at a later date to complete the remaining study activities (2 visits maximum). The following procedures will be performed during the study visit:

**Informed consent:** Written informed consent will be obtained from each subject at V1 by the investigator or designee. The UW IRB approved consent form will state that subjects may withdraw from this study at any time without any change in the quality of their medical care or loss of benefits.

**Brief medical history update:** A brief medical history update on the subject's past and present medical, surgical and medication history, asthma and allergy history, and smoking history, which was previously provided as part of their SARP participation.

**Review of current medications:** Current medications will be reviewed and updated.

**Physical exam:** Physical examinations will include a determination of vital signs and evaluation of the lungs, cardiovascular system, and a nasal-oropharyngeal examination.

**Vital signs (BP, P, RR and weight):** Vital signs will be measured and will include temperature, blood pressure, pulse, and respiration rate. We will also measure pulse oximetry, and height and weight.

**Urine Collection.** If the subject is a female of child-bearing potential, a urine pregnancy test will be conducted to exclude pregnancy prior to performance of certain study procedures.

**Exhaled nitric oxide (eNO):** Exhaled NO has recently been shown to be a useful marker of airway inflammation in asthma, in particular because its rise frequently precedes that of lung function decline and thus is valuable in preventative care (Hoffmeyer et al., 2009). Fraction of exhaled NO (parts per billion) will be measured in breath condensate, following American Thoracic Society guidelines (Silkoff et al., 1997; Anonymous, 1999), at a flow rate of 50 mL/s, with a rapid-response chemiluminescent analyzer (NIOX System; Aerocrine, Solna, Sweden; Silkoff et al., 2004).

**Spirometry (FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC, and FEF<sub>25-75%</sub>):** Spirometry will be conducted according to ATS guidelines. The variables FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC and FEF<sub>25-75</sub> will all be recorded from the "best" effort, defined as the effort with the highest sum of FEV<sub>1</sub> + FVC. FEV<sub>1</sub> will be used as the global indicator for spirometry, and this variable will be partitioned into FVC and FEV<sub>1</sub>/FVC components.

**Blood sample (up to 30 ml):**

a. CBC with differential: this will include a cell differential count of eosinophils, neutrophils, macrophages, and lymphocytes.

b. Measure of gene expression in peripheral inflammatory cells by RNA seq: Peripheral blood mononuclear cells (PBMC) from severe asthmatic subjects will be prepared on Ficoll density gradient from 20 ml of Lithium heparinized whole blood. 10 to 20 million PBMC will be

recovered and cultured in RPMI with 10 % FBS in 24-well plates for 4 h or 24 h. To evaluate peripheral blood T lymphocyte activatability, PBMC will be cultured with (for 4 h) or without (for 24 h) anti-CD3 plus anti-CD28 (each at 1 µg/ml). Peripheral blood monocyte activatability will be evaluated by LPS (1 µg/ml) stimulation for 4 h. In addition, sensitivity to corticosteroids will be analyzed by adding 10<sup>-9</sup>, 10<sup>-8</sup> and 10<sup>-7</sup> M of dexamethasone to PBMC 15 minutes before activation with anti-CD3 plus anti-CD28 and LPS. Cells will be harvested in buffer RLT (Qiagen) and stored at -80°C until they undergo total RNA extraction. Total RNA quality control and RNAseq will be performed by the Biotech Center at UW-Madison on total RNA from unactivated PBMC, and LPS- and anti-CD3/CD28-activated cells, using Illumina HiSeq 2500. SeqMAN NGen13 and Array Star13 softwares will be used to analyze gene expression. **Analysis:** Principal component analysis (PCA) will be performed to group the genes which expressions change concomitantly among the asthmatic subjects. The groups of genes will then be associated with markers of the neurocircuitry activity (MRI, questionnaires). The genes part of PCA and associated with the neurocircuitry activity will be further analyzed using the Ingenuity Pathway Analysis (IPA) system from Qiagen. PBMC treated with dexamethasone will be analyzed by RT-PCR, to measure expression levels of pro-inflammatory (IL-1, TNF, IL-6, IL-8), Th-2 (IL-13), Th-17 (IL17A) and Th-1 (IFN-γ) cytokines. Expression levels will then be analyzed for associations with the neurocircuitry activity.

#### c. plasma for RNA analysis

**Asthma and psychological questionnaires:** Participants will complete self-report questionnaires that assess life stressors, perceived stress, emotional style, and symptoms of anxiety and depression. These questionnaires may include the following: Beck Depression Inventory, Beck Anxiety Inventory, State-Trait Anxiety Inventory, Penn State Worry Questionnaire, Positive and Negative Affect scale, Perceived Stress Scale, and the Asthma Control Questionnaire.

**Cognitive function tests:** Tasks that assess cognitive function will be performed, which may include tests of memory, attention, and executive function such as those contained in the NIH cognitive assessment toolbox (<http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/cognition>) or the CANTAB cognitive test battery (<http://www.cambridgecognition.com/academic/cantabsuite/tests>). Stimuli in these tasks may include letters, numbers, pictures, words, shapes, symbols, or sounds. Tasks will be performed on a computer or tablet.

**Functional magnetic resonance (fMRI):** fMRI images will be acquired using on a GE 750 3.0 Tesla MRI scanner device with either an 8 or 32-channel head coil at the Waisman Lab for Brain Imaging and Behavior. fMRI images will be collected while participants view words or pictures, some of which may have emotional content or meaning. Participants will respond to these stimuli by pressing a button on an MRI-compatible response box. Stimuli will be presented using a projection system and eye-tracking technology for monitoring of eye position may be used during scanning. This system provides video-based, contact-free evaluation of gaze position. Additionally, functional resting state data may be collected during the fMRI scan, lasting between 5-12 minutes, and the participant will be asked to rest. This data would allow for comparisons in brain activation among rest and task states. A diffusion tensor image (DTI) scan may also be obtained as we have recently described (Hanson et al., 2012).

A Brain Array radiofrequency (RF) coil from Nova Medical (Wilmington, MA), model number NMSC077-3GE-MR750 for the 3T GE X750 MRI scanner will be used to collect MRI images. This multi-channel (receive-only) RF coil provides increased MRI signal sensitivity, improved

spatial localization, and the ability to scan more rapidly over existing RF head coil hardware on our system. This is important for this brain imaging project as it will enable us to obtain (1) more accurate brain images, (2) improved spatial resolution, and/or (3) faster scanning times. Though an Investigational Device and not FDA approved for clinical use, this particular coil device includes multiple features for safe operation involving human studies. This 32-channel head coil device is designed and constructed as a receive-only detector of RF signals that are emitted by the brain following the RF excitation generated by the GE MRI scanner, which is FDA approved. During the RF excitation by the scanner, the coil device is decoupled (made inactive) through redundant circuitry (described below), thus the coil device never transmits RF to the subject, so it has no impact on subject risk or safety.

More specifically, the coil design and construction include the following safety features: (1) High voltage breakdown (>2kV) housing. (2) Rugged construction to assure safe operation in case of rough handling. (3) Active detuning circuitry providing greater than 35 db isolation per element. (4) High power passive detuning circuits in case primary detuning circuitry fails. (5) Multiple common mode traps in all receive coil cables. (6) Minimum of 5mm spacing between coil conductors and patient contact. Additionally, the coil was designed and manufactured under an ISO 13485 certified quality management system. As part of this quality system, Nova Medical has conducted a Failure Means and Effects Analysis (FEMA) of this product and we feel that it is a non-significant risk under foreseeable normal conditions when used on the above referenced MR scanner.

In addition, we note the following important details regarding the application of this coil: (1) The device is not an implant. The placement is external to the body. (2) The device is not required for life support and will not be applied in that manner. (3) The device will not be used in diagnosing, curing, mitigating, or treating disease and will not impair human health. While MRI is used for diagnostic imaging, that is not the objective in this project. In this project, we will be using the coil to obtain images that will be used to quantify structural brain anatomy and perform functional mapping. These will not be used specifically for diagnostic purposes. (4) As discussed above, this coil device has been designed and constructed such that it does not significantly incur any additional risk to the health, safety or welfare of the subjects.

Also during the MRI, MRO pulse sequences will be used. The MRI physics team at the Waisman Laboratory for Brain Imaging and Behavior (WLBIB) has developed MRI pulse sequences that are available for research projects used by investigators on the 3- Tesla MRI scanner at the WLBIB at the University of Wisconsin-Madison. According to the FDA, these non-product pulse sequences are not FDA-approved and thus are considered investigational devices.

These pulse sequences are developed using the GE Healthcare EPIC pulse sequence development and software compiler tools provided by the scanner manufacturer and are based upon existing pulse sequence software that are part of their FDA approved product. All of these software changes are minor and designed to enable new features for research projects that are not available through the scanner product software. Also, the product pulse sequences often change with scanner software upgrades, thus it is easier to standardize imaging studies over time with research pulse sequences. These pulse sequence modifications were performed by individuals with MRI physics and pulse sequence software training. Pulse sequence changes include modification to the gradient or RF waveforms either in shape or timing, ordering of gradient waveforms, changes to gradient trajectories, and timing of data acquisition.

The GE EPIC software tools include safety limits for gradient switching (dB/dt) to minimize nerve stimulation and RF power (BI specific absorption ratio, SAR) to minimize tissue heating. The pulse sequences are developed to operate below the limits of the FDA. By staying below these limits, the operating conditions of the MRI device are generally deemed, in and of themselves, to make the MRT device a non-significant risk device. In addition, the MRI system monitors the RF power in real time to operate within the FDA safety specifications.

It has been determined that the above pulse sequences developed in the Waisman Laboratory for Brain Imaging and Behavior do not pose any significant risk to subjects being scanned at the Waisman Laboratory for Brain Imaging and Behavior GE 3-Tesla scanner. This software device should be considered a non-significant risk device due to the following:

1) The Device is not an implantable device. 2) The Device is not intended to support or sustain human life. 3) The Device is not for a use of substantial importance in diagnosing, curing, mitigating, or treating disease or otherwise preventing impairment of human health and it does not present a potential for serious risk to the health, safety, or welfare of a subject. As is the case with all investigational devices, the device cannot be represented as safe or effective for the purposes for which it is being studied. 4) The Device does not present a potential for serious risk to health, safety, or welfare of a subject. 5) The Device has been verified to operate, from a technical standpoint, below the limits of the FDA articulated in "Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices -FDA Guidance document, dated July 14, 2003." By staying below these limits, the operating conditions of the MRI device are generally deemed, in and of themselves, to make the MRI device a non-significant risk device.

The entire procedure, including anatomical and functional scanning protocols, may require up to 90 minutes; however, our experience to date indicates that a single scanning session can be completed in less than 60 minutes. Accurate co-registration of the multiple images acquired in a scan session requires that motion be minimized during the procedure. Foam cushions, positioned next to the head will be used to help the subject keep their head still during collection of neuroimaging data.

## **Safety**

**eNO:** There is no known risk associated with the exhaled nitric oxide procedure.

**Spirometry:** During spirometry the subject may feel short of breath during the 6-second exhalation measurement. If this occurs and does not go away on its own, the subject may be given two puffs of an albuterol inhaler.

**Risks of Albuterol:** Common side effects are headache, increased pulse rate, shakiness of hands. Uncommon side effects are awareness of heart pounding or racing, mouth and throat irritation and muscle cramps. Rare side effects are low blood potassium, irregular heartbeat or heart rhythm, hyperactivity and an immediate increase in wheeze after dosing. Very rare side effects include an allergic reaction (hives, swelling of the face, mouth, tongue, and breathing problems). These are the most common side effects of these medications, but there are other less common events that are not listed. There may be side effects or risks that are unknown at this time.

**Blood draw:** The risk of phlebotomy is minimal and includes bruising, local discomfort, bleeding, infection and fainting. The maximum total volume of blood to be obtained during this study is 30ml or two tablespoons. All attempts have been made to minimize all lab assays in order to make the most efficient use of the cells that are isolated.

**fMRI:** The confining conditions of the MR system can precipitate claustrophobia in a subject. Subjects are screened for possible claustrophobia before they are enrolled in the study and familiarized with the scanning environment in a mock scanner before completing the fMRI procedure. In addition, each subject will have access to a 'panic button' to indicate that they would like to be removed from the scanner. The Waisman Laboratory staff has a 7 second turnaround time in getting someone out of the scanner after the subject has pushed the 'panic button'. In addition, pulse oximetry will be monitored during scanning to ensure safety. Although the goal of the study is to complete all procedures in one visit, subjects will have the option to split their study participation into two days, or two separate visits (V1 and V2). Subjects splitting study participation into two visits will sign the consent form and undergo fMRI simulation at V1. If eligible after V1, the subjects will come back on a different day (V2) to complete the remaining study procedures. V2 will be scheduled according to the availability of the subjects, study staff, and study equipment.

Electronic implants, such as cardiac pacemakers, may be susceptible to interference from the magnetic and RF fields produced by the MR system. This interference may destroy or negatively affect operation of these devices. Since interference to cardiac pacemakers is observed in magnetic fields as low as 13 gauss, means have been provided to prevent persons with cardiac pacemakers or other implanted electronic devices from entering a zone where the magnetic field exceeds 5 gauss. The magnetic field of the MR system exerts a force on ferromagnetic objects within the field. This force can cause a ferromagnetic implant, such as an aneurysm clip, surgical clip, or prosthesis, to move or be displaced and cause injury or death. If the implant is large, sufficient currents can be induced in the metal by the magnetic field (eddy currents are induced by pulsed gradient fields) to cause heating of the implant. It is possible that subtle genetic or molecular changes could be caused by the magnetic fields produced by the MR system. To date, however, no harmful biological effects have been demonstrated at the magnetic field strengths and exposure times utilized by the MR system. At the present time, the likelihood of any significant biomagnetic effect is considered to be very low. The magnetic field near the MR system is strong enough to attract ferromagnetic objects with great force. Near the magnet this force can be strong enough to pull objects in and cause them to fly down the axis of the magnet. Such objects become projectiles that can cause injury or death. The user must establish a security zone to prevent ferromagnetic objects from coming into proximity of the magnet. Access to all areas exceeding the 5 gauss level will be controlled by warning signs, barriers, staffed entry locations, or adequate interrogation to assure avoidance of incidents. Access to the magnet room by any personnel will be closely controlled for safety of persons, in particular to prevent accidental introduction of ferromagnetic objects that could be attracted by the magnetic field generated by the MR system.

There is a remote chance that brain imaging may reveal findings that are of uncertain medical importance. Knowing this kind of information may be of some benefit. On the other hand, sometimes there is little benefit and it has some risks; for example, it could affect the subject's insurability or employability, or just make the subject worried. All scans will be reviewed by a team of radiologists headed by Dr. Howard Rowley, and any clinically significant findings will be reported to subjects by that team. Findings of clinical significance will be released to subjects. The participant may also choose whether or not their physician is informed of any clinically significant findings. . Findings will be reported within one month from date of scan.

**Risks related to women:** The rules for this type of research do not allow a woman who is pregnant to participate. All women of childbearing potential must have a negative urine pregnancy test before beginning any study procedures.

**Self-report measures (Questionnaires):** The questionnaires are not tests; there are no 'right' or 'wrong' answers. There are no known risks to answering the questionnaires. The questionnaires might be considered long with repeating questions. The subject can skip any question you feel uncomfortable answering.

**Cognitive function tests:** There are no 'right' or 'wrong' answers to the cognitive tests. There are no known risks to completing the tests. The tests might be considered long with repeating sections. The subject can skip any section that makes them uncomfortable completing or stop at any time.

**Risk of potentially identifying depressive symptoms:** There is a risk of identifying depressive symptoms during the psychological questionnaires. Should the study staff identify moderate depression scores; the subject will receive a list of community resources for psychological treatment. Should the study staff identify severe depression scores; the subject will receive a follow-up consultation with a licensed clinical psychologist. The study staff will contact the on-call psychologist to follow-up with the subject. The subject will be given the list of community resources for psychologist support and will be contacted by the on-call psychologist within 3 days.

**Breach of confidentiality:** There is a risk of breach of confidentiality. In order to protect against this risk, all data will be stored on a secure, password protected network. All data will be identified using unique identification numbers, not names, date of birth, or other identifiable information. In files where identifiable information is linked to the unique ID number, only staff members who specifically use this information for contacting participants, etc. will have access.

**Email address:** We are requesting the subject's email address so we can communicate study visit reminders or changes with them. Email is generally not a secure way to communicate about a subject's health as there are many ways for unauthorized users to access email. The subject is instructed to avoid sending sensitive, detailed personal information by email. Email should also not be used to convey information of an urgent nature. If the subject needs to talk to someone immediately, they are instructed to contact a pulmonary research study coordinator at 608-263-0524. Or can also contact the Principal Investigator of the study William Busse, MD at 608-263-6183. The subject does not have to provide their email address to participate in this study. The subject can also request at any time that email no longer be used to communicate about the study, without any loss or penalty.

### **Sample Size Calculation and Data Analysis:**

This is a pilot study. Our target sample size is total 30 individuals with severe asthma. The attrition rates in our prior research have been low (0-7%) and our power calculations assume that at least 22 subjects (12% attrition) complete the study. For continuous outcomes in general, including airway cellular and molecular inflammatory responses to stimulation, and measures of neural activity (e.g. % signal change in insula by fMRI), effect sizes for the relationship between neural activity and increases in inflammatory markers is expected to be large. In previous studies, we report associations between increases in activity in insula and ACC and peripheral measures of inflammation with effect sizes that range from .56 to .99. It should be noted that the extremely large effect sizes are, in part, a function of the small sample size and thus, the

confidence interval for the population statistic is quite large. Nonetheless, these relationships were replicated in a subsequent investigation with robust, though smaller, effect sizes. Based on these preliminary studies, we expect to have very high power (>.85) to detect an association of moderate magnitude (.5) between neural activity and peripheral biological markers across groups with the proposed sample size. Data from individuals with severe asthma will be compared with data from individuals with mild to moderate asthma, as well as non-asthmatics.

a. Comparison to other study populations. Data from subjects with severe asthma will be compared with data from individuals with mild-to-moderate asthma, as well as non-asthmatic subjects. These data have been collected under protocol 2014-0116. The data has been collected but not yet analyzed in relationship to other severities of asthma. The data and samples were collected in the same way the data and samples will be collected under the current study.

b. Utilization of SARP data. Subjects who enroll into this study will also have SARP (2012-0571) data that will be utilized. The SARP Data Coordinator Center and the SARP Steering Committee has a Data Sharing Policy. Briefly, all data collected by a specific site, can be utilized at that site for other purposes. A SARP Data Request Form is completed and submitted to the Steering Committee for approval. Information requested on the Data Request Form includes, requesters, site affiliation, main hypothesis, rationale, description of the data requested (time points and type of data (spirometry, questionnaires, etc)), and the format being requested. This request form is reviewed first by the Publications Committee and secondly by the Steering Committee. A Data Request will be made for SARP data to accompany any subject who enrolls into this study. Data to be requested for this comparison analysis will include demographics, lung function testing, questionnaire data, sputum cell count and differential, medication use, and other data as deemed appropriate by the PI or designee.

### **Data Storage:**

Clinical and lab data will be entered into the UW REDCap system. The REDCap server is housed in a state-of-the-art data center managed by the UW SMPH network staff. The levels of security for the server are fivefold and include: 1) Physical Security: server is located in a secure data center under control of UW School of Medicine and Public Health (SMPH) ITS, which is a dedicated computer machine room (passkey access only) containing emergency backup power, an uninterruptible power supply (UPS), and an automatic fire detection and suppression system. SMPH ITS does not have access to the DOM RedCap server; 2) Access controls: Data access is limited to DOM Faculty and staff approved individuals; 3) Domain access restrictions: access to DOM computing resources, including the DOM RedCap Server, is restricted to individuals with a logon ID for the DOM Domain. Logon IDs are issued only upon approval of the Administrator or Data Custodian (PI); 4) Authentication: Password protection is used at the network level for all transactions that allow entry and editing of data, provide access to EPHI data, or administrative activities, and; 5) Firewall: located behind UW-Madison SMPH firewall.

Data will be coded with a study specific patient identification number. There will be no information included in the database that will link the information in the database to the subject. The code link is kept in RedCap that is accessible only to the research coordinators who will be conducting subject visits. A link is necessary in order for the coordinator to be able to maintain contact information for subjects and to track reimbursement for accounting purposes.

The clinical data collected directly from each participant includes the following information: age, sex, race, ethnic classification, birth year, asthma history including family history and personal symptom history and triggers, allergy history including symptoms history and triggers and skin test results from the study, lung function, asthma medications, medical history relevant to allergic disease, smoking history and current smoking status, adverse events from time of consent until that individuals completion of the study .

There are also research data generated from study procedures or samples collected specifically for this study including blood cell numbers and types and other asthma and inflammation related markers. Participants will also have lung imaging data generated.

The coded raw MRI data is stored at the Waisman Brain Imaging Core in their password-protected centralized computing infrastructure. Central storage consists of 75TB of network attached, 15k rpm Serial Attached SCSI RAID6 disk arrays. Data is made available to Core workstations via nfs and smb/cifs security protocols. Each participant is given a unique number that corresponds to the subject's data and personal information. The codes that link both the number and participant data are stored on a password protected network with access allowed only to those that require it.

### **Residual Samples and Data**

We will use the samples collected in this study for the scientific investigations described above. Occasionally the planned experiments do not use the entire sample collected. The study investigator will ask permission of the subjects to keep their left over samples (along with clinical data associated with those samples) for future research. If the subject agrees to have their residual samples and data used for future research, their samples will be stored until they are gone. This may take several months or several years. Their samples will not be used for future genetic studies. Their stored samples and data will not contain any identifiable information, so there will be no way for researchers to link their samples and data to them and no way for them to withdraw their sample if they agree to this future use. They can withdraw the samples prior to them being banked by writing to the PI of the study, however, once the samples have been banked, they will be anonymous, and the subjects will not be able to withdraw them. If the subject does not agree to the future use of their residual samples they will be destroyed once the study has completed.

Data collected throughout this research study, including their health history, medications, pulmonary function testing, and results of tests that were conducted during the study will be stored with the samples. Primary future use of these samples and data will be for research related to respiratory disease and inflammation. The residual samples will be stored in Dr. Busse's laboratory with limited access (locked doors with key or keypad entry). Upon completion of the study, samples will enter a sample bank. New code numbers will be randomly generated and samples will be relabeled.

In addition to the data retained as part of the optional sample bank, we plan to keep the information collected for this study for use in future studies related to respiratory disease and inflammation. The information will be kept indefinitely, meaning we do not have any plans to destroy it. The information may be shared with other researchers at the UW-Madison and researchers outside of the UW-Madison. However, all of the information we keep in this separate data bank will have the subject's identifiable information removed from it, so no one will be able to link the information back to the subject. Because the information will not be identifiable, the subject will not be able to withdraw the data from future research uses.

## **Data Quality & Safety Monitoring Plan:**

### **A. Data Quality and Management**

1. Description of Plan for Data Quality and Management– The PI, or study staff, will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. When possible, data will be collected using programs that allow direct transfer to a spreadsheet, in order to avoid human error. When data must be manually entered, double-entry will be used to allow accuracy checks. Accuracy checks will be performed by a different individual than those involved in data entry.
2. Frequency of Data Review for these Studies – The frequency of data review for these studies differs according to the type of data and can be summarized in the following table:

<b>Data type</b>	<b>Frequency of review</b>	<b>Reviewer</b>
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Annually	Principal Investigator, DMC
Adverse event rates (injuries)	Annually	Principal Investigator, DMC
Out of range (non-clinically significant) laboratory data	Annually	Principal Investigator, DMC
MRI data	Each time a scan is collected	Dr. Howard Rowley's team

### **B. Subject Accrual and Compliance**

1. Measurement and reporting of subject accrual, adherence to inclusion/exclusion criteria – Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur monthly to assure that participants meet eligibility criteria and ethnic diversity goals outlined in the grant proposal.
2. We use the UW-ICTR for local data monitoring oversight. This plan includes the use of the ICTR OnCore clinical research management (OnCore-CRM) system which allows more efficient tracking of protocols and protocol subjects. ICTR OnCore-CRM is a secure, web-based, customizable information system that addresses issues related to the costs and complexities associated with conducting clinical trials. It provides fully integrated clinical data management, study administration, and financial management capabilities for a portfolio of clinical trials. The ICTR OnCore-CRM system is specifically designed to improve the efficiency and effectiveness of clinical research at large research centers by facilitating core activities such as study setup and activation, accrual, clinical data collection and analysis, data and safety monitoring, financial management, and regulatory compliance.

## **Adverse Event Reporting:**

### **Adverse Events**

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the study procedure or medicinal product. An AE can therefore be any clinically significant and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the study product or procedure. Unexpected side effects that are not clinically significant (NCS) will not be considered adverse events. Anticipated day-to-day fluctuations of the conditions or diseases will not be considered an adverse event, unless they:

1. fulfill the definition of a serious adverse event, or
2. unexpectedly worsen, requiring medical intervention other than the use of the rescue medication, or
3. result in discontinuation of subject from the study.

The Investigator and study staff will record adverse events representing (1) unexpected, clinically significant reactions or (2) abnormal worsening of expected symptoms on an adverse event form to be maintained in the subject's research chart, regardless of the relationship to investigational product or procedures. The form will include observation date and resolution date.

The common, uncommon, rare, and very rare side effects indicated in the Safety section of the protocol will be recorded as adverse events if deemed clinically significant by a study physician. If a study physician determines that a clinically significant side effect renders study activities unsafe, subjects may be temporarily or permanently excluded from study participation.

All adverse events will be graded by severity using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, May 28, 2009 listed below:

Grade 1= Mild adverse event

Grade 2= Moderate adverse event

Grade 3= Severe and undesirable adverse event

Grade 4= Life threatening or disabling adverse event

Grade 5= Death

#### Assessment of Causality

The principal or co-investigator will assess the relationship between the study and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the study will be considered and investigated.

The relationship between an adverse event and the study will be recorded on the study adverse event form. The NCI-CTCAE provides the following descriptors and definitions for assigning relationship. This NCI nomenclature will be used but causality will be judged by the investigators in the context of asthma.

Code	Descriptor	Definition
1	Unrelated	The event is clearly not related to the study
2	Unlikely	The adverse event is doubtfully related to the study
3	Possible	The adverse event may be related to the study
4	Probable	The adverse event is likely related to the study
5	Definite	The adverse event is clearly related to the study

#### Disease- or Study-Related Events or Outcomes

### **Definition of a Serious Adverse Event**

A serious adverse event (SAE) or reaction is defined (21 CFR 312.32 (a) ) as any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect or precaution. This includes, but may not be limited to any of the following events:

- Death: A death occurring during the study or which comes to the attention of the Investigator during the protocol-defined follow-up after the completion of therapy, whether or not considered treatment-related, must be reported.
- Life-threatening: Any adverse therapy experience that places the participant or participant, in the view of the Investigator, at immediate risk of death from the reaction as it occurred.
- Hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity.
- Congenital anomaly/birth defect.
- An event that required intervention to prevent permanent impairment or damage.

An important medical event that does not meet the criteria listed above may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether reporting is appropriate in other situations. These situations might include an important medical event that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition.

### **Study Related Non-serious and Unexpected or Expected Adverse Event Reporting**

All non-serious and unexpected adverse events will be kept in an adverse event log and reported annually. Other adverse events that are both not serious and expected will not be reported. These are the expected adverse events listed in the protocol and consent as possible results from a study procedure. The investigator will meet all local reporting requirements as dictated by the UW IRB.

Individual stopping rules have been outlined that address the intensity or persistence of these adverse events.

### **Individual and Overall Study Stopping Rules:**

Based upon the definition of Serious Adverse Event as described in 21 CFR 312.32.

#### **Individual Safety Stopping Rules**

Individuals will be stopped from the study if the following events occur:

- The subject requests that a particular procedure be stopped or expresses a desire to withdraw from the study.
- Failure to recover to within 80% of baseline FEV<sub>1</sub> at discharge from any study visit. Albuterol therapy may be employed to assist in recovery.
- Any serious and unexpected adverse event or any event that, in the opinion of the investigator, poses undue risk to the subject.

#### **Overall Safety Stopping Rules**

The study will be stopped if:

1. A research related death occurs in any subject.
2. An event occurs that the investigator feels warrants halting of the study.
3. The same or similar serious and unexpected adverse event associated with the study that occurs in at least two subjects. This may include a potentially life-threatening event, event requiring hospitalization, or any event that may potentially result in serious harm to other research subjects.
4. For non-serious adverse events, if more than 20% of research volunteers meet the individual safety stopping rules listed above, and are withdrawn from the study, the protocol will be halted. The NIH (and IRB if required) will be notified and appropriate modifications to the protocol will be made.

**Ethical Considerations:**

1. This study will be conducted in compliance with current Good Clinical Practice of the International Conference on Harmonization and the ethical principles of the Declaration of Helsinki. The study will begin only after the UW Health Sciences Institutional Review Board (IRB) has granted approval.
2. The UW IRB approved consent form will state that subjects may withdraw from this study at any time without any change in the quality of their medical care or loss of benefits.
3. Each subject is required to sign a HIPAA Research Authorization at the time of consent. This authorization clearly outlines who will have access to specified protected health information. The information obtained in these studies is not particularly sensitive. However, data will coded by subject identification numbers and any key linking this code number to subject identifiers will have access limited to the investigator and key study personnel all of whom have had HIPAA training required by the University of Wisconsin. Data will be stored in files and a computer database, both in a locked location with access only by study personnel. Data obtained from the study may be published in scientific journals or presented in scientific meetings but results will be coded so individuals are not identified. Records will be retained by the Principal Investigator for at least two years after the investigation is discontinued and the FDA is notified. This is in accordance with US FDA Regulation (21CFR 312.62 (c) ).

Calendar of Events:

<b>Procedure</b>	<b>Visit(s)</b>		
	<b>V1</b>	<b>V1a</b>	<b>V2</b>

	Day 1 <sup>(x)</sup>	Day 1 <sup>(y)</sup>	Day 2 <sup>(y)</sup>
Informed Consent Process	X		
MRI Simulation	X		
Medical History		X <sup>AN</sup>	X <sup>AN</sup>
Review of Concomitant Medications		X <sup>AN</sup>	X <sup>AN</sup>
Physical Exam		X <sup>AN</sup>	X <sup>AN</sup>
Pregnancy Test		X <sup>AN</sup>	X <sup>AN</sup>
Vital Sign Measurement		X <sup>AN</sup>	X <sup>AN</sup>
eNO		X <sup>AN</sup>	X <sup>AN</sup>
Spirometry		X <sup>AN</sup>	X <sup>AN</sup>
Blood Draw <sup>A</sup>		X <sup>AN</sup>	X <sup>AN</sup>
Eligibility Review		X <sup>AN</sup>	X <sup>AN</sup>
Questionnaires <sup>B</sup>		X <sup>AN</sup>	X <sup>AN</sup>
Cognitive Function Tests		X <sup>AN</sup>	X <sup>AN</sup>
fMRI Scan		X <sup>AN</sup>	X <sup>AN</sup>
Adverse Event Symptom review		X <sup>AN</sup>	X <sup>AN</sup>

Calendar Foot Notes	
<b>(x)</b>	V1 - Consent, MRI Screen, MRI Simulation
<b>(y)</b>	All procedures marked "As Needed" must occur once, all on the same visit. All <b>AN</b> procedures will take place at either V1a or V2. (N/A for subjects deemed ineligible at V1)
<b>A</b>	Measurement of gene expression (PBMC) via RNA sequencing; RNA analysis of plasma
<b>B</b>	Asthma and Psychological Questionnaires: BDI, BAI, STAI, PSWQ, PANAS, PSS, ACQ
<b>AN.</b>	As Needed