Study Protocol

Symptom Clusters in Children with Exacerbation-prone Asthma

Protocol Date: August 3, 2023

NCT04002362

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STUDY PROTOCOL

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Version 5
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Summary of Protocol Changes

Version 4.1, 1/17/22

- Changed the types of blood tubes for blood sample collection (to make laboratory processing easier). Blood draw volumes remain the same.

Version 4, 5/18/21

- Changed age of eligibility from 8-17 years to 6 up to 21 years of age
- Expanded definition of “exacerbation” for eligibility purposes
- Added option for a single study visit with telephone/email follow-ups and review of medical records
- For participants who consent to the 48 week follow-up, added option for visits 3, 4 and 5 to be completed by telephone

Version 5, 8/3/23

- Added two additional specific aims and their analysis plans to the study protocol that will be funded through an Administrative Supplement to the parent grant.

A. Background

Asthma symptom control is suboptimal in the majority of children in the United States,[1-3] despite widespread availability of asthma controller medications and standardized treatment guidelines.[4, 5] While deaths from asthma have declined,[6] 53.7% of children with asthma continue to experience an exacerbation each year[7] and the associated public health burden is substantial. Indeed, national surveillance data in children highlight disturbing trends of decreased primary care visits for asthma,[8] increased emergency department visits for asthma exacerbations,[9] and rising asthma costs estimated at $82 billion annually.[10-13] School absenteeism[14, 15] and decreased productivity in working parents[12] are also consequences of childhood asthma that negatively impact affected children and their families.[16, 17]

While the factors responsible for poor asthma symptom control are complex and include limited access to care,[18] poor adherence to preventative asthma medications[3, 19] and exposures to environmental allergens and irritants such as tobacco smoke,[20-22] it is also recognized that children with exacerbation-prone asthma are a heterogeneous group[23, 24] with differing clinical outcomes and longitudinal disease trajectories.[25, 26] **Symptoms (defined as subjective sensations)** can also be quite varied within and among affected children. Whereas some children have persistent, troublesome respiratory symptoms, others have respiratory

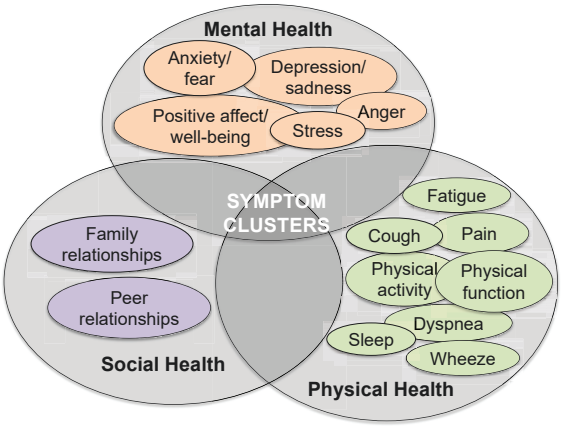

Figure 1. Symptom domains (large circles) and potential symptom clustering (overlap areas) in children with exacerbation-prone asthma.

symptoms only with upper respiratory infections.[26, 27]. Mental health symptoms and social health symptoms have been inadequately characterized in this population, but some children with asthma also report depression and anxiety[28, 29] and impaired family functioning and relationships[30, 31] that may further worsen asthma outcomes. However, prior studies are limited by a narrow focus on individual symptoms in isolation. To date, there has been no attempt to identify **symptom clusters (defined as two or more concurrent symptoms independent of other clusters) (Figure 1)** in children with exacerbation-prone asthma.

Poor understanding of symptom clusters is a major shortcoming in asthma symptom science. In other chronic disorders such as cancer, compared with a single symptom, symptom clusters of pain, fatigue, sleep disturbance and mood disturbance significantly worsen patient-reported outcomes of functional status and quality of life.[32] There is also emerging evidence that interventions for one symptom within a cluster (i.e., cognitive-behavioral therapy for pain) reduce the severity of other symptoms within that cluster (i.e., fatigue and sleep disturbance).[33] Because children with exacerbation-prone asthma rarely report a single symptom, greater knowledge of the assessment (and ultimately management) of symptom clusters in these children has the potential to significantly improve individualized treatment and clinical outcomes.

B. Study hypothesis/aims

We propose a 48-week cohort study (N=173) to test the overarching hypothesis that symptom clusters and their associated inflammatory and metabolic pathways predict corticosteroid treatment responsiveness (primary objective outcome) and quality of life (patient-reported secondary outcome) in children 6 up to 21 years with exacerbation-prone asthma.

Aim 1. Identify and phenotype symptom clusters and assess their temporal trajectories in children with exacerbation-prone asthma.
Hypotheses: A cluster of children with the poorest physical, mental, ***and*** social health symptoms will be identified by latent class analysis; this cluster will have stable symptom presentations over time.

Aim 2. Determine associations between symptom clusters and clinical outcomes in children with exacerbation-prone asthma.
Hypotheses: Children with the poorest physical, mental, ***and*** social health symptoms will have the poorest corticosteroid treatment responsiveness and quality of life.

Aim 3. Identify inflammatory and metabolic pathways underlying symptom clusters in children with exacerbation-prone asthma.
Hypotheses: Symptom clusters will be distinguished by systemic and airway inflammatory cytokine profiles and metabolomic biomarkers.

Dr. Fitzpatrick is also receiving supplemental funding to pursue two additional aims related to climate change. Climate change poses a significant threat to respiratory health in patients with asthma. The effects are direct, through extreme heat or humidity, and indirect, through

synergistic disturbances in air quality and airborne allergens. For example, rising temperatures accelerate production of pollutants such as ozone, fine particulate matter (PM2.5), polycyclic aromatic hydrocarbons, and volatile compounds, which alter airway function and host defense through epigenetic modifications in immune cells, airway eosinophil infiltration and epithelial inflammation. Heavy metals, nitrates and sulfur pollutants can also agglomerate on the surface of pollen grains and may increase allergenic potential and absorption of these toxicants once inhaled.

The effects of climate change and air pollution are not equal. **Children are more susceptible.** Children have a higher respiratory rate and minute ventilation, which increases pollutant inhalation, and more narrow airways, which amplify particulate trapping, absorption, and epithelial inflammation and oxidative stress. The negative effects of climate are increased further in **socially vulnerable populations**. Black and Hispanic children are more likely to live in lower opportunity neighborhoods due to systemic structural racism and historic red lining practices, are exposed to more pollutants, and have more severe asthma exacerbations attributable to air pollution. They are also projected to have a 1.3-fold risk of living in a neighborhood with the highest projected increases in asthma due to increased PM concentrations. In these neighborhoods, fears about crime and safety and limited recreational green space also force children indoors. However, poorer housing standards allow for increased infiltration of outdoor pollutants into the home. Impaired ventilation or filtration also results in 2-5 higher pollutant concentrations when compared to what is experienced outdoors.

Through supplemental funding, the study team will also test the central hypothesis that climate change-induced disruptions in air quality worsen asthma inflammation, symptoms, and longitudinal outcomes in school-age children with asthma and that these effects are mediated by social vulnerability. Two additional aims to be pursued are below:

Aim 4. Determine associations between climate, air quality, symptoms, and outcomes in children with exacerbation-prone asthma during two climate seasons.
Hypothesis: Children with greatest social vulnerability will have more air pollution exposure, increased symptoms, poorer lung function, more exacerbations and decreased QOL during each season.

Aim 5. Determine the biological impact of climate and pollutants in children with exacerbation prone asthma. Children with greatest social vulnerability and the highest air pollution exposure will have differentially expressed cytokines and metabolomic features.

This project involves a multidisciplinary team with a history of collaboration and is aligned with the NINR Strategic Plan to "*Advance Symptom Science Research*" in chronic conditions through biobehavioral research.[32, 34] The study population, children with exacerbation-prone asthma, has also been understudied in this regard. This project is expected to: 1) refine knowledge regarding the identification and clinical utility of symptom clusters in these children, and 2) identify mechanisms underlying symptom clusters that can be targeted in future interventional studies, to reduce the high morbidity and improve quality of life.

C. Significance

Importance and barriers. Asthma currently affects more than 6 million children less than 18 years in the United States (8.3% prevalence) and the majority of these children have poor symptom control.[7] In 2016, more than 3.2 million children with asthma experienced an exacerbation necessitating unscheduled care.[7] Exacerbations consist of narrowing of the airway lumen, inflammation, and mucus plugging that can be deadly. Even in non-fatal cases, exacerbations may lead to progressive loss of lung function.[35-37] To date, the specific factors (and associated symptom profiles) that contribute to asthma exacerbations in children are unclear.[38] Major barriers in the pediatric asthma field include inadequacies of animal models in recapitulating human asthma, a paucity of basic/translational studies, developmental differences that prohibit extrapolation of data from adults to children, and few studies of long-term outcomes in affected children.[39]

Knowledge gap. In June 2015, the NINR/Office of Rare Disease Research/NCATS held a workshop on "Advancing Symptom Science Through Symptom Cluster Research." While the majority of evidence presented at this workshop originated from oncology patients, it was recognized that symptom clusters also occur in other chronic conditions such as asthma and result in decrements in functional status and quality of life.[32] Studies of symptom clusters (and ultimately development of personized strategies for symptom management) could advance care. However, this area of research is only emerging; existing studies tend to be cross-sectional in nature, ignoring the temporal component of disease progression.[32] To date, there has also been no attempt to identify and phenotype symptom clusters in asthma. Limited symptom science in asthma is a major shortcoming given the chronicity and burden of the disorder that this study is designed to address.

D. Innovation

Novel concepts. Whereas other studies have focused on single symptom manifestations (i.e., wheeze or anxiety) in children with exacerbation-prone asthma, this would be the first study to:

- Identify and phenotype **symptom clusters** in children with exacerbation-prone asthma using validated, age-appropriate PROMIS measures and a novel statistical analytical technique in symptom science research, latent transition analysis, to address temporal symptom trajectories,
- Examine whether identified symptom clusters predict objective and patient-reported clinical outcomes (corticosteroid treatment responsiveness and quality of life) that are important to both physicians and affected patients, and
- Link symptom clusters with inflammatory/metabolic pathways that could be targeted with future interventions.

The results of this study could have a significant impact on the assessment and management of children with asthma through identification of high-risk children who might benefit from personalized medicine approaches. While national asthma treatment guidelines do recognize the importance of respiratory symptoms, these guidelines provide no guidance regarding the assessment and management of non-respiratory symptoms in affected children.[4, 5] To address these critical knowledge gaps, we will perform extensive clinical characterization and state-of-the-art laboratory analyses of children with exacerbation-prone asthma. We will also use an adaptation of the conceptual framework proposed by Brant et al.[40] that incorporates temporality **(Figure 2)**. Our framework proposes that: 1) children with exacerbation-prone asthma and a unique mixture of covariates possess an equally unique set of inflammatory/metabolic pathways that are each associated with symptoms, 2) those symptoms synergize positively or negatively in a symptom cluster that may or may not evolve over time, and 3) symptom clusters impact asthma outcomes.

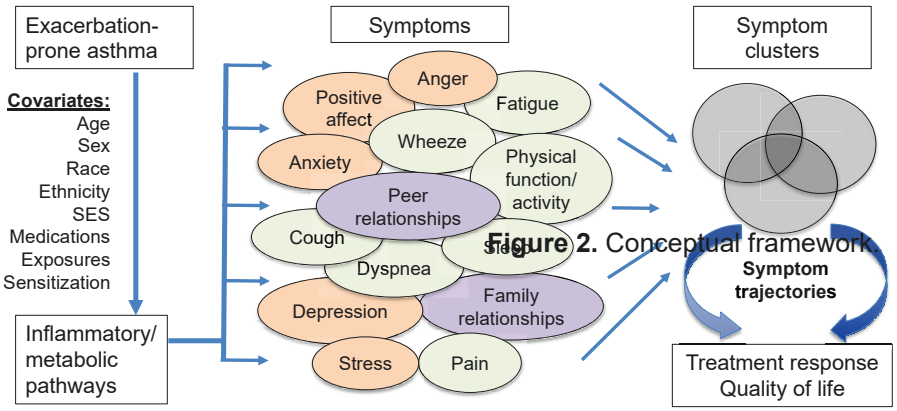

Figure 2. Conceptual framework.

<u>Application of novel technology.</u> The technology and the statistical techniques that we will utilize for this study are cutting-edge. We can multiplex up to 100 biomolecules with magnetic bead technology in 35 microliters of plasma.[41] With only 2.5 microliters of plasma (from a starting volume of only 40 microliters), we can perform chemical partitioning with hydrophilic interaction liquid chromatography (using a novel Merck SeQuant ZIC-HILIC column that is well-known for high-performance selectivity, reproducibility and robustness) for hundreds of small molecules including amino acids and metabolites. This may lead to identification of new biomarkers and opportunities for technology transfer. Our core laboratory facilities are also state-of-the-art (see letters of support). Ultimately, the analyses performed will capitalize upon a rich sample of highly characterized children with exacerbation prone-asthma. Thus we will have the rare opportunity to link symptom clusters with inflammatory and metabolic pathways and key outcomes of clinical relevance to both physicians and patients.

E. Study population

We will enroll a cohort of 173 children age 6 up to 21 years with exacerbation-prone asthma. Participants will be given the option to either: 1) complete a single study visit, with telephone/email contacts and a review of their electronic medical record for up to 48 weeks after enrollment, or 2) complete the 48-week cohort study with scheduled study visits. Participants who select option 2 with follow-up over 48 weeks will also have the option to complete visits 3, 4 and 5 by telephone.

Eligibility criteria include the following:

- Age 6 to less than 18 years at the enrollment visit
- Physician diagnosis of asthma, and
- History of an asthma exacerbation in the previous 12 months, defined as either:
  - Treatment with systemic corticosteroids,* or
  - Increase in rescue medication use (i.e., abuterol or inhaled corticosteroid) for 24 hours or more, or
  - One or more missed school days due to asthma symptoms, or
  - An unscheduled visit for asthma at either a physician's office, urgent care, hospital emergency room, or
  - Hospitalization for asthma.

  **In the event of a recent exacerbation treated with systemic corticosteroids or requiring hospitalization, the first study visit will be postponed until two weeks after the last dose of systemic corticosteroids (i.e., prednisone or prednisolone).*

These criteria should ensure a study population with sufficient disease burden, since several prior studies in children have identified these risk factors as significant predictors of poor asthma control and poor future outcomes.[42] Indeed, several prior studies in children have identified a recent exacerbation as the strongest risk factor for subsequent exacerbations regardless of disease severity or use of controller medications.[26, 43-46] In the third iteration of the NHLBI Severe Asthma Research Program (**SARP**, Fitzpatrick co-investigator), exacerbations occurred in 39.7% of enrolled children by one year (unpublished data), similar to reports in other cohorts.[47, 48] In SARP, an exacerbation in the previous year was also associated with an 8.94-fold increase in the odds of future exacerbation by one year (95% CI: 4.37, 18.69). Poor asthma symptom control at baseline was similarly associated with increased odds of future exacerbation (OR 2.65, 95% CI: 1.44, 4.82), but there was no additive effect with prior exacerbation. Therefore, we have elected to enroll participants irrespective of asthma symptom control. This will not only facilitate enrollment of participants, but will also promote symptom heterogeneity (which is essential for symptom clustering) and the likelihood of biomarker detection (since inflammation will also be heterogeneous).

Exclusion criteria for Option 1, a single study visit with telephone/email and electronic medical record follow-up for up to 48 weeks, include:

- Pregnancy,
- Current smoking,
- Congenital disorders or deformities of the chest wall, lungs or airways,
- History of premature birth <35 weeks gestation, or
- No medical care provider for asthma.

Exclusion criteria for Option 2, a 48-week cohort study with scheduled study visits, include:

- Previous allergic reaction to systemic corticosteroids,
- Hepatic, biliary, or renal disease that can interfere with drug metabolism/excretion,

- Chronic medical disorders that may increase the risk of drug-related injury, including osteogenesis imperfecta (increased risk of fracture with corticosteroids), or Crohn's disease, ulcerative colitis, juvenile rheumatoid arthritis, clotting disorders, or Factor deficiency (increased risk of bleeding with corticosteroid therapy),
- Pregnancy,
- Current smoking,
- Congenital disorders or deformities of the chest wall, lungs or airways,
- History of premature birth <35 weeks gestation,
- Unwillingness to receive triamcinolone,
- No medical care provider for asthma, or
- Planning to relocate before study visit 2.

F. Rationale for heterogeneous study population

In children, asthma symptoms are complex and result from a variety of triggers including allergens, irritants, pollutants, microbes, viruses, psychological stressors and emotions.[43, 44] These triggers induce airway changes such as mucus production, hyperresponsiveness, smooth muscle hypertrophy and constriction that result in respiratory symptoms (and consequent physical, mental and social health symptoms). However, triggers act through different pathways **(Figure 3)** and likely yield different symptom clusters. For example, allergen sensitization is associated with Type-2 (i.e., Th2) eosinophilic inflammation and is thought to be a pivotal risk factor for persistent and severe wheezing [49, 50] and airflow obstruction.[51] By contrast, children with non-Type-2 neutrophilic inflammation tend to wheeze only with respiratory viruses and often have cessation of asthma symptoms during later childhood.[52] Children with non-Type-2 inflammation also have increased IL-8 expression during the course of the exacerbation,[53-55] which may reflect innate immune impairment in response to viral[56] or bacterial[57] exposures.

Nonetheless, the paradigm of "Type-2" versus "non-Type-2" inflammation is not quite as simplistic as depicted and there can be synergy of triggers and pathways. For example, in addition to being a pivotal risk factor for asthma persistence, Type-2 inflammation has also been associated with increased severity of upper and lower respiratory tract infections and a greater risk of hospitalization for human rhinovirus infection[58, 59] Psychological stress can also skew T cells toward a Th2 phenotype, since norepinephrine suppresses Th1 cytokines (i.e., IFN-γ) via binding to adrenergic receptors.[60] Additionally, stress promotes DNA methylation and alters expression of numerous genes that regulate behavioral, autonomic, neuroendocrine, and immunological responses.[61] Chronic stress further exerts direct effects on airway smooth muscle through downregulation of the β2-adrenergic receptor,[62] possibly through dysfunction of the pituitary adenylate cyclase-activating polypeptide 1 pathway which has been previously implicated in children with anxiety[63] and asthma.[64] **We therefore anticipate that the synergy of physical respiratory symptoms with mental and social health symptoms augments inflammation and results in poorer asthma outcomes** compared to children with respiratory symptoms alone. Identification of symptom clusters and their biological features may ultimately advance knowledge of inflammation (and treatment) in children with exacerbation-prone asthma.

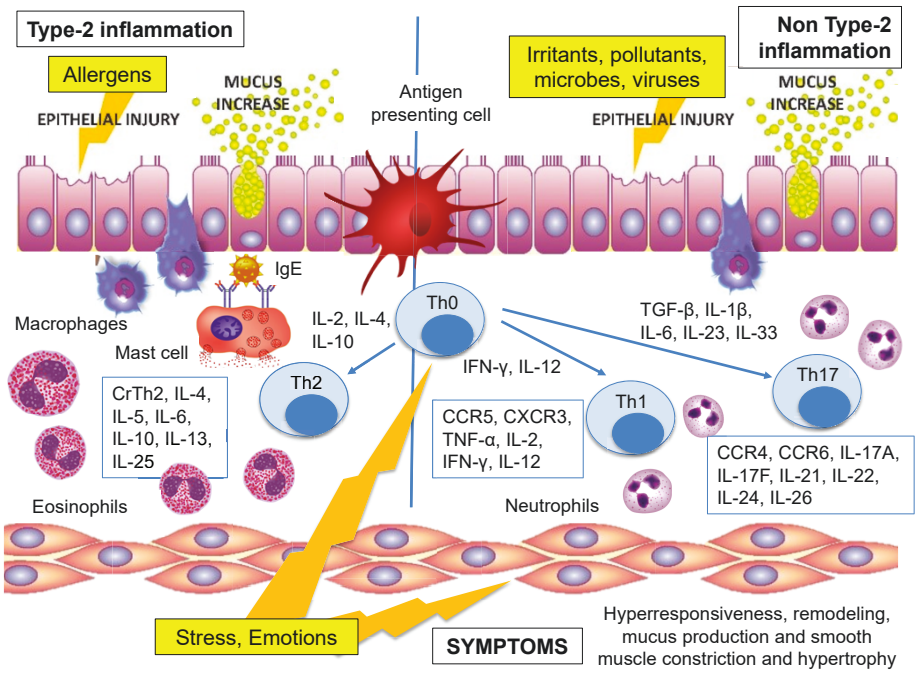

Figure 3. Asthma triggers (yellow) and pathways contributing to symptoms/symptom clustering.

G. Rationale for inflammatory measures

In clinical practice, many children with asthma have ongoing airway inflammation and poor symptom control despite best therapy attempts with controller medications such as inhaled corticosteroids. Inflammation is driven by white blood cell release of cytokines and chemokines, which in turn, promote generation of reactive oxygen species **(ROS)** and free radicals that regulate inflammation through complex interactions with cysteine residues on the surface of proteins. These protein modifications may or may not be reversible[65] and could result in sustained inflammation and tissue damage. For example, nitration and chlorination of catalase results in impairment of the enzymatic catalyzation and breakdown of hydrogen peroxide,[66] whereas nitration of manganese superoxide dismutase and glutathione-S-transferase inhibits

detoxification of superoxide and other electrophilic substances.[67, 68] Another example involves glutathionylation of the p50 subunit of the transcription factor, nuclear factor kappa B, which is associated with repressed DNA binding activity.[69]

There is also a wide body of literature demonstrating the inhibitory effects of ROS on corticosteroid responsiveness, through direct actions on the glucocorticoid receptor **(GR)**, resulting in a sustained pro-inflammatory state.[70] Ligand binding of the GR in the cytoplasm results in translocation of the activated GR complex into the nucleus, where it binds to the DNA glucocorticoid response element and regulates gene transcription through recruitment of histone deacetylase **(HDAC)** complexes.[71, 72] Whereas increased histone acetyltransferase activity promotes acetylation of histones and inflammatory gene transcription, removal of acetyl groups by HDACs results in gene silencing.[73, 74] Like any protein, the GR contains several cysteine residues that maintain its structure and function; these are also highly sensitive to ROS and can form disulfide bonds that inhibit ligand binding to the receptor[75] or cause the GR to assume a folded conformation,[76] inhibiting nuclear translocation and DNA binding.[77, 78] These actions might account for prior observations of GR dysfunction (with unattenuated proinflammatory cytokine release and increased levels of glucocorticoid hormones) in patients with severe asthma[79-82] and also in patients with chronic stress and anxiety,[83] depression[84], and fatigue.[85] **We anticipate that inflammation will be greatest in the cluster of children with the poorest physical, mental, *and* social health symptoms; we also anticipate that this inflammation (from symptom synergy) will be less responsive to systemic corticosteroid treatment, resulting in persistently poor quality of life.**

H. Rationale for triamcinolone acetonide injection

At the completion of the baseline visit, a subset participants who consented to the 48 week cohort study will receive intramuscular triamcinolone acetonide (Kenalog,® 1 mg/kg, up to 40 mg maximum) administered deep in the gluteal muscle by a trained registered nurse. Corticosteroids are the cornerstone of treatment for asthma;[4, 5] intramuscular triamcinolone was selected as the treatment of choice over oral prednisolone or inhaled corticosteroids to allow for characterization of corticosteroid insensitivity without the confounding effects of poor adherence or inadequate drug delivery or absorption. We further considered the pharmacology of corticosteroids. Because triamcinolone has 20% more glucocorticoid activity than prednisolone,[86] triamcinolone 1 mg/kg will ensure that participating children have a sufficient dose of corticosteroid to achieve the desired potential anti-inflammatory effects. In previous studies of adults, a single dose of 80 mg of triamcinolone significantly improved lung function and asthma symptoms in the majority of patients.[87-89] Given several previous studies in children[90-95] and our own published studies of triamcinolone responsiveness in children with asthma,[79, 96, 97] a dose of 1 mg/kg of triamcinolone should elicit a clinical response. Because inclusion is limited to children with a recent exacerbation (many of whom will have poor symptom control and all of whom are at risk for future exacerbation), all participants have the potential to benefit from this procedure. The safety of our dosing strategy has also been well established. In our previous study (Fitzpatrick R01NR012021),[96] the triamcinolone injection was well tolerated and no unexpected adverse events were reported. Expected adverse events

within 24 to 48 hours of triamcinolone receipt included tenderness (25%), warmth (3%), and mild itching (2%) at the injection site. No activity limitation from injection pain was reported and there were no reports of subcutaneous atrophy following the injection. Other unrelated adverse events included dizziness (n = 1), atopic dermatitis (n = 1), facial rash (n = 1), headache (n = 1), nausea and vomiting (n = 1), pharyngitis (n = 1), and cough (n = 1). The distribution of adverse events did not differ according to asthma severity or treatment response group, and each event resolved within 14 days.[96] In the proposed study, the one-time administration of 1 mg/kg of triamcinolone (40 mg maximum) has documented safety and will result in a significantly smaller cumulative corticosteroid dose than that used by other pediatric investigators.[90-92]

I. Overview of study design

We will enroll a cohort of 173 children age 6 up to 21 years with exacerbation-prone asthma. Participants will be given the option to either: 1) complete a single study visit, with telephone/email contacts and a review of their electronic medical record for up to 48 weeks after enrollment, or 2) complete the 48-week cohort study with scheduled study visits **(Appendix 1)**. Participants who select option 2 with follow-up over 48 weeks will also have the option to complete visits 3, 4 and 5 by telephone.

Option 1: A single study visit with telephone/email and electronic medical record follow-up for up to 48 weeks

Visit 1 (enrollment) will involve informed consent and participant characterization for the purpose of symptom cluster identification. After this visit is completed, the participant's medical record will be reviewed for up to 48 weeks to assess for asthma exacerbations requiring medical treatment. Telephone/email contacts will also be attempted at 24 weeks and 48 weeks after this visit to ensure that asthma exacerbations treated outside of a healthcare facility or other asthma exacerbations that are managed at home are accounted for.

Option 2: A a 48-week cohort study with scheduled study visits

Visit 1 (enrollment) will involve informed consent and participant characterization for the purpose of symptom cluster identification and receipt of systemic triamcinolone acetonide. Visit 2 will involve assessment of triamcinolone responsiveness. Visits 3, 4 and 5 are follow-up visits to assess the temporal stability of the symptom clusters. Visits 3, 4 and 5 can be completed in person or over the telephone.

This research is considered a clinical trial, because it involves an intervention (triamcinolone injection), and the study is designed to evaluate the efficacy of the intervention on symptoms and quality of life. The Emory Office of Clinical Research will facilitate the registration, periodic updates, and results reporting within ClinicalTrials.gov for the duration of the study.

J. Outcome measures

Asthma exacerbations are an outcome of interest for all participants.

For participants enrolled in the 48-week cohort study, the primary outcome is corticosteroid treatment responsiveness, assessed 14 days after systemic triamcinolone administration (at visit 2). Responsiveness will be determined from the mean difference in the Asthma Control Questionnaire **(ACQ)** score between visit 1 (baseline) and visit 2 (14 days), with a reduction of 0.5 considered a minimally important difference.[98, 99]

The secondary outcome for participants enrolled in the 48-week cohort study is quality of life, assessed by the PROMIS Pediatric Asthma Impact Scale **(PAIS)** at visits 3, 4 and 5. As with all PROMIS measures, the PAIS is scored on the T-score metric, with higher scores reflecting more of the concept being measured. On the T-score metric, 50 is the mean of the reference population and 10 is the standard deviation; thus scores of 40 and 60 are one standard deviation lower and higher than the mean of the reference population, respectively.

K. Visit-specific procedures

Detailed procedures are listed below and also appear in the Appendix (Appendix I).

Option 1: A single study visit with telephone/email and electronic medical record follow-up for up to 48 weeks

Baseline visit (Visit 1), study week 0

1. Informed consent obtained
2. Eligibility determined based upon inclusion and exclusion criteria
3. Brief physical examination (by RN) including height and weight performed
4. Medical history obtained
5. Urine pregnancy testing
6. Administer Demographic questionnaires
7. Asthma control evaluation (Asthma Control Questionnaire and Asthma Control Test)
8. Administer PROMIS symptom questionnaires
9. Measure exhaled nitric oxide
10. Perform spirometry with oscillometry (with bronchodilator reversibility)
11. Perform venipuncture for:
    a. Total IgE (Children's Healthcare of Atlanta)
    b. Inhalant allergen specific IgE (Children's Healthcare of Atlanta)
    c. Complete blood count with differential (Children's Healthcare of Atlanta)
    d. Plasma and white blood cells for aim 3 (Fitzpatrick laboratory)
12. Perform sputum induction with samples stored for aim 3 (Fitzpatrick laboratory)

Telephone/email contact at 24 weeks and 48 weeks

1. Medical history obtained
2. Asthma control evaluation (Asthma Control Questionnaire, Asthma Control Test, Pediatric Asthma Impact Questionnaire)

Option 2: A 48-week cohort study with scheduled study visits

Baseline visit (Visit 1), study week 0

1. Informed consent obtained
2. Eligibility determined based upon inclusion and exclusion criteria
3. Brief physical examination (by RN) including height and weight performed
4. Medical history obtained

5. Urine pregnancy testing
6. Administer Demographic questionnaires
7. Asthma control evaluation (Asthma Control Questionnaire and Asthma Control Test)
8. Administer PROMIS symptom questionnaires
9. Measure exhaled nitric oxide
10. Perform spirometry with oscillometry (with bronchodilator reversibility)
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    b. Inhalant allergen specific IgE (Children's Healthcare of Atlanta)
    c. Complete blood count with differential (Children's Healthcare of Atlanta)
    d. Plasma and white blood cells for aim 3 (Fitzpatrick laboratory)
12. Perform sputum induction with samples stored for aim 3 (Fitzpatrick laboratory)
13. Triamcinolone acteonide injection

Steroid Responsiveness visit (Visit 2), Study Week 2 (window +7 days)

1. Brief physical examination (by RN) including height and weight performed
2. Medical history obtained
3. Urine pregnancy testing
4. Asthma control evaluation (Asthma Control Questionnaire and Asthma Control Test)
5. Administer PROMIS symptom questionnaires
6. Measure exhaled nitric oxide
7. Perform spirometry with oscillometry (with bronchodilator reversibility)
8. Perform venipuncture for:
    a. Complete blood count with differential (Children's Healthcare of Atlanta)
    b. Plasma and white blood cells for aim 3 (Fitzpatrick laboratory)
9. Perform sputum induction with samples stored for aim 3 (Fitzpatrick laboratory)

Follow-up visits (Visits 3 and 4), Study Week 16, 32 (window +4 weeks)

1. Brief physical examination (by RN) including height and weight performed*
2. Medical history obtained
3. Urine pregnancy testing*
4. Asthma control evaluation (Asthma Control Questionnaire and Asthma Control Test)
5. Administer PROMIS symptom questionnaires
6. Measure exhaled nitric oxide*
7. Perform spirometry with oscillometry*

*procedure will not be performed if follow-up occurs by telephone

Final follow-up visit (Visit 5), Study Week 48 (window +4 weeks)

1. Brief physical examination (by RN) including height and weight performed*
2. Medical history obtained
3. Urine pregnancy testing*
4. Asthma control evaluation (Asthma Control Questionnaire and Asthma Control Test)
5. Administer PROMIS symptom questionnaires

6. Measure exhaled nitric oxide*
7. Perform spirometry with oscillometry (with bronchodilator reversibility)*
8. Perform venipuncture:*
    a. Complete blood count with differential (Children's Healthcare of Atlanta)
    b. Plasma and white blood cells for aim 3 (Fitzpatrick laboratory)
9. Perform sputum induction with samples stored for aim 3 (Fitzpatrick laboratory)*

*procedure will not be performed in follow-up occurs by telephone

L. Procedural details

Asthma control evaluation. Asthma control and associated respiratory symptoms will also be assessed with the Asthma Control Questionnaire **(ACQ)** and the Asthma Control Test **(ACT)**. The ACQ can be completed by children down to 6 years with strong measurement properties when administered by a trained interviewer.[98] The ACQ is sensitive to short-term changes in asthma control and has well-documented reliability and cross-sectional and longitudinal validity in diverse asthma populations across a wide range of severities.[100] This 7-item questionnaire includes questions related to daytime and nocturnal symptoms, short-acting bronchodilator use, and lung function during the clinic visit on that day. A higher score indicates poorer asthma control.[101] The ACT can be completed directly by children 12 years and older; children 6-11 years will complete the Childhood ACT **(C-ACT)**. Both tools have been extensively validated and have excellent measurement properties.[4, 102-107] For both tests, individual items about respiratory symptoms (5 and 7 in total for ACT and C-ACT, respectively) are summed; scores of 19 or less reflect uncontrolled asthma.

PROMIS symptom questionnaires. Symptoms will be assessed with validated PROMIS measures for children. PROMIS measures will be completed directly by children age 8-21 years, with caregiver assistance for children 6-7 years. The following PROMIS short-form measures will be utilized:

- Pediatric Life Satisfaction (4a)
- Pediatric Meaning and Purpose (4a)
- Pediatric Physical Stress Experience (4a)
- Pediatric Physical Activity (4a)
- Pediatric Positive Affect (4a)
- Pediatric Psychological Stress Experiences (4a)
- Pediatric Family Relationships (4a)
- Pediatric Asthma Impact (8a)
- Pediatric Anger (5a)
- Pediatric Anxiety (8a)
- Pediatric Depressive Symptoms (8a)
- Pediatric Fatigue (10a)
- Pediatric Peer Relationships (8a)

Spirometry with Oscillometry. Spirometry for measurement of lung function will be performed with a portable spirometer (MGC Diagnostics® CPFS/D USB™) according to technical standards.[108] The best of three forced vital capacity maneuvers will be interpreted according to population reference standards.[109] Oscillometry will be performed in cooperative participants (typically age 3 and older) with the TremoFlo C-100 device (Thorasys). Participants will wear a noseclip and take tidal breaths through a mouthpiece for 20 seconds. Airway resistance and reactance will be recorded.

Bronchodilator reversibility testing. Participants will receive 4 inhalations of albuterol from a metered dose inhaler (MDI) and valved holding chamber and spirometry will be repeated 15 minutes later.

Exhaled nitric oxide. Exhaled nitric oxide will be measured with a commercial device (NIOX VERO®, Circassia) using recommended standards.[110] The procedure is non-invasive and considered to be an indirect measurement of airway inflammation. Participants will be instructed to take in a deep breath and blow air out at a constant pressure. One measurement will be taken and recorded.

Venipuncture. Blood will be collected by venipuncture as outlined in the table below. Participants will have the option to receive anesthetic cream prior to venipuncture.

Test	Where performed	Test code	Visits	Collection tube	Blood collection volume
Total IgE and Specific IgE	Children's	TIGE, INHALA Panel	1	Gold top SST	1.5 mL
Complete blood count with differential	Children's	CBCD	1,2,5	Lavender EDTA	1 mL
Cytokines, metabolomics	Fitzpatrick lab		1,2,5	Lavender EDTA Blue tempus	~6 mL ~3 mL (+6 mL solution)

Table 2. Blood collection procedures

Urine collection for pregnancy testing. Urine will be collected in females of child-bearing potential by clean-catch methods for the purpose of pregnancy testing. Unused urine will be discarded.

Sputum induction. Sputum induction will be performed in participants 12 years and older after pre-treatment with 4 inhalations of albuterol sulfate delivered through a MDI and holding chamber. 3% saline (substitutions permitted) will be inhaled through an ultrasonic nebulizer for a maximum of 12 minutes, with breaks every 2 minutes. To ensure safety, the procedure will only be performed on children with a post-bronchodilator FEV1 of >70% predicted; spontaneous sputum samples may be collected on children with an FEV1 below

these limits. A physician will be available during the challenge; study staff will calculate and record the FEV1 value that equals both a 10% and 20% fall in lung function based upon the recorded post-bronchodilator FEV1 values. Participants will not discharged until their FEV1 is within 10% of their post bronchodilator FEV1 and will be given albuterol sulfate (2 inhalations as needed) for this purpose. Sputum samples will be processed by the Fitzpatrick laboratory.

Triamcinolone acetonide injection. Each participant who meets inclusion/exclusion criteria will receive triamcinolone acetonide intramuscularly at the end of visit 1. Children 6-21 years will receive 1 mg/kg triamcinolone acetonide (up to 40 mg maximum). Triamcinolone acetonide will be administered as a single intramuscular dose deep in the gluteal region.

M. Non-study drugs

Because this study is not a treatment trial, any medication considered necessary for the medical management and welfare of the participant may be given at any time. Participation in this study will also not prohibit access to new or novel therapies. Medications will not be withheld.

N. Drug supplies

The study intervention, triamcinolone acetonide (Kenalog® for injection), is FDA-approved for the treatment of asthma in children. There is no IND associated with this study. The drug will be administered consistent with the instructions listed in the accompanying package insert.

The following drug supplies will be provided by the research pharmacy:

- Triamcinolone acetonide (Kenalog® for injection), 1 mg/kg, 40 mg max
- Albuterol sulfate (90 mcg/inhalation) for bronchodilator reversibility testing and sputum induction
- 3% saline for sputum induction (substitutions and compounding permitted)

O. Study failure

Given the observational nature of this study, there are no specified criteria for study failure. Communication with each participant's primary caregiver will be ongoing.

P. Availability of study personnel

The study physician (Dr. Claudia Morris) will be available for discussion with families 24 hours/day should uncertainty or questions arise regarding the study. Communication regarding the study, receipt of triamcinolone, and laboratory results will be sent to the child's primary care provider. At all study visits, coordinators will emphasize the importance of maintaining contact with the child's primary care provider.

Q. Recruitment and Retention

Prospective enrollees will be enrolled at Children's Healthcare of Atlanta, which serves an urban population with diverse demographic features including race, ethnicity and socioeconomic status. Eligible participants will be identified from within the Children's Healthcare of Atlanta system by ICD-10 codes for wheezing and asthma. We will obtain a HIPAA waiver from the Emory and Children's Healthcare of Atlanta IRBs for this purpose (i.e., identification of eligible participants). Once the list of eligible participants is generated, IRB-approved mailings will be sent to these families informing them of the study and the phone number/email address of the study coordinator to use if they would like more information. We will also run printed advertisements in Atlanta Parent Magazine and contact previous study patients/caregivers (who have given us permission to do so) to see if they are interested. We anticipate no problems with our recruitment approach since the burden of asthma and wheezing disorders is significantly higher in Georgia versus other states. Indeed, the current asthma prevalence in children in Georgia is 12.1% compared to a pooled estimate of 9.0% in 38 other states.[111] Our planned recruitment strategies were also previously utilized in our NHLBI AsthmaNet studies and yielded a large number of eligible participants who were interested in research. Emory University, under the leadership of Dr. Anne Fitzpatrick, was one of the lead recruitment sites for multiple NHLBI longitudinal asthma studies.

Retention of research participants is essential for this longitudinal study. In our experience, the best retention method for participants in long-term clinical studies is education and continual "reminders." For all participants enrolled in our clinical research studies, including the study proposed here, newsletters are distributed every 3 months (by mail or email) that include educational material about respiratory health, bulletins of important issues that affect patients (flu vaccines), and study updates. We also have several additional methods to encourage continued involvement in our studies, including distribution of materials with our logos and contact numbers and other continual "reminders" of study involvement (refrigerator magnets, pens, mugs, t-shirts or blankets). Other retention efforts will focus on ease of the majority of visits and informational rewards (such as asthma education). Visits will be at times convenient to the parents, many of whom are employed. Appointments after school will also be available. Study team members will be available to answer questions about asthma. The study physician will also be available by phone during off-hours to aid in the management of adverse events including asthma exacerbations.

Participants will also be paid for their time and participation in the study. While we will enroll 173 children, we expect some dropout to occur. Study visits will be pro-rated accordingly, and families will be paid at the end of each visit. Parking passes are also provided so that families do not have to pay for parking. In the event that transportation is a challenge, we also pay for taxis to pick up participants at their homes and drive them to the study site.

R. Risks and benefits

Asthma exacerbation. The inclusion criteria require that all participants have a history of asthma exacerbation, and therefore we can expect a similar pattern of illness (i.e., a high probability of subsequent exacerbation) in the year of study enrollment. While we do not anticipate that children enrolled in this study will have significant worsening of asthma symptoms as a result of the study procedures, children enrolled in this trial may develop an asthma exacerbation of sufficient severity to warrant emergency medical care. Hospitalization will be considered a Serious Adverse Event, and be reported to local IRBs in the usual manner.

Parent-completed questionnaires (medical history, demographics) and child-completed questionnaires (asthma control, PROMIS symptoms). Participants will complete a variety of questionnaires at each study visit. There are minimal risks to answering these questionnaires, although they may be considered long with repeating questions. For certain questions (such as those that ascertain family education or income level), participants will be allowed to decline from responding. Some of the questionnaires pertaining to depression and anxiety may also highlight significant yet undiagnosed mental health disorders. We will encourage all children to discuss their concerns with their parents, teachers or physicians. In more severe cases, we will also identify available support services through Children's Healthcare of Atlanta and share these with participating children and their families. In severe cases where suicidal ideation is stated or presumed, emergency referrals will be made in accordance with Children's Healthcare of Atlanta policies and procedures.

There is a rare risk that information from a study can be disseminated in ways that can risk the privacy of a person with attendant social and occupational harm. However, this risk is not as great for conditions like asthma, which are common. Regardless, care will be taken to ensure confidentiality. Research records will be held in locked cabinets or secure storage rooms. All transmission of data to the study co-investigators for analysis is by study ID code only. Publication of results will involve aggregate data only so that individual participants cannot be identified. On occasion it may be necessary, for legal reasons or for good clinical practice, for third parties such as the FDA, IRB, or NIH to review medical records that are identified by name. However, this is not a common occurrence, and every effort will be made by the investigators to provide confidentiality for such audits.

Urine pregnancy testing. Women of child-bearing potential will submit a urine sample at each study visit for pregnancy testing. Pregnancy is an exclusion since intramuscular triamcinolone acetonide is classified as category C for pregnant women and may be associated with virilizing effects on the human fetus. In the event that a female participant becomes pregnant during this study, she will be withdrawn immediately and referred to her primary physician for further treatment. The perinatal outcomes of any pregnancies will be reported. This will be considered an adverse event and will be reported to the local IRB. The ascertainment of sexual activity, use of birth control, and administration of pregnancy tests to girls age 11 and older is a sensitive issue. In the NHLBI's Childhood Asthma Management Program (CAMP),[112] parents were informed in the initial consent process that the study

coordinators or physicians may ask the participants confidential questions about their health or administer pregnancy tests. Our medical team and research staff are versed in the sensitive acquisition of this information from adolescents and exchange experiences and effective communication techniques. Whether a pregnancy needs to be reported to the participant's parent (legal guardian) is a matter of local hospital policy and state law that will not be addressed as a separate study policy, but we strongly advocate the practice of informing participant's parents with the consent of the participant and referral for appropriate prenatal care.

Spirometry with Oscillometry. Participants will undergo spirometry with oscillometry (lung function tests) at each study visit. These tests can cause children to become light-headed or dizzy. The risk of syncope is minimized by having participants sit during the procedure. These tests may also result in shortness of breath or cough. Treatment (albuterol sulfate) will be available if these symptoms occur. However, in the Childhood Asthma Management Program,[113] in approximately 12,000 spirometry tests in children age 6-15 years, there were no serious adverse events.

Bronchodilator reversibility testing. Children will undergo spirometry with bronchodilator reversibility testing at visits 1, 2 and 5. This involves administration of 4 inhalations of albuterol sulfate through a metered dose inhaler with a mouthpiece. Side effects of albuterol administration may include nervousness, hyperactivity, increased heart rate, nausea, or headache. These side effects are temporary and should resolve within 15 to 30 minutes.

Exhaled nitric oxide determination. Exhaled nitric oxide testing will be performed at each study visit. Exhaled nitric oxide testing can cause similar risks as spirometry but these risks are less intense and are even more rare. Exhaled nitric oxide tests involve breathing into a device at a flow rate that is held constant by an in-line resistance valve. As a result, participants occasionally perceive an inability to freely exhale, or similar to spirometry, may complain of light-headedness.

Venipuncture. Blood will be collected by venipuncture for the following: 1) total and allergen-specific IgE, 2) complete blood count with differential, 3) cytokine analyses, and 4) metabolomic analyses. The blood draw will involve a maximum of 12 mL (about a tablespoon) and this volume is well below our local IRB guidelines of no more than 1 mL/kg. Therefore, the risk of anemia is extremely small. This blood will be collected with a butterfly needle and aliquoted into the necessary tubes to avoid participant burden related to multiple needle insertions. A maximum of three attempts will be made to obtain the blood sample. The volume of blood is not expected to be an issue. To minimize risk to the participants, fasting will not be required. Risks of venipuncture can include brief pain during the needle insertion, local bruising at the collection site, and small hematomas. Rarely, children can get light-headed or even faint. Venipuncture can also cause anxiety in children. Therefore all pediatric venipunctures will only be performed by pediatric phlebotomists who are trained in pediatric blood collection methods, which differ from those in adults by the use of topical anesthetic creams and small gauge

needles. In our experience from several local and multi-center pediatric asthma studies which involve blood draws, the most effective strategy for dealing with children is honest explanation of the procedure and associated feelings/sensations (such as “pressure, prick, pinch”), how long those sensations last, strategies to overcome those sensations (singing, thinking about “happy” thoughts), and other coping strategies.

Sputum induction. Sputum induction will be performed in participants 12-21 years of age at visits 1, 2 and 5 for cytokine and metabolomic assays. Induced sputum can lead to increased wheezing, coughing and dyspnea and thus, sputum induction will be halted if participants become increasingly symptomatic or do not tolerate the procedure. Our manual of procedures includes frequent measurements of peak expiratory flow to prevent excessive bronchospasm during sputum induction and the safety of this procedure in moderate-severe obstructive lung disease (asthma) has been published.[114] Additionally, the following safety procedures will be followed: 1) a physician will be available during the procedure, 2) all participants will be pre-treated with 4 inhalations of albuterol before the procedure, and 3) only participants with a post-bronchodilator forced expiratory volume in one second **(FEV1)** of >70% predicted will undergo the procedure. Spontaneous sputum samples may be collected on children with an FEV1 below these limits. Study staff will calculate and record the peak flow and FEV1 value that equal both a 10% and 20% fall in lung function based upon the recorded post-bronchodilator peak flow and FEV1 values. Children will not be discharged until FEV1 is within 10% of their post bronchodilator FEV1.

Triamcinolone injection. Triamcinolone acetonide injectable suspension (Kenalog®, 40 mg/mL) provides a synthetic corticosteroid with anti-inflammatory action. Triamcinolone acetonide is FDA-approved and indicated for children 6 years of age and older with asthma. The suggested initial dose for children 6-12 years of age is 40 mg, although dosage depends more on the severity of symptoms than on age or weight. For this protocol, children will receive 1 mg/kg in one intramuscular dose (up to 40 mg maximum). Although local side effects of intramuscular administration have been reported, including pigmentation changes and subcutaneous and cutaneous atrophy, these side effects are minimized by deep injection into the gluteal muscle. All triamcinolone acetonide injections will be administered by an experienced pediatric registered nurse. Systemic side effects with a single triamcinolone acetonide injection (40 mg max) are rare and triamcinolone acetonide injection is thought to be generally safe in children. Recent studies of much higher doses of intramuscular triamcinolone acetonide in children with nephrotic syndrome (~2 mg/kg)[115] and difficult-to-treat asthma (60 mg)[90, 91] have revealed little side effects and good tolerance of the medication, with no reported incidence of cataracts, diabetes, added weight gain, or arterial hypertension. Although repeated use of triamcinolone was associated with slightly decreased growth velocity during the active treatment phase (2 mg/kg injection each month for up to 36 months), growth velocity returned to normal after discontinuation of triamcinolone therapy.[115] Nonetheless, participating children will be screened thoroughly by the study physician for the presence of any co-morbid conditions that may increase the risk of triamcinolone acetonide administration. Children receiving triamcinolone acetonide will be educated about signs of potential complications and will have

24-hour access to an on-call pediatric physician. Participants will also undergo a comprehensive evaluation 2 weeks after the triamcinolone acetonide is administered.

Triamcinolone acetonide is a pregnancy category C medication and will not be administered to pregnant adolescents due to potential virilizing effects on the fetus.

Children receiving triamcinolone acetonide injection can experience localized pain at the injection site. This risk will be minimized through the application of topical ethyl chloride anesthetic prior to injection.

Potential benefits. While some study participants may receive no direct benefit from participating in this study, others may benefit from the close monitoring of their respiratory health, specialized asthma education, and general evaluation of their condition, including aeroallergen sensitization profiles. Some families also achieve psychological benefit from participating in an important research study and from interaction with the study staff. There is a possibility that the knowledge obtained from participation in this study (i.e., allergen sensitization profiles, lung function results, symptom profiles and corticosteroid treatment responsiveness) will directly benefit the majority of the participating children and their families.

All research procedures will be completed free of charge to the research participants and their families. All research data will also be shared with the caregivers (or the legal guardians). Data that may influence the clinical care of the participant, such as allergen sensitization reports and complete blood counts, will also be shared with the participant's primary care physician. Every child enrolled in this research protocol will also undergo a medical respiratory evaluation at several time points and the information obtained from this study may be useful in the development of specialized respiratory care. It is also possible that the knowledge obtained from this study, such as identification of biomarkers, may assist in the creation of novel asthma therapies in the future.

S. Data and Safety Monitoring Plan

This protocol has a Data and Safety Monitoring Plan that was reviewed and approved by the NIH/NINR and the IRB. This Plan is available as an attachment in the IRB application and will adhere to all aspects of the protocol. The DSMP attachment provides an overall framework for monitoring including :1) Monitoring Entity, 2) Procedures for Monitoring Safety and Minimizing Risk, 3) Data Quality, Management, Confidentiality and Security, 4) Adverse Events and Unanticipated Problems, and 5) Assessment of External Factors or Relevant Information.

T. Sample size

The sample size is 173 participants age 6 up to 21 years, which accounts for a conservative attrition rate of 15% (final N=147). The following assumptions were used for sample size calculations:

- Identification of 4 symptom clusters resulting in 3 degrees of freedom,

- >80% desired power for a chi-square test of multiple proportions,
- Two-tailed significance level of 0.05 if the effect size (W) is small-to-medium by Cohen's convention[116] and equivalent to W=0.275 (small effect: W=0.10, corresponding odds ratio=1.49; medium effect: W=0.30, corresponding odds ratio=3.45), and
- Sample size allocation ratio of 1-2 per cluster group and sample sizes of at least 20 per group.

The sample size calculations with the above parameters should achieve at least 80% power as shown in **Table 3.**

Drop-out	df	Power if effect size is:				
		0.250	0.275	0.300	0.325	0.350
10% (N=156)	3	0.749	0.833	0.897	0.941	0.969
	4	0.703	0.795	0.867	0.920	0.956
	5	0.664	0.760	0.840	0.900	0.942
12.5% (N=151)	3	0.734	0.820	0.886	0.933	0.963
	4	0.687	0.780	0.855	0.911	0.949
	5	0.648	0.744	0.826	0.889	0.934
15% (N=147)	3	0.721	0.808	0.877	0.926	0.959
	4	0.674	0.767	0.844	0.903	0.943
	5	0.634	0.731	0.814	0.878	0.927

Table 3. Estimated power.

For the secondary outcome, The sample size of 173 children necessary for the primary outcome should achieve >85% power to detect a difference of 0.2 between group means (considered a small effect size *d*)[116] with standard deviation of 0.20. Power >80% is achieved if the mean difference between groups and associated standard deviation is slightly larger but still small (0.25-0.30). In our prior study of triamcinolone administration to children with asthma,[96] mean (± standard deviation) quality of life scores (on a different instrument, the Asthma Quality of Life Questionnaire) were 6.17 (± 0.75) and 5.07 (± 0.99), respectively. If similar differences are noted between two symptom cluster groups, we should have >90% power for the primary outcome and >95% for the secondary outcome if the groups have at least 20 participants. With smaller group sizes of only 13 participants, power would be >81% and >89% for the primary and secondary outcomes, respectively.

U. Statistical analysis plan

Plan for parent R01 study (aims 1-3)

Latent transition analysis **(LTA)** is a cluster-based model that allows for integration of repeated data.[117] Discrete latent classes of individuals are identified based on their shared characteristics across a set of observed categorical variables and models are allowed to change over time between classes. Parameters estimated in LTA include class membership probabilities and item response probabilities, which are used to characterize the group structures. LTA also produces a transition probability matrix, estimating probabilities of membership in the same group at each time point (entries along the diagonal) and probabilities of transitioning to a different group over time (entries off the diagonal).[118, 119] Parameters are combined mathematically into a likelihood function, and the set that maximizes the likelihood function determines the membership of each individual in one of the latent classes.

Because LTA is aimed at characterizing transition over time, it requires variability in all variables. Therefore, we will perform LTA with patient-reported symptoms (global, mental, social and physical health categories) and measures of asthma disease activity (ACQ, ACT, lung function, exhaled nitric oxide, blood eosinophils) measured at baseline and visits 3, 4 and 5 **(see Appendix 1)**. Age, sex, race, ethnicity, socioeconomic status, medications, exposures and allergic sensitization measured at the baseline visit will be considered stable and treated as covariates in the LTA model.[120] All participants will be included in the analysis since missing data for the variables are handled in the procedure, with data assumed to be missing completely at random.[121] The test of the null hypothesis that data are missing completely at random (Missing Completely At Random test in the SAS LTA procedure) will be evaluated; if non-significant, missingness does not affect the interpretation of the results. The number of classes will be statistically determined using the Bayesian information criterion **(BIC)** and class prevalence if BICs are of a similar magnitude, to avoid a solution leading to low prevalent groups (<5%). To assess the stability of the group structure, goodness of fit of the "constrained" LTA model in which identical item–response probabilities across time are estimated (i.e., the same cluster structure is forced into multiple time points) will be compared with the "unconstrained" LTA model in which item response probabilities are estimated at each time point. To further address sex as a biological variable, models will also be fitted by sex, with statistical comparison of results observed in boys and girls. All analyses will be performed with SAS software version 9.4 (SAS Institute) using the PROC LTA procedure.[122, 123]

The primary outcome will be assessed after assignment of individuals to the latent classes that they had the highest probability of belonging to (based on the posterior probability provided by the model at baseline). A straightforward analysis of the primary outcome will follow and involves comparison of the proportion of children achieving a minimally importance difference in ACQ of at least 0.5 in each of the latent classes via a Chi-Square test.

The secondary outcome, quality of life, will be compared with generalized estimating equations at visits 3, 4 and 5 using latent class assignment as the predictor. Analyses will be evaluated in light of pertinent covariates such as age, sex, race, ethnicity, socioeconomic status, medications, exposures and allergic sensitization. A 0.05 significance threshold will be utilized for secondary analyses without adjustment for multiple comparisons.

Metabolomic data, cytokine data, and amino acid data will be quantitative, linear mixed effects models will be used to determine the association between these symptom cluster group assignment.

Plan for supplemental R01 funding (aims 4-5)

Aim 4 will determine associations between neighborhood air quality indices (predictor variables) and patient-reported asthma control and symptoms, lung function, exacerbation rates and QOL (outcome variables) during two separate seasons (spring/summer versus fall/winter) in children stratified by high- versus low-to-moderate social vulnerability. Prespecified outcomes are continuous and will be evaluated with linear regression. Analyses will be stratified by social

vulnerability (high versus low-to-intermediate) and season (spring/summer versus fall/winter). Models will be generated for air quality variables on the visit date and for each of the five lag days prior to the visit. Given the relatively small number of participants and high number of air quality predictor variables, we will use ElasticNet to select the most important variables in Python. Variables with a Pearson correlation coefficient >0.8 will be removed to minimize redundancy. Models will be evaluated considering pertinent covariates such as age, asthma medications and tobacco smoke exposure. To further address sex as a biological variable, models will also be fitted by sex, with statistical comparison of results observed in boys and girls. Analyses will assume a type 1 error rate of 0.05 without adjustments for multiple testing (exploratory study). For exploratory dichotomized outcomes that are not pre-specified, such as exacerbation occurrence or QOL scores below a threshold, partial least squares regression and discriminant analysis could be performed to determine the air quality features that provide the largest separation between outcomes. Temporal stability of the results would be assessed by repeating analyses during the second season.

Aim 5 will determine whether children with greatest social vulnerability and the highest air pollution exposure will have differentially expressed cytokines and metabolomic features. Cytokine and metabolomics data will be integrated using the R package Multiple Co-inertia (MCIA) to identify novel biomarkers of climate-related outcomes. These co-inertial analyses (CIA) are similar to the sparse Partial Least Squares (sPLS) approach used to integrate multi-omics data in the analyses pipeline from the popular package mixOmics by maximizing the covariance between eigenvectors and efficiently determining key features driving variance in paired multi-omics samples. Unlike sPLS, CIA allows us to project multi-omic data in the same space, reducing the need to generate several paired multi-omic integration modules.

V. Timeline

	Pre-award	Year 1 first 3 months	Year 1 last 9 months	Year 2	Year 3	Year 4	Year 5
Start-up activities							
Institutional Review Board approval	X						
Georgia CTSA study approval (for utilization of the Pediatric Research Center)	X						
Develop study case report forms		X					
Develop study database		X					
Develop participant characterization Manual of Procedures		X					
Develop laboratory sample processing Manual of procedures		X					
Study team training		X					
Participant-related activities							
Creation of advertisements, mailings		X					
Screening and recruitment		X	X	X	X	X	
Participant enrollment			X	X	X	X	
Participant follow-up (48 week study)			X	X	X	X	X
Laboratory procedures							

Cytokine analyses			X	X	X	X	X
Metabolomic analyses			X	X	X	X	X
Statistical analyses							
Preliminary analyses for progress reporting						X	
Primary/secondary outcome analyses							X
Dissemination							
Research conference presentation						X	X
Manuscript development					X	X	X
Grant submission							
Future related studies							X

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Appendix I. Schedule of study procedures for the 48 week cohort study (option 2). Procedures that will not be performed if telephone follow-up vistis are requested are indicated by an (X).

6-21 years with diagnosed asthma and an asthma exacerbation in the past 12 months

	Visit (week) (window)	**Visit 1***	**Visit 2** (2) (+7 days)	**Visit 3** (16) (+4 weeks)	**Visit 4** (32) (+4 weeks)	**Visit 5** (48) (+4 weeks)
		Baseline	Steroid response	Follow-up	Follow-up	Follow-up
	Procedure					
	Consent	X				
Eligibility	Vital signs, physical exam	X	X	(X)	(X)	(X)
Eligibility	Medical history review	X	X	X	X	X
Eligibility	Urine pregnancy testing	X	X	(X)	(X)	(X)
Characterization	Demographic questionnaires	X				
Characterization	Asthma evaluation (ACQ/ ACT)	X	X	X	X	X
Characterization	PROMIS questionnaires	X	X	X	X	X
Characterization	Spirometry	X	X	(X)	(X)	(X)
Characterization	• Bronchodilator reversibility	X	X			(X)
Characterization	Exhaled nitric oxide	X	X	(X)	(X)	(X)
Characterization	Venipuncture	X	X			(X)
Characterization	• Total IgE, specific IgE	X				
Characterization	• Complete blood count/diff	X	X			(X)
Mechanisms	• Blood samples stored (aim 3)	X	X			(X)
Mechanisms	Sputum induction	X	X			(X)
Mechanisms	• Sputum sample stored (aim 3)	X	X			(X)
	Triamcinolone injection	X				

* Can be split into two visits (V1A and V1B, with triamcinolone at V1B)