Study Title: Effects of Interleukin (IL)- 4R-alpha Inhibition on Respiratory Microbiome and Immunologic Correlates in Severe Asthma

PI: Yvonne Huang, MD

NCT: NCT05036733

Protocol Version IRB Approval Date: 9/8/2021

Effects of Il-4rα Inhibition On The Respiratory Microbiome And Immunologic Correlates In Patients With Severe Asthma: A Pilot Study

Principal Investigator: Yvonne Huang, MD; UM Division of Pulmonary/Critical Care Med
Co-Principal Investigator: Alan Baptist MD; UM Division of Allergy/Clinical Immunology
Co-Investigator: Njira Lugogo; UM Division of Pulmonary/Critical Care Med

I. Overview

This prospective pilot study will address the following main research question: *"Does IL-4Rα (dupilumab) inhibition of Type 2-mediated immune responses alter the respiratory microbiome within a 4 month period of treatment initiation and follow up, and if so, what are the specific microbiological changes and immune response correlates of these changes?*" We will compare pre- and post-dupilumab effects on the microbiomes, immune responses and metabolome of severe asthma patients who are clinically eligible for dupilumab treatment. Eligible patients will be screened and enrolled prior to start of therapy and return for visits at 1 month and 4 months after initiation of treatment. The overall goal of this study is to understand biological responses related to dupilumab treatment among severe asthma patients who have clinical indication to be treated. We will collect biospecimens (nasal, sputum, stool, blood) to examine dupilumab-related changes in the microbiome, measured immune responses, and clinical outcome measures. Research methods will include microbiome profiling of respiratory and stool samples, cytokine measurements from nasal epithelial lining fluid, and metabolite analysis of stool samples.

II. Background

Inhibition of the IL-4 receptor α (IL-4Rα) by dupilumab has been shown to significantly improve clinical outcomes and disease severity measures in Type 2 (T2)-driven phenotypes of severe asthma, as well as atopic dermatitis (AD), and chronic rhinosinusitis with nasal polyposis (CRSwNP) [1-4]. These findings are accompanied by demonstration of dupilumab-induced suppression of T2-related allergic responses, both in the blood and in organ-specific compartments such as lesional AD skin and nasal samples in CRSwNP. These include measures of total IgE, TARC, and eotaxin-3 [5,6], and of nasal polyp tissue levels of the same plus eosinophilic cationic protein and PARC (CCL18) [4]. Gene expression analysis of lesional AD skin identified congruent findings with Th2-associated mediators, but also decreased expression of genes involved in other immune cell pathways, such as dendritic cells and Th17 cells [6]. Thus, biomarkers of effective suppression of T2-related immune responses are evident both systemically and in affected tissue compartments. Further analyses of the latter may reveal localized interactions that contribute to differences between severe asthma patients in disease phenotype and therapeutic response. Indeed, despite recent advancements in anti-T2 directed therapies, not all severe asthma patients clinically respond to such treatments. Thus, more research to understand this is needed, as was noted in a recent NEJM editorial [7].

Published data on changes in airway immune responses with dupilumab treatment are sparse, but similar suppression of T2-mediated inflammation would be expected. Although T2-high inflammation plays a key role in many with poorly controlled asthma, at the same time the potential ramifications of long-term suppression of T2-related immune responses are uncertain, particularly

from a microbiological perspective. Increased presence of fungi in the airways is associated with Th2 responses in individuals with allergic airway disease [8], suggestive of a causal role, and eosinophils serve important antimicrobial and other regulatory functions [9,10]. Further, a wealth of evidence exists implicating perturbed human microbiota patterns (gut, nasopharyngeal; both bacterial and fungal) as a risk factor for allergy and asthma [11]. Relevant to this proposal, we have found in recent multi-center investigations of the airway microbiome in asthmatic adults that attributes of the airway microbiota differ significantly between those with T2-high versus T2-low asthma [12,13]. Specifically, airway bacterial burden is significantly lower in those with higher T2 airway inflammation. This finding translates into differences in the compositional structure of airway microbiota, including differences in the proportional abundance of specific bacteria, when compared to T2-low asthmatic subjects. Conversely, we have found that T2-low asthmatic subjects harbor higher airway bacterial burden and enrichment in a greater number of different bacteria. Similar trends were observed in recent analysis of induced sputum data from the same subjects in this NHLBI AsthmaNet study [14]. Separately, studies in mice have found that levels of lung inflammatory cytokines (IL-4, IL1-α) correlate with variation in lung microbiota composition and that such interactions likely have bidirectional influence [15].

Given this evidence, we propose a pilot study, leveraging the inherent value in using a specific immunologic intervention, to explore the question: “Does IL-4Rα inhibition of T2-mediated immune responses alter the respiratory microbiome within a 4 month period of treatment initiation and follow up, and if so, what are the specific microbiological changes and immune response correlates of these changes?” We will compare pre- and post-dupilumab effects on the respiratory microbiome and determine potential associations between dupilumab-related changes on the microbiome, measured immune responses, and clinical outcome measures. Since dupilumab is systemically administered, we will similarly explore gut microbiome characteristics related to dupilumab-associated changes in clinical and immunologic measures.

III. Objectives

Primary objective

- To determine changes in the respiratory microbiome after inhibition of IL-4Rα in patients with asthma at 1 and 4 months after initiation of treatment

Primary Endpoint (Biological) Measures

- Changes in measured features of respiratory microbiota composition (bacteria, fungi), profiled from induced sputum and nasal samples. Such features include alpha- and beta-diversity ecologic measures, which reflect microbiota composition within and between-samples, respectively. Examples include Shannon and phylogenetic diversity indices, richness and evenness metrics (alpha-diversity), and Unifrac or Bray-Curtis distance measures to compare across samples (beta-diversity). Additional readouts of the microbiome to be analyzed include bacterial burden by quantitative PCR, abundance profiles of microbial community members, and microbial gene functions annotated from metagenomic sequence data.
- Changes in similarly calculated measures of microbiota composition in stool samples collected before and after dupilumab initiation (baseline and 4 months)

Secondary Objectives

- To examine correlations between changes in measured features of the respiratory microbiome and changes in blood and airway immune responses with dupilumab treatment
- To determine relationships between changes in measured features of the respiratory microbiome and changes in clinical outcome measures including lung function and asthma control scores with dupilumab treatment
- To determine if combinations of correlated immune markers and microbiota patterns predict improvement or lack of improvement in clinical outcome measures related to dupilumab treatment
- To explore associations between gut microbiome composition, aeroallergen sensitization, severe asthma features and response to dupilumab treatment.
- To explore the relationship between gut microbiota compositional, the intestinal metabolome, and response to dupilumab.
- To explore and compare dupilumab-related, compartment-specific changes in the respiratory microbiome to that observed in the gut microbiome

Secondary Endpoint (Clinical) Measures

Clinical outcome measures (all visits)

- Spirometry: FEV1/FVC ratio, FEV1, and FVC pre- and post-bronchodilator.
- Fractional exhaled nitric oxide (FeNO)
- Asthma Control Test [16]
- Asthma quality of life questionnaire (mini-AQLQ) [17]
- SNOT-22 [18, 19]
- Asthma medication use and dosage
- Asthma exacerbations requiring at least 3 days of oral corticosteroids

Immune and Other Microbial Biomarkers - blood, induced sputum, nasal epithelial lining fluid (NELF), stool, exhaled air

- Serum total IgE and specific IgE to aeroallergen panel (ImmunoCap)
- Complete blood count with cell differential
- Sputum cell differential counts
- Sputum, NELF cytokines (Mesoscale Discovery; 30-plex panel: Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, TNF-α, TNF-β, VEGF-A)
- Sputum antimicrobial peptide expression (RT-PCR): defensins, lysozyme, lactoferrin, LL-37, and secretory leukocyte protease inhibitor
- Total bacterial burden in sputum samples by 16S rRNA Q-PCR or droplet digital PCR; with determination of both relative and absolute abundances of taxa.
- Respiratory microbial functional gene annotations from metagenomic sequencing
- Fecal metabolites by global/untargeted and targeted short-chain fatty acid assays

Primary hypotheses:

Hypothesis 1. Dupilumab-induced suppression of T2 inflammation in severe asthma is associated with changes in respiratory tract bacterial burden at 1 month and 4 months after treatment initiation (16S rRNA qPCR of bacterial DNA in induced sputum)
Hypothesis 2. Dupilumab-induced suppression of T2 inflammation in severe asthma is associated with changes in the compositional structure of respiratory and/or gut microbiota, detectable at 1 month and 4 months after initiation of treatment. (bacterial and fungal microbiota profiles and related measures of microbial diversity in induced sputum and stool)
Hypothesis 3. Dupilumab-induced changes in specific biomarkers (e.g. T2 and non-T2-related cytokines; targeted gene expression) correlate with changes in the respiratory and/or gut microbiome (phylogenetic diversity, specific compositional and functional profiles)
Hypothesis 4. Dupilumab-related improvement or lack of improvement in clinical outcomes (lung function, asthma control scores) over a 4-month period are associated with changes in specific characteristics of the respiratory and/or gut microbiome.

IV. STUDY OVERVIEW, INCLUSION/EXCLUSION CRITERIA, PROCEDURES

Dupilumab-eligible severe asthma patients will be identified and recruited from asthma and allergy clinics at the University of Michigan. Severe asthma patients are seen and cared for by Pulmonary and Allergy faculty at two main outpatient clinic locations. All severe asthma patients who meet clinical prescribing criteria for dupilumab are considered eligible and will be recruited for this study. The study coordinator and/or physician investigator will meet with subjects to provide information about the study and invite them to participate in this 3-visit study occurring over a 4-month period. We will enroll 15 severe asthma patients who complete all 3 visits. The funder of the grant for this project (Regeneron) will provide dupilumab for this study.

Inclusion criteria:

1. Adults ages 18 or older with physician-diagnosed/-managed severe asthma who are clinically eligible for dupilumab (clinical criteria below).

2. Current treatment with a medium-to-high-dose inhaled glucocorticoid (fluticasone propionate at a total daily dose of ≥440 µg or equipotent equivalent) plus up to at least one additional controller (e.g., a long-acting β2-agonist or leukotriene receptor antagonist)

3. Eosinophilic asthma phenotype (blood eosinophil level >300) or asthma requiring daily oral corticosteroids

4. Asthma that is uncontrolled, as defined by a score on the Asthma Control Test of 19 or lower, OR a worsening of asthma in the past year that led to an asthma hospitalization, ED visit, or 3 days of oral corticosteroids

5. Severity of asthma that, in the opinion of the subject's asthma care specialist, requires dupilumab for control

Exclusion Criteria:

1. Patients with diagnosis of other chronic lung diseases (e.g. COPD, idiopathic pulmonary fibrosis, Churg-Strauss syndrome, ABPA, etc.)

2. Current smoker or reported smoking within 1 month of the screening visit (tobacco or any inhaled recreational product)

3. Greater than 10 total pack-year of cigarette smoking history

4. Treatment with oral corticosteroids for an asthma exacerbation 1 month prior to screening or during the screening period

5. Use of any biologic therapy for asthma within the past 3 months

6. Respiratory or GI illness within 1 month prior to screening or during the screening period

7. Treatment with antibiotics for acute infections within six weeks prior to screening or during the screening period.

8. Pregnancy at enrollment or during the study

9. Known hypersensitivity to dupilumab or its excipients

Procedures

The following lists the study procedures subjects will be asked to perform. Some are indicated only for visit-specific criteria as detailed in subsequent sections and summarized in **Table 1.**

1. Full and abbreviated medical history intake and physical exam by study physician
2. Review of inclusion/exclusion criteria at each visit to ensure criteria still met.
3. Questionnaires
    - Asthma Control Test (ACT; 5 items; Appendix 1*;* [16])
    - Mini-Asthma Quality of Life Questionnaire (mAQLQ; 15 items; Appendix 2 [17])
    - SNOT-22 (22 items; Appendix 3 [18,19])
3. Blood draw (for clinical lab tests and plasma tube for Huang lab processing)
4. Nasal epithelial lining fluid (NELF) collection (Appendix 4)
5. Spirometry pre- and post-albuterol administration
6. Oral rinse/tongue scraping prior to sputum induction
7. Sputum induction
8. Stool collection (two samples: one for metabolite analysis- EasySampler kit, and one for microbial using Zymo; Appendix 5)

Table 1. Schedule of visits and procedures (V1-V3 conducted at Medical Clinical Research Unit)[1]

Visit	**Screening**	**V1**	**TC1**[4]	**V2**	**TC2**[4]	**V3**
		2-4 wks post-screen	Brief Phone call	1 month post-V1	Brief phone call	4 months post-V1
Dupilumab treatment status	No drug	Start drug	On drug	On drug	On drug	On drug
Procedures						
COVID-19 testing per Michigan Medicine policies[2]		X		X		X
Review of inclusion/exclusion criteria[3]	X	X		X		X
Full or short history/exam[3]		X		X		X
ACT questionnaire		X		X		X
SNOT-22 questionnaire		X		X		X
Mini-AQLQ		X		X		X
Exhaled nitric oxide (FeNO)[3]		X		X		X
Nasal epithelial lining fluid (NELF) collection[4]		X		X		X
Spirometry pre- and post-bronchodilator		X		X		X
Blood draw/Phlebotomy lab		X		X		X
Complete blood count with differential		X		X		X
Serum total IgE		X				
Respiratory Allergen panel (specific IgE tests)		X				
Plasma collection for research lab assays		X				X
Oral saline rinse/tongue scraping		X		X		X
Sputum induction		X		X		X
Stool collection materials (distribution, instruction)		X				X
Administer/provide dupilumab doses at study visit		X		X		X

1 Subjects should not eat for at least 4 hours prior to visits to limit intolerance of spirometry or sputum induction due to a full stomach. Subjects should not have caffeine (as is in coffee, teas, colas, and chocolate) for at least 6 hours prior to visits, for accuracy of measurements in lung function tests.

2 Per current policy (as of Jan 2021) at Michigan Medicine, subjects must undergo COVID-19 testing (nasal swab) and have a negative test within 96 hrs prior to aerosol-generating procedures. Testing will be arranged by study team for each visit. If the test is positive for COVID-19 or test result is not available, the study visit will need to be rescheduled.

3 A physician must see the subject at each visit.

4 FeNO measurement and NELF collection should be performed before spirometry and sputum induction.

5 Brief telephone call by study coordinator to (a) ensure subjects are self-administering dupilumab doses between study visits, (b) address any interim questions, and (c) remind them of upcoming in-person study visit. The TC encounters are important to maintain contact with subjects and ensure adherence and follow through during the course of the study

V. VISIT-SPECIFIC DETAILS

SCREENING.
This is the first encounter with eligible patient at which time information about the study is provided by the study coordinator. This may occur by phone or email. A short list of questions will be asked to confirm eligibility. If on the basis of this screen, an individual is eligible to participate the study, a copy of the informed consent will be provided for subject review prior to Visit 1. Signed informed consent will be collected at Visit 1. (*Anticipated total time by study coordinator: 10 minutes.)*

VISIT 1.

Preparations

1. V1 should be scheduled within 2-4 weeks after screening. Subjects will be reminded by study coordinator to take their usual asthma treatments as prescribed including on the day of the visit. Coordinate V1 scheduling with physician investigator availability to perform history and physical exam.

2. Coordinate with Research Pharmacy preparation of dupilumab syringe for administration to subjects after completing all visit testing. Ensure appropriate orders are submitted in advance of V1 date. Also ensure subject will have subsequent doses of dupilimab prepared for self-administration at home between V1 and V2.

3. Notify Lesa Begley in Huang Lab (674-9414) the day before the visit so that the lab can prepare necessary materials to collect and process samples. Ensure a bucket of ice is available to place collected samples for transport to Huang lab.

Visit procedures

4. Study coordinator must don appropriate protective personal equipment (PPE) for the study visit and keep on during the duration of the visit (gloves, masks, safety goggles or face shield for eye protection; corrective glasses do not suffice).

5. If not previously collected, obtain signed informed consent from the subject. Confirm no changes in continued eligibility of subject since screening, review inclusion/exclusion criteria (*study coordinator or physician time: 5-10 min*).

6. Perform procedures as listed in **Table 1**. Plan the order of procedures accordingly with the following performed by the study coordinator. Questionnaires should be completed first (*time*: 10 minutes). FeNO measurement (*time*: 3-5 minutes) and NELF collection (*time*: 10 minutes) should be performed next, before spirometry pre- and post-bronchodilator (*time*: 45 minutes including 15min wait pre-/post-bronchodilator), oral rinse/tongue scraping collection (*time*: 2 minutes), and sputum induction (*time*: 15 minutes). Blood draw at phlebotomy lab (*time:* 5 min) can be done anytime during the visit.

   - **FeNO measurement** will be performed using NiOx Vero machine per device instructions.

- **NELF collection (**instructions and schematic of sample collection provided by UNC Alexis Lab/Spirovation; Appendix 4)

    - The NELF procedure involves sitting the patient comfortably upright, and the head extended backwards
    - Place the absorbent end of the nasosorption strip device on the lateral wall of the nasal cavity, onto the lateral/inferior surface of the inferior turbinate
    - Hold the strip in place by pressing a finger against the nostril for 60 seconds to absorb nasal secretions
    - Remove the device (moist with nasal mucosal lining fluid) from the nostril and place it on wet ice until storage
    - Repeat these steps in the other nostril using a fresh nasosorption strip

7. Perform spirometery pre- and post-bronchodilator. Collect oral rinse/tongue scraping sample, then perform sputum induction assuming the subject's post-bronchodilator FEV_1 meets safety criteria per Sputum Induction MOP. Record lung function (FEV1) every 2 minutes per protocol during sputum induction. Notify Huang lab personnel when specimens are ready for pick-up.

8. After the above procedures are completed, 1st dose of dupilumab (600 mg subcutaneously, loading dose) should be administered to the subject in MCRU. Subjects will be monitored post-injection for 30 minutes after their first injection. For high-risk patients, defined as those with prior history of anaphylaxis to any medication, very poor asthma control or low lung function, this will be extended for up to 60 minutes post-injection.

9. Prior to end of visit:
    - Ensure subjects have received subsequent doses of dupilumab (300 mg subcutaneously) and instruction on how to self-inject dupilumab every 2 weeks. The 2nd injection and subsequent injections between study visits should be administered at home, per usual prescribing criteria. (*time*: 10 min)
    - Schedule V2 which should be at least 4 weeks after Visit 1.
    - Ensure subjects know how to contact study team in case of interim medical events or changes in health status before Visit 2

After-visit tasks by study coordinator and physician PI (Dr. Huang).

Data entry into REDCAP by coordinator with integrity review, plus physician interpretation:

- Questionnaire items/results (ACT, SNOT-22, miniAQLQ): 15 minutes/5-10 minutes
- Spirometry pre-/post-bronchodilator and calculation of change: 10 minutes/5 minutes
- FENO result: 2 minutes/1 minute
- All individual blood test results: 20 minutes/5-10 minutes

TELEPHONE CALL 1.

1. The purpose of TC1 is to check in with the subjects around 2 weeks after Visit 1 and review the following:

- Coordinator should inquire about any interim health issues and ensure subject knows and has self-administered 2nd dose of dupilumab (every 2 weeks).
- Remind subjects to continue to take all their other usual asthma treatments as prescribed including on the day of upcoming Visit 2.
- Arrangements for COVID-19 testing should be made so that results are available prior to Visit 2 (within 96 hrs).

VISIT 2.

Preparations

1. Visit 2 should take place approximately 4 weeks after Visit 1.

2. Coordinate with Research Pharmacy for preparation of dupilumab injection at visit**. This should include dose to be administered at Visit 2 and subsequent doses that the subject will self-administer at home every 2 weeks, until Visit 3**. Therefore, enough doses should be ensured to cover the 3-month period until the last visit.

3. Notify Lesa Begley the day before the visit so that the lab can prepare necessary materials to collect and process samples. If a stool specimen is brought in by the subject, the sample will be transported to Dr. Huang's lab.

Visit procedures

4. Perform Visit 2 procedures as indicated Table 1. Plan the order of procedures accordingly, as before. All are performed by the study coordinator. Anticipated order and estimated times: Questionnaires first (*time*: 10 minutes). FeNO measurement (*time*: 3-5 minutes) and NELF collection (*time*: 10 minutes) next, followed by spirometry pre- and post-bronchodilator (*time*: 45 minutes including 15 min required wait pre-/post-bronchodilator measurement), oral rinse/tongue scraping collection (*time*: 2 minutes), and sputum induction (*time*: 15 minutes).

5. Prior to discharging the subject:
    - Remind subject to continue home dupilumab, every 2 weeks until Visit 3.
    - Ensure that the subject has all doses of dupilumab needed for the next 3 months.
    - Provide subjects with a stool collection packet and instruct them not to collect specimen until the day of Visit 3 to be returned in person.
    - Schedule Visit 3, which should occur 3 months after Visit 2.
    - Arrange COVID-19 pre-procedural testing per institutional policies.

After-visit tasks by study coordinator and physician PI (Dr. Huang).

Data entry into REDCAP by coordinator with integrity review, plus physician interpretation:

- Questionnaire items/results (ACT, SNOT-22, miniAQLQ): 15 minutes/5-10 minutes
- Spirometry pre-/post-bronchodilator and calculation of change: 10 minutes/5 minutes
- FENO result: 2 minutes/1 minute

TELEPHONE CALL 2.

1. Like TC1, the purpose of TC2 is to check in with the subject at around 6 weeks post-Visit 2 (halfway between V2 and V3)

    - Coordinator inquires about any interim health issues and ensure subject is continuing home administration of dupilumab every 2 weeks.
    - Remind subjects to continue to take all other usual asthma treatments as prescribed per their primary physician.

2. Within 1-2 weeks before Visit 3, coordinator should:
    - Arrange for COVID-19 pre-procedural testing per current institution policies

VISIT 3.

Preparations

1. Visit 3 occurs ~ 6 weeks after TC2 and 3 months after Visit 2

2. Notify Lesa Begley the day before the visit so that the lab can prepare necessary materials to collect and process samples. If a stool specimen is brought in by the subject, the sample will be transported to Dr. Huang's lab.

Visit procedures

3. Perform Visit 3 procedures as indicated Table 1. Plan the order of procedures accordingly as before. Questionnaires first (*time*: 10 minutes). FeNO measurement (*time*: 3-5 minutes) and NELF collection (*time*: 10 minutes) next, followed by spirometry pre- and post-bronchodilator (*time*: 45 minutes including 15 min required wait pre-/post-bronchodilator), oral rinse/tongue scraping collection (*time*: 2 minutes), and sputum induction (*time*: 15 minutes). Blood draw at phlebotomy lab (*time:* 5 min) can be done anytime during the visit.

4. Prior to discharging the subject:
    - Remind subject to discuss/coordinate with their primary physician if prescription of dupilumab to be continued for clinical reasons.
    - If subject has not yet returned stool sample for Visit 3 timepoint, remind them to do so within 1-3 days of Visit 3.

After-visit tasks by study coordinator and physician PI (Dr. Huang).

Data entry into REDCAP by coordinator with integrity review, plus physician interpretation:

- Questionnaire items/results (ACT, SNOT-22, miniAQLQ): 15 minutes/5-10 minutes
- Spirometry pre-/post-bronchodilator and calculation of change: 10 minutes/5 minutes
- FENO result: 2 minutes/1 minute
- All individual blood test results: 20 minutes/5-10 minutes

VI. Rationale for Data Collection and Procedures

1. *Medical History and Exam*: Medical history intake and physical exam will be conducted by the study physician at each visit. A full H&P will be performed at Visit 1, with shorter H&P appropriate at Visits 2 and 3.
2. *Inclusion/Exclusion criteria*: Reviewed in full at Visit 1 and re-checked at each visit to ensure no new exclusionary criteria met.
3. *Questionnaires* will be used in this study to collect information about asthma severity/control (ACT), asthma-related quality of life (mAQLQ), and sinus symptomatology (SNOT-22). These data will allow for analysis of clinical symptoms and asthma control in conjunction with molecular data generated on the airway and gut microbiomes, and immune mediators measured before and after initiating dupilumab. These validated instruments have been used extensively in asthma clinical trials and outcomes research.
4. *Exhaled nitric oxide measurement* (FeNO). FENO is a recognized biomarker for monitoring Type 2 airway inflammation in asthma. Its use in the clinical management of asthma is supported by clinical asthma guidelines.
5. *NELF sample collection.* The purpose of collecting nasal epithelial lining fluid is that it is a simple non-invasive way of sampling the respiratory mucosa to measure immune cytokines. NELF has been used in a number of research investigations to profile mucosal immune mediators relevant to asthma.
6. *Spirometry pre- and post- bronchodilator*. A standard clinical test for the presence of airflow obstruction and assessment for reversibility. FEV1 and FVC will be determined before and after 4 puffs of albuterol. This will be performed by experienced study coordinators using existing equipment for research use.
7. *Blood draw:* This will be performed by a clinical phlebotomist or nursing staff
8. *Complete blood count (CBC) with differential.* Results will be used to determine peripheral blood eosinophil counts, to gauge biological response to treatment.
9. *Total and specific IgE.* Clinical tests of allergy-related immune responses. We will measure both total serum IgE and respiratory allergen specific IgE based on ImmunoCap assays
10. *Plasma* will be stored at -80°C for future analysis of other immune or metabolic response markers.
11. *Oral saline rinse/tongue scraping:* This involves having the subject swish and gargle 10mL of sterile saline than spit into a collection container. This is followed by applying a commercially available plastic tongue scraper to remove any film on the tongue into the same collection container. This is performed prior to sputum induction and will be processed for microbiome analyses as biological controls.
12. *Sputum induction:* This will follow a standardized manual of procedures involving inhalation of 3% saline mist for 12 minutes. Subjects will be instructed to periodically cough up sputum into a specimen cup and FEV1 is monitored every 2 minutes using a handheld PiKo-6 meter per a standardized protocol. If the FEV1 declines ≥20% of baseline, the procedure would be stopped and albuterol administered with follow-up monitoring. The sputum will be portioned into aliquots to be processed for different analytic purposes, including cell count/differential analysis, microbial community profiling, mediator analyses.
13. Stool sample collection. This will be processed for analysis of gut microbiome composition and intestinal metabolites and their relationships to clinical measures of treatment response.

VII. Recruitment

Subject with severe asthma who are clinically eligible for potential treatment with dupilumab will be recruited from pulmonary asthma and allergy clinics at the University of Michigan. Identified eligible patients will be screened by the study coordinator to confirm eligibility and interest in participating.

VIII. Human Subjects and Risks

A. Subjects:

1. General Description: inclusion/exclusion criteria are as described earlier in this document.
2. Gender/Minority Inclusion: No subject will be excluded based on gender or ethnicity.
3. Exclusion of children: Children (<18 years of age) are excluded from this study as the research aim relate to hypotheses about the effects in adult asthmatics of dupilumab treatment on host-microbiome interactions and clinical outcomes.

B. Potential Risks and Procedures for Minimizing Risks: Potential risks can be categorized as "Likely", "Infrequent", or "Rare".

Likely Risks

- *Venipuncture risks:* Drawing blood may cause temporary discomfort from the needle stick, bruising, and infection. Dizziness may occur and subjects may be asked to lie down for venipuncture if they indicate this history.
- *Spirometry/peak flow risks:* Breathing fast and hard into a spirometer or peak flow device can be associated with cough or lightheadedness. Such symptoms are usually temporary and resolve within a few minutes without intervention.
- *Albuterol use risks:* Albuterol is an approved drug that broncho-dilates the airways and widely used to treat asthma symptoms. Albuterol use may cause muscle tremors, palpitations, rapid heart rate, and headache. If these symptoms do occur, they are temporary and usually disappear within 1 hour.
- *Sputum induction risks:* The sputum induction manual of procedures for this study is adapted from those used in prior NHLBI asthma clinical research studies. The risks associated with this test include an unpleasant taste in the mouth, asthma-like symptoms (chest tightness, wheezing, shortness of breath), and occasionally nausea. Infrequently, the procedure may result in an asthma exacerbation requiring treatment. To prevent asthma-like symptoms, all subjects will have received 4 puffs of albuterol prior to undergoing induction. FEV1 will be monitored during the procedure which may be terminated for sustained decline in FEV1. Additional albuterol can be administered per to reverse bronchoconstriction which is rarely refractory to additional albuterol.

Infrequent Risks

- *Sputum induction resulting in asthma exacerbation:* Subjects will be pre-treated with albuterol and their lung function closely monitored during the procedure, as outlined in the sputum induction MOP. Sterile ampoules of 3% saline will be used for the procedure. If a decrease in FEV1 of ≥ 20% occurs during the procedure, subjects will be given additional albuterol and the FEV1 must improve to ≥ 90% of their baseline value prior to being discharged from the visit. If

the FEV1 does not improve to this value, further evaluation and treatments may be required, as determined by the study physician.

Rare

- *Sputum induction:* There is a theoretic possibility that the coughing associated with sputum induction could cause a failure of any prior hernia surgeries a subject may have had. Such surgeries include repair of hiatal hernia, other surgery for gastro-esophageal reflux ("heartburn"), or surgery to repair hernias elsewhere in the abdomen, groin, or pelvis. If a subject has such history on evaluation, they will be given the opportunity to discuss the study related procedures with their surgeon prior to proceeding.
- *Breach of confidentiality risks:* Participation in any research carries the risk of breach of confidentiality because participants provide personal information. Measures implemented in this study to protect subject privacy in collected research data and samples include the following: Use of servers and electronic database repositories to record clinical and demographic data that are HIPAA-compliant as approved by the University of Michigan (e.g. REDCAP, MBox). Study-related electronic and paper data files are made accessible only to essential study research staff involved in particular aspects of the study. Master key linking subject identifiers and assigned subject ID codes are accessible only to the PI and lead study coordinator(s). For sample storage, processing and data analyses, only coded subject identifiers will be used.

C. Asthma Exacerbations

If a subject notifies the study team of symptoms that suggest an asthma exacerbation, they will be referred to their primary asthma provider (or directed to urgent care if necessary) for clinical evaluation and determination of any treatments. A clinically adjudicated asthma exacerbation that required treatment intervention will be documented in the study record when the study team is made aware of this information.

IX. Adverse Events and Data Safety Monitoring Plan

An adverse event shall be defined as any detrimental change in the subject's condition, whether it is related to asthma or to an unrelated illness or condition. Adverse events related to asthma exacerbations will be managed as noted above. An adverse event is deemed serious if it presents a significant hazard, contraindication, or side effect. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose. Serious adverse events deemed potentially related to study procedures will be reported to the University of Michigan's Institutional Review Board and to study funders according to respective policies or reporting guidelines. Adverse events due to therapy or concurrent illnesses *other* than asthma may be grounds for withdrawal as determined by study PIs if the illness is considered significant enough to affect study findings or if the subject is no longer able to effectively participate in the study.

It is not considered an adverse event if subjects experience minor intercurrent illnesses during the study. Examples of minor intercurrent illnesses of relevance include acute rhinitis, sinusitis, upper respiratory infections, or accompanying gastrointestinal symptoms. Documentation recorded will include information on the nature, severity, duration of the illness and any new medications

required to treat the illness. Medications are allowed for treatment of these conditions as per clinical guidance of the subject's personal physician.

Documentation of adverse events will be recorded on a Clinical Adverse Event Report Form and will include the following information: dates and description of the event/illness, assessment performed by the study team, any necessary treatments administered or prescribed for the event/illness (medications, doses, and dose frequency/duration), need for emergency transfer or hospitalization was required, and outcome.

Adverse Events and their reporting are further defined as follows:

Definition and Reporting

Adverse events include the following:

• *Clinical Adverse Events*: Any unintended worsening in structure or function of the body; any illness that occurs during the trial. These events are documented on a Clinical Adverse Event Report form.

• *Laboratory Adverse Events*: Occurrences of abnormal laboratory tests or other test results. These events are documented on the Laboratory Adverse Event Report form. The study physician will offer to communicate the test results, if desired by the subject, to their primary care physician.

• *Serious Adverse Events*: Any experience that poses a significant hazard to a participant is considered a serious adverse event. With respect to human clinical experience, a serious adverse event includes any experience that meets at least one of the following criteria:
1. Results in death
2. Is life threatening (places the participant at immediate risk of death from the event as it occurred)
3. Results in a significant or persistent disability/incapacity
4. Requires inpatient hospitalization or prolongation of an existing hospitalization
5. Results in a congenital anomaly/birth defect
6. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. Examples include allergic bronchospasm requiring intensive treatment in an emergency department or at home, or the identification of other blood test abnormalities that do not require immediate inpatient management.

Serious adverse events are reported on the Serious Clinical Adverse Event Report form and noted also on the regular Clinical Adverse Events form.

Each time the study team has contact with a participant, whether for a scheduled visit or phone contact, impromptu visit, or unexpected phone call, the possibility of adverse events should be reviewed. This would include reviewing prior to the contact (if applicable), the participant's file for any ongoing adverse events at the last visit/contact, updating a reported adverse event if an ending date/outcome becomes available, or identifying any new adverse events to report.

If there have been no adverse events since the prior visit or last subject contact, then indicate 'No' in the appropriate section of the respective visit/encounter form.

Events that are ongoing at the time a participant completes or leaves the study should be left open for stop dates (i.e. report as 'ongoing'). The participant should be probed for any stop dates that are now known to close out previously recorded events. All adverse event report forms will be reviewed quarterly (or sooner as necessary) per the Data Safety Monitoring Plan below and reported as indicated.

Data Safety Monitoring Plan:
Adverse events will be reviewed every 3 months as needed by the study team physicians (Huang, Baptist, Lugogo) and study coordinators. In case of uncertainty or disagreements among the above individuals regarding adjudication of possible study-related AES, or serious AEs, the team will seek additional input from non-study clinicians (e.g. Dr. Michael Coffey of the Pulmonary Division)

Potential Benefits Gained from Data

The described risks of the study represent minor increases over minimal risk. Spirometry and sputum induction are used in clinical care setting, where the incidence of serious adverse events related to these procedures is very low. The anticipated scientific benefits of this study are increased knowledge of the biological effects of dupilumab on host-microbiome interactions, how such effects may impact clinical responses to the treatment and identify potential new mechanisms. We believe the potential benefits of the study outweigh the minor increase over minimal risk of the proposed testing procedures.

X. Sample Processing/Analysis *(Overview).* Respiratory samples will be processed for microbiota analysis by 16S rRNA and shotgun DNA sequencing, measurement of airway bacterial burden by quantitative PCR, cytokine measurements, sputum inflammatory cell counts/differential and airway gene expression studies (targeted). Blood will be analyzed for complete blood count with differential analysis, total IgE, respiratory allergen specific IgE, and blood cytokine mediators. Stool will be processed for gut microbiome and metabolomic analyses (global and targeted for short chain fatty acids). These data will be generated using University of Michigan Clinical Lab and Research Core facilities (Microbiome Core, Advanced Genomics Core, Metabolomics Core), as well as subcontract collaboration with UNC-Chapel Hill (Spirovation).

XI. Data Management and Analysis.
A HIPAA-compliant, passcode-secured REDCAP database for the study will be created. The study team (coordinator, PI) will enter all clinical test results. This database will reside on University of Michigan servers approved to store HIPAA-related research information. Access is given only to study-specific research team members for whom the PI provides permission. Subjects will be assigned a code using a study-specific identifier for downstream sample processing and data analyses. All samples for molecular sequencing/cytokine analysis will be identified only by a unique code.

All sequencing data (large data files) will be processed/stored on platforms maintained by University of Michigan Advanced Research Computing (https://arc.umich.edu/systems-services), which is part of UM Information and Technology Services. Specifically, we will store coded data

(no HIPAA identifiers) on Turbo and utilize the Great Lakes high-performance cluster to process the raw sequence data and perform analyses of the metagenomic sequence data. Several of the analysis pipelines to be used are already or can be installed for microbial metagenomic analysis. UM-DropBox (HIPAA-compliant) will be used to store intermediary and final result files that need to be easily accessed for non-computationally intensive analyses, discussions and write-ups by the research team.

XII. Data Analysis

This is a pilot, proof-of-concept study. There is no available published data (at the time of writing) that addresses the same research questions to perform a sample size calculation based on changes in microbiome readouts (primary objective/endpoint) related to dupilumab intervention. However, we have previously reported significant changes in the airway microbiome, related to a prospective treatment intervention (fluticasone propionate), in comparisons that also involved small numbers of subjects. Specifically, in an earlier NHLBI AsthmaNet Proof-of-concept study, we examined the effects of randomized, placebo-controlled fluticasone intervention (6 weeks) on the airway microbiome in subjects with mild asthma. We identified fluticasone-associated changes in the airway microbiome between steroid-responders vs. non-responders, in an initial analysis of paired protected bronchial brushings from 16 subjects [13]. In an analysis of induced sputum from the same study, we recently reported changes in the airway microbiome related to fluticasone intervention, which differed from placebo [14]. Notably, we observed a significantly greater change in airway microbial community structure in ICS non-responders (n=8) compared to responders (n=12).

To address our exploratory hypotheses in this pilot study, we will first generate the following readouts of microbial community composition: (1) read counts or relative abundance data for each microbial species determined from sequence alignments to reference databases. These readouts can be at the level of ASVs (amplicon sequence variant) or OTUs (operational taxonomic unit), which can also be collapsed into higher taxonomic levels for analysis, e.g. genus or family-level comparisons; (2) within-sample and between-sample measures of microbial diversity, i.e. alpha- or beta-diversity. Alpha-diversity measures will include Shannon, inverse Simpson, richness, and the phylogeny-based Faith index. Beta-diversity measures will include calculation of Bray-Curtis and Unifrac distance measures, the latter being a phylogeny-based calculation of between-sample differences in overall microbial composition. We will also determine 16S rRNA gene copy numbers in induced sputum by quantitative PCR, which serves as a proxy measure of ‘bacterial burden’ often used in the airway/lung microbiome literature. Other generated molecular data will be used to explore secondary questions related to whether dupilumab intervention effects change in predicted microbial functions (KEGG orthologue modules and pathways), intestinal metabolites, and whether changes in the airway or gut microbiome correlate with changes in inflammatory markers and immune mediators measured in sputum, blood and NELF.

For Hypothesis 1, we will compare sputum bacterial burden (normalized 16S rRNA gene copy number) before and after treatment (baseline vs month 1; baseline vs. month 4; all three timepoints), using paired t-test if normally distributed (Wilcoxon sign-rank test if non-normal distribution) and repeated measures ANOVA for all three timepoints.

For Hypothesis 2, we will compare alpha-diversity measures before and after treatment (baseline vs month 1; baseline vs. month 4; all three timepoints), using paired t-test, Wilcoxon sign-rank test or rm-ANOVA. For beta-diversity -based measures of overall microbial composition, we will use Bray-Curtis or Unifrac distance matrices to perform (i) pairwise PERMANOVA tests (R package vegan; adonis2 or in QIIME2 (q2-longitudinal) [20,21]; (ii) linear mixed-effects models with a random intercept and slope for each individual; (iii) Procrustes analysis with Mantel test. We will also explore other available tools for time-series microbiome analysis such as MDSINE [22]. Differences in specific species (ASVs, OTUs) between time points will be analyzed using DESeq2 which applies a variance-stabilizing transformation to the data. Other methods using log-ratio transformation (e.g., CLR) of species counts will also be explored.

For Hypothesis 3, we will examine correlations between change in biomarkers (e.g., cytokines, inflammatory cell counts, targeted gene expression) and changes in the airway or gut microbiome. The latter based on either specific compositional readouts (ASV, OTUs) or in terms of overall microbial community structure. The following approaches will be used: (i) initial correlation analyses performed between paired data at each time point (e.g., specific biomarker dataset vs. microbial ASVs), using Spearman correlations, with adjustments for multiple comparisons by q-values; (ii) linear mixed-effects models, e.g. levels of immune markers as predictors of post-treatment microbiota diversity or abundances of each microbiota member; fixed effects would include age, sex, BMI, and chronic treatments including corticosteroids or macrolides (type, dose, route); (iii) tests for biomarker association with overall microbial community structure, using distance-based PERMANOVA (adonis2) at each time point or in a paired design; (iv) additional computational approaches based on methods for high-dimensional dataset comparisons, as described below.

For Hypothesis 4, which focuses on microbial correlations with clinical outcome measures rather than biomarkers, we will apply same approaches as described for Hypothesis 3. In addition, we will explore the following analysis methods (also applicable to Hypothesis 3): (i) feature-volatility action algorithm, which performs longitudinal feature selection based on random forest regression, implemented in q2-longitudinal in QIIME2; (ii) tools in the R mixOMICS package which bundles access to several sparse multivariate modeling methods that aim to identify key features that explain a biological or clinical outcome of interest.

Related to this, and to leverage tools for examining microbiome data with other -omics/high-dimensional data we will apply tools such as DIABLO (available in mixOMICS package). DIABLO performs data integration coupled with discriminant analysis approach to identify combinatorial features predictive of a binary outcome (e.g., is there a combination of microbial and metabolite features that predict improved asthma control or not? or that associate with an immune biomarker of interest?) As an example, we have recently used DIABLO to explore microbial and metabolite features associated with COPD outcome measures in a multicenter study. In addition, we will determine co-occurrence relationships between microbiota (SparCC [24] or SPIEC-EASI [25]) to identify highly correlated species and determine if ‘hubs’ of microbiota associate with particular cytokines or metabolites, following by analysis for clustering patterns of microbial-immune relationships [26]. Given the likely heterogeneity of measures in the molecular data types and the small number of subjects, we will apply corrections for multiple comparisons where indicated.

References

1. Guttman-Yassky E, Bissonnette R, Ungar B, Suárez-Fariñas M, Ardeleanu M, Esaki H, Suprun M, Estrada Y, Xu H, Peng X, Silverberg JI, Menter A, Krueger JG, Zhang R, Chaudhry U, Swanson B, Graham NMH, Pirozzi G, Yancopoulos GD, D Hamilton JD. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol. 2019 Jan;143(1):155-172.
2. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, Zhu H, Hamilton JD, Swanson BN, Khan A, Chao J, Staudinger H, Pirozzi G, Antoni C, Amin N, Ruddy M, Akinlade B, Graham NMH, Stahl N, Yancopoulos GD, Teper A. Efficacy and Safety of Dupilumab in Glucocorticoid-Dependent Severe Asthma. N Engl J Med. 2018 Jun 28;378(26):2475-2485.
3. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, Ford L, Sher L, FitzGerald JM, Katelaris C, Tohda Y, Zhang B, Staudinger H, Pirozzi G, Amin N, Ruddy M, Akinlade B, Khan A, Chao J, Martincova R, Graham NMH, Hamilton JD, Swanson BN, Stahl N, Yancopoulos GD, Teper A. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med. 2018 Jun 28;378(26):2486-2496.
4. Jonstam K, Swanson BN, Mannent L, Cardell LO, Tian N, Wang Y, Zhang D, Fan C, Holtappels G, Hamilton JD, Grabher A, Graham NMH, Pirozzi G, Bachert C. Dupilumab reduces local type 2 pro-inflammatory biomarkers in chronic rhinosinusitis with nasal polyposis. Allergy. 2018 Nov 28. PubMed PMID: 30488542.
5. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, Wang L, Kirkesseli S, Rocklin R, Bock B, Hamilton J, Ming JE, Radin A, Stahl N, Yancopoulos GD, Graham N, Pirozzi G. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med. 2013 Jun 27;368(26):2455-66.
6. Hamilton JD, Suárez-Fariñas M, Dhingra N, Cardinale I, Li X, Kostic A, Ming JE, Radin AR, Krueger JG, Graham N, Yancopoulos GD, Pirozzi G, Guttman-Yassky E. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. J Allergy Clin Immunol. 2014 Dec;134(6):1293-1300.
7. Drazen JM, Harrington D. New Biologics for Asthma. N Engl J Med. 2018 Jun 28;378(26):2533-2534.
8. Porter PC, Lim DJ, Maskatia ZK, Mak G, Tsai CL, Citardi MJ, Fakhri S, Shaw JL, Fothergil A, Kheradmand F, Corry DB, Luong A. Airway surface mycosis in chronic TH2-associated airway disease. J Allergy Clin Immunol. 2014 Aug;134(2):325-31.
9. Wong CK, Cheung PF, Ip WK, Lam CW. Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. Am J Respir Cell Mol Biol. 2007 Jul;37(1):85-96.
10. Wen T, Rothenberg ME. The Regulatory Function of Eosinophils. Microbiol Spectr. 2016 Oct;4(5).
11. Huang YJ, Marsland BJ, Bunyavanich S, O'Mahony L, Leung DY, Muraro A, Fleisher TA. The microbiome in allergic disease: Current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. J Allergy Clin Immunol. 2017 Apr;139(4):1099-1110.
12. Huang YJ, Nariya S, Harris JM, Lynch SV, Choy DF, Arron JR, Boushey H. The airway microbiome in patients with severe asthma: Associations with disease features and severity. J Allergy Clin Immunol. 2015 Oct;136(4):874-84.
13. Durack J, Lynch SV, Nariya S, Bhakta NR, Beigelman A, Castro M, Dyer AM, Israel E, Kraft M, Martin RJ, Mauger DT, Rosenberg SR, Sharp-King T, White SR, Woodruff PG, Avila PC, Denlinger LC, Holguin F, Lazarus SC, Lugogo N, Moore WC, Peters SP, Que L, Smith LJ, Sorkness CA, Wechsler ME, Wenzel SE, Boushey HA, Huang YJ; National Heart, Lung and Blood Institute's “AsthmaNet”. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. J Allergy Clin Immunol. 2017 Jul;140(1):63-75.
14. Durack J, Christian LS, Nariya S, Gonzalez J, Bhakta NR, Ansel KM, Beigelman A, Castro M, Dyer AM, Israel E, Kraft M, Martin RJ, Mauger DT, Peters S, Rosenberg SR, Sorkness C, Wechsler M, Wenzel S, White SR, Lynch SV, Boushey HA, Huang YJ. for NHLBI AsthmaNet. Distinct associations of sputum and oral microbiota with atopic, immunologic, and clinical features in mild asthma. *J Allergy Clin Immunol* 2020 Nov; 146(5): 1016-1026.
15. Dickson RP, Erb-Downward JR, Falkowski NR, Hunter EM, Ashley SL, Huffnagle GB. The Lung Microbiota of Healthy Mice Are Highly Variable, Cluster by Environment, and Reflect Variation in Baseline Lung Innate Immunity. Am J Respir Crit Care Med. 2018 Aug 15;198(4):497-508.

16. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, Kosinski M, Pendergraft TB, Jhingran P. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol. 2006 Mar;117(3):549-56.
17. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J 1999; 14: 32 -38.
18. Hopkins C, Gillett S, Slack R, et al. Psychometric validity of the 22-item Sinonasal Outcome Test. Clin Otolaryngol 2009; 34: 447–454
19. Piccirillo JF, Merritt MG, Richards ML. Psychometric and clinical metric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg 2002;126: 41–47.
20. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7(5):335-6.
21. Bokulich NA, Dillon MR, Zhang Y, Rideout JR, Bolyen E, Li H, Albert PS, Caporaso JG. q2-longitudinal: longitudinal and paired-sample analyses of microbiome data. mSystems. 2018; 3:e00219-18.
22. Bucci, V., Tzen, B., Li, N. *et al.* MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. Genome Biol 2016; 17(1): 121.
23. Rohart F, Gautier B, Singh A, Lê Cao K-A. mixOmics: An R package for `omics feature selection and multiple data integration. PloS Comput Biol 2017; 13(11): e1005752.
24. Friedman J, Alm EJ. Inferring Correlation Networks from Genomic Survey Data. PLoS Comput Biol 2012; 8(9): e1002687.
25. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput Biol. 2015;11(5):e1004226.
26. Clauset A, Newman ME, Moore C. Finding community structure in very large networks. Phys Rev E Stat Nonlin Soft Matter Phys. 2004 Dec;70(6 Pt 2):066111.

APPENDICES for IRB.

Appendix 1. Asthma Control Test (ACT)

The Asthma Control Test (ACT) is a patient self-administered tool for identifying those with poorly controlled asthma. It is based on a 4-week recall of symptoms and daily functioning captured in 5 items on the questionnaire. These include assessment of the frequency of shortness of breath, general asthma symptoms, use of rescue medications, the effect of asthma on daily functioning, and overall self-assessment of asthma control. Each item is scored on a 5-point scale, with higher scale values indicative of, depending on the question, infrequent/absent symptoms or better asthma control.

The ACT score is the sum of the values for all 5 items. The scores range from 5 (poor control of asthma) to 25 (complete control of asthma), with higher scores reflecting greater asthma control.

An ACT score >19 indicates well-controlled asthma; the minimal clinically important difference in score is 3.

Appendix 2. Mini-Asthma Quality of Life Questionnaire Control (mAQLQ)

The shorter and simpler mAQLQ consists of 15 questions and was developed to meet the need for greater efficiency in large clinical trials and group patient monitoring. The mAQLQ is a patient self-administered test with questions covering 4 domains: Symptoms (5 questions), Activity Limitations (4 questions), Emotional Function (3 questions), and Environmental Stimuli (3 questions). The recall time for the mAQLQ is 2 weeks, so patients must base their answers on their experiences during the previous 2 weeks.

To score the mAQLQ, there is an overall score and a mean score for each domain.
Overall score (average of 15 questions): add up responses to all questions, and divide by 15.
Mean for each domain: add up responses to each item and take the average for each domain.

Symptoms:	items 1, 4, 6, 8, 10	(sum & divide by 5; variable label- mAQLQ-S)
Activity Limitations:	items 12, 13, 14, 15	(sum & divide by 4; variable label- mAQLQ-AL)
Emotional Function:	items 3, 5, 9	(sum & divide by 3; variable label- mAQLQ-EF)
Environmental Stimuli:	items 2, 7, 11	(sum & divide by 3; variable label- mAQLQ-ES)

Appendix 3. SNOT-22 Questionnaire.

Appendix 4. Nasal Epithelial Lining Fluid (NELF) collection (diagram, instructions).

Appendix 5. Stool Collection Instructions.