

Rare Diseases Clinical Research Network

A Prospective, Multicenter Study to Compare and Validate Endoscopic, Histologic, Molecular, and Patient-Reported Outcomes in Pediatric and Adult Patients with Eosinophilic Esophagitis (EoE), Gastritis (EG) and Colitis (EC)

Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR)

Protocol Number: CEGIR7801

Sponsored by:

**National Institute of Allergy and Infectious Diseases (NIAID)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Office of Rare Diseases Research (ORDR), National Center for Advancing
Translational Sciences (NCATS)**

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INVESTIGATOR SIGNATURE PAGE	
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Title: A Prospective, Multicenter Study to Compare and Validate Endoscopic, Histologic Molecular, and Patient-Reported Outcomes in Pediatric and Adult Patients with Eosinophilic Esophagitis (EoE), Gastritis (EG) and Colitis (EC)	
Study Sponsor: <div style="text-align: center;"> National Institute of Allergy and Infectious Diseases (NIAID) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Office of Rare Diseases Research (ORDR), National Center for Advancing Translational Sciences (NCATS) </div>	
<p><i>INSTRUCTIONS:</i> <i>The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, upload the signed document to the DMCC E-regulatory binder, IoRA cubby.</i></p> <p><i>Please contact, Christina Carpenter if you have any questions:</i></p> <div style="text-align: center;"> Christina L. Carpenter, Research Project Manager Data Management and Coordinating Center (DMCC) Health Informatics Institute; University of South Florida, 3605 Spectrum Blvd., Suite 100 Tampa, FL 33612 </div>	
<p>I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document <i>Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance</i> dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.</p> <p>In accordance with the National Institutes of Health (NIH) Federal-wide Assurance 00005897: “This investigator assures that all of its activities related to human subject research, regardless of funding source, will be guided by the ethical principles of The Belmont Report.” Additionally, the investigator assures that all activities of this registry will be guided by the ethical principles of The Belmont Report, and Title 45 Part 46 of the Code of Federal Regulations and all of its subparts (A, B, C, and D).</p> <p>As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID.</p>	
<div style="border-top: 1px solid black; margin-bottom: 10px;"> <p>Site Principal Investigator (Print)</p> </div> <div style="display: flex; justify-content: space-between;"> <div style="border-top: 1px solid black; width: 45%; margin-bottom: 10px;"> <p>Site Principal Investigator (Signature)</p> </div> <div style="border-top: 1px solid black; width: 45%; margin-bottom: 10px;"> <p>Date</p> </div> </div>	

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CCHMC	Cincinnati Children's Hospital Medical Center
CFR	Code of Federal Regulations
CHCO	Children's Hospital Colorado
CHOP	Children's Hospital of Philadelphia
COI	Conflict of Interest
COM	Clinical Outcome Measure
CRF	Case Report Form
CRO	Contract Research Organization
CRPC	Central Review Pathology Committee
DHHS	Department of Health and Human Services
EC	Eosinophilic Colitis
EDP	EoE Diagnostic Panel
EG	Eosinophilic Gastritis
EoE	Eosinophilic Esophagitis
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
HPF	High Power Field
HUP	Hospital of University of Pennsylvania
ICF	Informed Consent Form
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IU	Indiana University School of Medicine/IU Health
LCH	Lurie Children's Hospital
MOP/MOO	Manual of Procedures/Manual of Operations
N	Number (typically refers to participants)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NW	Northwestern University
OHRP	Office for Human Research Protections

OHSR	Office of Human Subjects Research
PHI	Protected Health Information
PI	Principal Investigator
PPI	Proton Pump Inhibitor
PRO	Patient Reported Outcomes
QA	Quality Assurance
QC	Quality Control
RCHSPB	Regulatory Compliance and Human Subjects Protection Branch
RCHSPP	Regulatory Compliance and Human Subjects Protection Program
RHC	Riley Hospital for Children
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TMC	Tufts Medical Center
UCSD	University of California, San Diego
UNC	University of North Carolina

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Protocol Summary

Full Title:	A Prospective, Multicenter Study to Compare and Validate Endoscopic, Histologic, Molecular, and Patient-reported Outcomes in Pediatric and Adult Patients with Eosinophilic Esophagitis (EoE), Gastritis (EG), and Colitis (EC)
Short Title:	OMEGA
Conducted by:	Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR)
Sample Size:	N= 600 EoE, 300 EG, and 150 EC patients (total for all participating sites), 150 non EGID for Normal controls
Accrual Ceiling:	This study plans to continue enrollment for the duration of funding.
Study Population:	<p>Males or Females ≥ 3 years of age; presence of symptoms including but not limited to:</p> <ul style="list-style-type: none">• EoE- abdominal pain, vomiting, heartburn, feeding/eating problems, dysphagia, food impaction• EG- abdominal pain, vomiting• EC- bloody/non bloody diarrhea, tenesmus, abdominal pain <p>Mucosal eosinophilia:</p> <ul style="list-style-type: none">• EoE- ≥ 15 eosinophils/HPF• EG- ≥ 30 eosinophils/HPF in 5 HPFs• EC- ≥ 65 eosinophils/HPF
Accrual Period:	The projected date of enrollment for this study is January 2015. We will accrue subjects for 5 years or longer if funding is renewed.
Study Design:	Prospective longitudinal cohort study. This study is observational in nature and no treatments or products are being studied.
Study Duration:	Subjects enrolled and followed for the duration of funding (5 years) and longer if funding is renewed.
Primary Objective:	<p><u>Specific Aim 1-</u> Determine the correlation of EoE, EG, and EC clinical outcome measures (COMs) with mucosal eosinophilia.</p> <p>Primary Objective-To perform a prospective, multicenter, pediatric and adult longitudinal study to determine the correlation of COMs</p>

(PROs, QoL and endoscopy) with the established biomarker of peak mucosal eosinophil count (Phase 1).

Specific Aim 2- Determine the correlation of the molecular profile for EoE, EG, and EC with clinical outcome measures (COMs) and mucosal eosinophilia.

Primary Objective-To perform a prospective, multicenter, pediatric and adult exploratory study to compare the transcriptomes (EoE-transcriptome, EG-transcriptome and EC-transcriptome) with their respective peak mucosal eosinophil counts and respective COMs generated in Aim 1.

Secondary Objectives: Specific Aim 1

Secondary Objective-To determine how the EoE, EG and EC COMs and tissue histology change over time in order to determine a better understanding of the natural history of the disease and response to therapy. (Phase 2)

Exploratory Objectives: Specific Aim 2

Secondary Objective- To perform an exploratory study to compare the three transcriptomes to each other in order to identify patterns of similarity and differences in order to understand the etiological relationship of these diseases and potential therapeutic strategies moving forward.

Creation of DNA repository

Exploratory Objective- To isolate and study some of the proteins, RNA, and DNA (the material contained in genes) from the blood such as the level of eosinophil attraction proteins (eotaxins) and eosinophil growth factors (such as interleukin 5). DNA will be banked at CCHMC for future studies including genome wide association analyses (GWAS), candidate gene analyses and sequencing. Results will be deposited into dbGAP if required by NIH.

1 Key Roles

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2 Background Information and Scientific Rationale

2.1 Background Information

2.1.1 Summary of Relevant Studies

Over the course of the last decade, investigators have taken a stepwise approach to define the diagnostic criteria of EoE and to develop metrics for assessing disease activity. These approaches, so far limited to EoE, have included reaching a consensus about the diagnostic threshold of tissue eosinophil levels, as well as the identification of tissue based transcript signatures that differentiate EoE from control individuals and correlate with disease activity and pathological components, and finally the development of outcome metrics that quantify tissue, endoscopic and clinical parameters. A summary of some of these developments is presented below:

1. *Consensus Recommendation*- the first Consensus Recommendations published in *Gastroenterology*¹ as well as second and third revisions published in *Journal of Allergy and Clinical Immunology*² and *American Journal of Gastroenterology*³, respectively. Each of these peer-reviewed revisions has utilized newly published data to continually revise and reshape clinically relevant and scientifically sound recommendations for the practitioner and researcher.
2. *Clinical Outcome Measure (COM) tools*- investigators have developed COMs. These metrics include the Pediatric EoE Symptom Score (PEESS)⁴, Pediatric Quality of Life (PedsQL™) EoE module⁵, EoE Symptom Activity Index (EESAI), REGID Provider Questionnaire and endoscopic scoring system (EREFS)⁶.
3. *Molecular Criteria for Disease Diagnosis*- Investigators have demonstrated that a panel of EoE related genes (EoE-transcriptome) has high sensitivity and specificity for diagnosing and monitoring EoE⁷. Importantly, the results of this gene panel align when assessed using either fresh tissue or formalin fixed paraffin embedded (FFPE) specimens, which significantly increases its applicability.
4. *Correlation of transcriptome with COMs*- Based on the EoE-transcriptome and the PEESS Patient Reported Outcome (PRO), investigators have preliminarily correlated increased immunoinflammatory gene expression and the cardinal clinical symptom of dysphagia in pediatric EoE (unpublished data). For example, as discussed below, genes involved in inflammation, including those that encode for eosinophil and mast cell products and interleukin receptors, correlate with dysphagia.

2.1.2 Summary of Epidemiological Data

Diverse clinical presentations, unknown pathogenesis and lack of treatments distinguish these rare diseases- Eosinophilic esophagitis (EoE), gastritis (EG) and colitis (EC) are each considered a rare disease on the basis of the estimated prevalence of less than 200,000 each in the US^{2, 8-10}. Each constitutes a chronic inflammatory disease characterized by gastrointestinal (GI) symptoms and dense mucosal eosinophilia. These diseases share the histologic finding of robust mucosal eosinophilia and have variable presentations. For example, while EoE is characterized by symptoms of feeding difficulties and vomiting in young children and food impactions and dysphagia in teenagers and adults, EG patients have upper abdominal pain, nausea and vomiting and EC patients present with diarrhea, hematochezia and lower abdominal

and rectal pain¹¹⁻¹³. To date, the diagnosis of each of these diseases rests on the finding of dense mucosal eosinophilia in the proper clinical setting when all other causes of mucosal eosinophilia are ruled out. Currently, little is known regarding the natural history and pathophysiological mechanisms, especially for EG and EC, and FDA approved drugs do not exist for any of these diseases.

Variability in correlation of mucosal eosinophilia with GI symptoms- Elevated eosinophil levels are required for diagnosis of these diseases and it has generally been accepted that eosinophil counts correlate with disease activity. However, few studies have directly addressed whether eosinophils are the best histological component that correlates with clinical symptoms and these studies have been primarily limited to EoE¹⁴. Proving the fundamental assumption that tissue eosinophil counts correlate with disease activity will indeed be a major focus of the proposed study.

A number of issues are likely contributing to the uncertainty about the best tissue features to monitor for correlation with clinical symptoms. These include 1) lack of validated metrics to assess whether symptoms or PROs align with tissue histology measures; 2) lack of prospective, randomized, placebo controlled trials that have utilized the same primary outcome variables; 3) lack of studies that have cohort sizes that are truly powered to find correlations between symptoms and tissue histology elements; 4) the intermittent nature of symptoms and the ability of patients to institute behavioral and lifestyle changes to compensate for their symptoms¹⁵; and 5) inconsistent measurements of tissue eosinophil counts in various studies¹⁶.

In this study, we will overcome many of the limitations of prior studies by (1) utilizing a series of uniform clinical outcome measures (COMs); (2) conducting a large multi-site prospective trial with well-defined entry criteria, and (3) standardizing the way in which tissue eosinophil counts and other histological features are measured.

Complexity of tissue eosinophil counts as diagnostic feature of EG and EC-The normal esophagus is void of mucosal eosinophils¹⁷. As such, the histological assessment of EoE is relatively straightforward. In contrast, the diagnosis of EG and EC are particularly complex as eosinophils are normal resident cells in the gastrointestinal mucosa¹⁷⁻¹⁹. For all of these diseases, increased mucosal eosinophils are not pathognomonic for EoE, EG, or EC as they can be seen in other diseases including reflux esophagitis, food allergy, inflammatory bowel diseases and infections²⁰. As such, definitions of key clinical and histological features, especially for patients with EG and EC are urgently needed. Currently, based on limited data and clinical experiences, it has been proposed that the diagnostic criteria for EG and EC includes the histological finding of at least twice the normal peak number of eosinophils per high power field (eos/hpf) reported in different regions of the non-diseased stomach and/or colon²¹⁻²³. During the course of studies defined herein, we seek to advance the field by determining the relationships between COMs and mucosal eosinophilia that will ultimately assist in developing diagnostic criteria, understanding mechanisms, and identifying endpoints for treatment efficacy.

Lack of understanding of pathophysiology- Pathophysiological mechanisms defining EoE have made great strides in the last decade^{24, 25}. For instance, a number of studies identified dysregulation of the allergic arm of the immune system in the pathogenesis of EoE²⁶. This etiology is supported by the reversibility of EoE following dietary avoidance of specific foods²⁷, the reoccurrence of EoE upon re-introduction of the removed foods²⁸, the induction of the disease in mice by exposure to both food and aero-allergens²⁹, and genome-wide transcriptome analysis of esophageal tissue that implicated an interplay between the innate and adaptive immune responses³⁰⁻³². EoE has a strong hereditary component with a large sibling risk ratio ($\lambda_s \sim 80$)³².

Early genetic analyses have identified susceptibility loci in the regions that contain candidate genes expressed in epithelial cells and strongly implicated in antigen recognition (*TSLP*, thymic stromal lymphopoietin) and inflammatory cell recruitment / activation (*CCL26*, eotaxin-3)^{26, 30, 31}. The Th2 cytokine IL-13 programs transcription of key EoE-related genes and pathways and TGFβ1 has been proposed to be a regulator of EoE pathogenesis^{33, 34}. In contrast, our understanding of EG and EC is in its nascency with current murine model studies that suggest roles for eosinophil associated cytokines eotaxin-1 and IL-5³⁵. Thus, while early evidence suggests that these diseases occur as a result of immune dysfunction, specific targets and biomarkers remain to be fully recognized.

2.2 Rationale

A number of issues are likely contributing to the uncertainty about the best tissue features to monitor for correlation with clinical symptoms. These include 1) lack of validated metrics to assess whether symptoms or PROs align with tissue histology measures; 2) lack of prospective, randomized, placebo controlled trials that have utilized the same primary outcome variables; 3) lack of studies that have cohort sizes that are truly powered to find correlations between symptoms and tissue histology elements; 4) the intermittent nature of symptoms and the ability of patients to institute behavioral and lifestyle changes to compensate for their symptoms⁶; and 5) inconsistent measurements of tissue eosinophil counts in various studies¹⁶.

In this study, we will overcome many of the limitations of prior studies by utilizing 1) a series of uniform clinical outcome measures (COMs); 2) conducting a multi-site prospective trial with well-defined entry criteria; and 3) standardizing measurements of tissue eosinophilia and other histological features.

3 Study Objectives

3.1 Primary Objective

Specific Aim 1- Determine the correlation of EoE, EG, and EC clinical outcome measures (COMs) with mucosal eosinophilia.

Primary Objective-To perform a prospective, multicenter, pediatric and adult longitudinal study to determine the correlation of COMs (PROs, QoL and endoscopy) with the established biomarker of peak mucosal eosinophil count (Phase 1).

Hypotheses: The overriding hypothesis of this aim is that clinical outcome measures (COMs) will correlate with the standard biomarker of mucosal eosinophilia in subjects with EoE, EG, and EC. In addition, we will test a series of other hypotheses to help determine phenotypes associated with EoE, EG and EC. The overall aim of these studies will be to measure clinical features of pediatric and adult EoE, EG, and EC in a cross sectional and longitudinal study and correlate these findings with the standard biomarker, mucosal eosinophilia. Clinical endpoints include pediatric and adult specific COMs such as patient-reported outcomes (PROs), quality-of-life (QoL) scales, endoscopic scores, and the histological endpoint peak will be mucosal eosinophil count in the respective organ (EoE-esophagus, EG-stomach, EC-colon).

Specific Aim 2- Determine the correlation of the molecular profile for EoE, EG, and EC with clinical outcome measures (COMs) and mucosal eosinophilia.

August 8, 2016

Primary Objective-To perform a prospective, multicenter, pediatric and adult exploratory study to compare the transcriptomes (EoE-transcriptome, EG-transcriptome and EC-transcriptome) with their respective peak mucosal eosinophil counts and respective COMs generated in Aim 1.

Hypothesis: We hypothesize that there is a disease specific mRNA expression profile in EoE, EG and EC that will associate with tissue eosinophil counts and COMs.

3.2 Secondary Objectives

Specific Aim 1

Secondary Objective-To determine how the EoE, EG and EC COMs and tissue histology change over time in order to determine a better understanding of the natural history of the disease and response to therapy. (Phase 2)

3.3 Exploratory Objectives

Specific Aim 2

Secondary Objective- To perform an exploratory study to compare the three transcriptomes to each other in order to identify patterns of similarity and differences in order to understand the etiological relationship of these diseases and potential therapeutic strategies moving forward.

Creation of DNA repository

Exploratory Objective- To isolate and study some of the proteins, RNA, and DNA (the material contained in genes) from the blood/saliva such as the level of eosinophil attraction proteins (eotaxins) and eosinophil growth factors (such as interleukin 5). We will bank the DNA for future studies including genome wide association analyses, candidate gene analyses and sequencing. Results will be deposited into dbGAP if required by NIH.

3.4 Substudy Objectives

For a subset of 30 subjects, the histology scoring tool will be tested for inter- and intra-observer reliability at baseline and end of treatment for acceptable variability.

3.5 Substudy Objectives

For a subset of 144 to 150 subjects we will conduct a prospective, observational study that will quantitatively characterize the microbiome of pediatric and adult subjects with Eosinophilic Esophagitis (EoE), Eosinophilic Gastritis (EG), and Eosinophilic Colitis (EC) along with a group of normal adult and pediatric controls, by characterizing alpha and beta diversity at the taxonomic level within and between these groups.

4 Study Design

4.1 Description of the Study Design

Specific Aim 1: Primary Objective (Phase 1)-We will conduct a prospective, observational study that will measure COMs and peak mucosal eosinophilia in pediatric and adult subjects with EoE, EG and EC. Subjects will be recruited by investigators located at multiple academic and research medical centers. Subjects will be enrolled who have a confirmed diagnosis of EoE, EG or EC regardless of

their treatment status, as we are interested in the relationship between mucosal inflammation, whether present or not, with the COMs.

Three groups of patients will be recruited; 1.) patients with an established diagnosis of EoE, EG or EC 2.) patients who have a new diagnosis of EoE, EG or EC and 3) healthy controls that are having endoscopy and/or colonoscopy (this group is for the mechanistic microbiome study). The second group will be enrolled within 4 weeks of the diagnostic endoscopy and prior to any treatments for their disease. Recruited patients will then 1.) sign informed consent, 2.) undergo standard of care (SOC) evaluation to capture defined clinical data, 3.) complete questionnaires, and 4.) analysis of their formalin-fixed, paraffin-embedded (FFPE) esophageal, gastric, or colonic biopsy (obtained as part of SOC) to measure peak eosinophil count and associated features. At the time of SOC endoscopy, subjects who have consented to have research biopsies collected will provide two to four additional biopsies for RNA and microbiome analysis (this will be repeated at each SOC endoscopy during the course of the study). At the time of the SOC endoscopy post enrollment (microbiome sub-study participants are not in study after the first SOC procedure is completed), subjects who have consented to donate blood/saliva/stool for this study will provide 10-15 mls of blood and/or 2 ml of saliva for DNA analysis and stool for storage and for microbiome analysis (see sub studies). At the time of endoscopy, one photograph image will be captured from the site of the research biopsies.

Correlation of COMs with peak eosinophil counts will lead to development of an understanding of optimal COM to measure disease activity. If an endoscopy is to be scheduled, the endoscopic score will be assigned at the time of the procedure. If the endoscopy has been already completed, the score will be performed on the recorded video/pictures as described below. Instructions and administering the COMs will be coordinated at each enrollment site.

Creation of DNA repository

Exploratory Objective- Subjects who consent to donate blood/saliva/ stool for this study will have their sample used to isolate and study some of the proteins, RNA, and DNA (the material contained in genes) from the blood such as the level of eosinophil attraction proteins (eotaxins) and eosinophil growth factors (such as interleukin 5). DNA will be banked at CCHMC for future studies including genome wide association analyses, candidate gene analyses and sequencing. Results will be deposited into dbGAP if required by NIH. Stool will be stored and used for studies not limited to assessment of microbiome.

Specific Aim 1: Secondary Objective (Phase 2)- In Phase 2 of this study, subjects enrolled in Phase 1 will continue to be followed and assessed at each of the following time points: 1.) annually, 2.) at the time of any change in SOC treatment and 3.) at the time of any endoscopic procedure. In scenarios 1, or 2, only PRO and QoL COMs will be completed, and in scenario 3, all COMs and histological analysis will be analyzed. All patients will be undergoing SOC during this time; SOC treatment will be the choice of the attending physician and may include systemic or topical steroids, diet, or immunosuppression. This is not an interventional study and thus *treatment will be monitored, but not prescribed* as a part of this protocol. This is a natural history study, and since SOC *maintenance* treatments and paradigms do not yet exist, we will only monitor COMs and histology over time and not dictate treatments. We have taken this approach for several reasons including the capture of “real world” data such that study findings are broadly applicable to the EoE, EG, and EC populations at large, to allow entry of the largest cohort of subjects independent of therapeutic intervention, and to better understand patient adherence to a prescribed therapeutic regimen as it relates to disease course.

Specific Aim 2: Primary Objective- We hypothesize that there will be largely unique gene profiles in each disease. We will utilize well characterized EoE, EG, and EC subjects and control subjects for gene expression analysis. We will perform genome-wide screening in the EG and EC populations since it is not clear what the gene expression profile is in these diseases. The EoE genome wide

expression data is already well identified so we will not repeat this analysis on the esophageal samples, at least initially. Our first objective will be to identify disease specific transcript profiles that distinguish EG from controls and EC from controls. We will then correlate the pattern of gene expression in each disease with tissue eosinophil level for each disease. Furthermore, we will use the histology scoring tools described in Aim 1 to assess the correlation of dysregulated genes and other EoE-, EG-, and EC-related tissue features. During this process, the stringency levels (such as FDR, fold change, P value, etc.) will be interactively adjusted to yield a balance between gene of interest (GOI) pool size and positive prediction. The final leads selection should also be performed in the light of a comprehensive pathway analysis and principle component analysis to guarantee multiple biological processes coverage and minimal redundancy. The resulting GOI pool will be subsequently validated by qPCR and/or protein detection, at least for some of the major GOIs. Based on the identified disease specific genes, and those that track with specific disease characteristics such as tissue levels of eosinophils, mast cells, remodeling, mucus, and cellular hyperplasia, we will embed a set of diagnostic genes into a PCR-based high throughput fluid card, as we recently reported for the development of the EoE Diagnostic Panel (EDP)⁷. An EG diagnostic panel (EG-DP) and EC diagnostic panel (EC-DP) has potential to transform the field as it has potential for rapid, sensitive, specific diagnosis, and can provide information not readily apparent on initial microscopic analysis, as reported for the EoE-DP. It is noteworthy to point out that this potentially transformative undertaking could be scaled for general usage in the field.

Specific Aim 2: Secondary Objective- Determine if specific transcripts within the EoE, EG and EC DPs are reflective of disease activity as determined by COMs. In this aim, we hypothesize that specific genes will correlate with and reflect EoE, EG, and EC disease activity as assessed by the COMs described in Aim 1. We will compare the transcriptomes to COMs to determine which genes best correlate with specific clinical outcomes; this will provide insight into potential pathogenic etiology of specific clinical manifestations and has the potential to predict clinical phenotypes. Preliminary studies by Rothenberg and colleagues have interrogated the EoE-DP for its association with PEESV2.0 questions. In particular, each domain of the PEESV2.0 was correlated with the full set of genes in the EDP, using a cohort of 44 EoE patients with variable levels of esophageal eosinophils. This investigation has shown that the dysphagia domain correlated with a subset of genes. In particular, among the subset of genes were eosinophil associated genes (Charcot Leyden Crystal [CLC] and IL5Ra) and mast cell genes (carboxypeptidase A3 [CPA3]), which achieved statistically significant association. This important and potentially paradigm shifting preliminary finding highlights the proof of concept that gene expression can reflect clinical symptoms and demonstrates the ways in which we will dissect meaningful molecular-clinical data. We are well positioned to employ this innovative approach in our study since we have ready access to tissue specimens and the capabilities to perform molecular analysis, and related bioinformatics processing. Using unsupervised machine learning algorithms, we will determine whether patient samples can be accurately classified according to diagnosis based on their specific gene expression signatures. Signatures will be analyzed using a suite of gene set enrichment programs to identify metabolic pathways and/or gene networks that are enriched in these 3 rare diseases. These studies will provide preliminary data for more detailed studies focused on follow-up validation studies which will likely include studies of identified candidates as potential biomarkers of EoE, EG and EC. If our hypotheses are proven to be correct, they have potential to transform this field as we will test a number of subsequent hypotheses including 1) EoE, EG and EC are characterized by Th2 immune profiles; 2) EG and EC transcriptomes will be different than that from normal controls and 3) specific elements of the EoE, EG and EC transcriptomes will correlate with eosinophil levels (and possibly other features of inflammation). In essence, identification of the DP for each of these diseases has potential to improve monitoring of patients during therapeutic intervention and identify molecular markers with therapeutic significance.

4.2 Study Endpoints

4.2.1 Primary Endpoint

Specific Aim 1

Primary Endpoints

- Peak mucosal eosinophil count at the time of enrollment from endoscopic biopsies obtained from the esophagus for EoE, stomach for EG, and colon for EC. The peak eosinophil count will be obtained from mucosal samples of each organ in 1 high power field.
- COM scores (see table 1) at one year intervals and clinically indicated visits and procedures.
- Correlation of peak mucosal eosinophil counts at the time of enrollment with the COM scores at one year intervals and at clinically indicated visits and procedures.

Table 1- COM metrics- (see below for descriptions)

PROs- Symptoms and QOL					
Metric	Rationale	Age	EoE	EG	EC
PEESSv2.0 Child/Teen Report	Assesses EoE symptoms	Peds	X	X	
PEESSv2.0 Parent Report	Assesses EoE symptoms	Parent	X	X	
PedsQL™ – Parents of Toddlers 2 – 4 years	Assesses EoE quality of life	Parent	X		
PedsQL™ – Young Children 5 – 7 years	Assesses EoE quality of life	Peds	X		
PedsQL™ – Parents of Young Children 5 – 7 years	Assesses EoE quality of life	Parent	X		
PedsQL™ – Children 8 – 12 years	Assesses EoE quality of life	Peds	X		
PedsQL™ – Parents of Children 8 – 12 years	Assesses EoE quality of life	Parent	X		
PedsQL™ –Adolescents 13 – 18 years	Assesses EoE quality of life	Peds	X		
PedsQL™ – Parents of Adolescents 13 – 18 years	Assesses EoE quality of life	Parent	X		
EEsAI	Assesses EoE symptoms	Adult	X		
EoE-QoL-A	Assesses EoE quality of life	Adult	X		
Likert Dyspepsia	Assesses gastric symptoms	Peds		X	
SODA	Assesses gastric symptoms	Adult		X	
PUCAI	Assesses colitic symptoms	Peds			X
SCCAI	Assesses colitic symptoms	Adult			X
PROMIS Peds Profile 25	Assesses general quality of life	Peds	X	X	X
PROMIS Adult Profile 29	Assesses general quality of life	Adult	X	X	X
Endoscopy					
Metric	Rationale	Age	EoE	EG	EC

EREFS	Assesses esophagitis	Both	X		
Lanza score	Assesses gastritis	Both		X	
UCCIS	Assesses colitis	Both			X
Histology					
Metric	Rationale	Age	EoE	EG	EC
<i>EoE Biopsy Evaluation</i>	Assesses esophageal eosinophilia and histologic features	Both	X		
<i>EG Biopsy Evaluation</i>	Assesses gastric eosinophilia and histologic features	Both		X	
<i>EC Biopsy Evaluation</i>	Assesses colonic eosinophilia and histologic features	Both			X
Exploratory					
Metric	Rationale	Age	EoE	EG	EC
EoE, EG, and EC transcriptome panel	Assesses gene expression for transcript phenotypes	Both	X	X	X

PRO-Symptoms and QOL

Pediatric EoE Symptom Score (PEESS)- PEESV2.0 is a pediatric PRO that contains patient and parent proxy instruments that can be easily understood. Scoring is based on two domains that evaluate frequency and severity of the symptoms reported by the patient (see Appendix). PEESV2.0 will be used to assess EoE symptoms. In addition, the PEESV2.0 will be used to assess pediatric EG as symptoms are similar to those present in EoE.

EoE Symptom Activity Index (EEsAI)- The EEsAI is an adult symptom module that has been content and face validated and represents an EoE-specific PRO. The EoE-QOL is an adult 37 item, 5 factor HRQL specific for adult EoE that has demonstrated excellent internal consistency.

Likert dyspepsia scale- Since no instruments are available to date for pediatric EG, we will use the Likert dyspepsia scale as an instrument to assess for related health.

Severity of Dyspepsia Assessment (SODA)- Since instruments are not available to date for adult EG, we will use the severity of dyspepsia assessment (SODA) scale as a validated instrument to assess dyspepsia related health.

Pediatric Ulcerative Colitis Activity Index (PUCAI)- The PUCAI is a validated pediatric objective measure used to standardize reporting of clinical ulcerative colitis activity. The index is composed of a 6-point scale that includes abdominal pain, rectal bleeding, stool consistency, number of stools, nocturnal stools and activity level. Scores range from 0-85 with a score of 45 being used for most studies indicating disease activity.

Simple Clinical Colitis Activity Index (SCCAI)- The SCCAI is a validated symptom based index (score 0–19) used to assess ulcerative colitis. It shows good correlation with other measures of ulcerative colitis disease activity. The SCCAI will be used to assess adult EC as symptoms are similar to those present in EC.

PedsQL™ 3.0- This instrument is a disease-specific measure of health-related quality of life for pediatric patients diagnosed with EoE.

EoE-QoL-A- The EoE-QoL-A is a self-reported questionnaire designed to assess disease-specific health related quality of life in adult EoE patients. Questions are designed to evaluate established domains of Health Related QOL, such as social functioning, emotional functioning, and disease impact on daily life experiences. It is a 37-item measure with five subscales: eating/dietary impact, social impact, emotional impact, disease anxiety and choking anxiety.

PROMIS- The PROMIS assessments selected for this study are multi-purpose, short-form health surveys with 29 and 25 questions respectively, that will be used for adult and pediatric EoE, EG, and EC. It yields a profile of general well-being.

Endoscopy

Endoscopic Reference System (EREFS)- The EREFS is a visual scoring tool developed to detect endoscopic features of EoE. EREFS was developed by CEGIR gastroenterologists and has been validated in adult subjects with active EoE by gastroenterologists with good inter-observer agreement. At time of endoscopy, an EREFS score will be assigned to the five central esophageal features of EoE (edema, rings, exudates, furrows, stricture). Training of endoscopists will be provided by Dr. Hirano as to the use of the scoring system by means of a presently developed video library. Dr. Hirano has developed a pictorial atlas with scoring criteria that will be provided to each investigator for reference. A score will be recorded for analysis with separate components for inflammatory and fibrostenotic features. If a procedure was completed prior to study entry, the endoscopic pictures and report will be used to generate an EREFS score.

Lanza Score-The Lanza score is an endoscopic grading system used to evaluate for gastritis. It has been used in therapeutic trials and is based on a 0-7 with 0=no lesions and 7= >3 mm ulcer scale. Since no system has been developed/used to date for EG, this score will be used to assess both pediatric and adult EG.

Ulcerative Colitis Colonoscopic Index of Severity (UCCIS)-This score was developed and validated for assessment of ulcerative colitis (UC). It is based on 6 components including vascular pattern, granularity, ulceration, and friability. Interobserver agreement is good to excellent and is correlated with clinical activity indices (R=0.52, p,0.001).

Histology

Pediatric and adult subjects with upcoming clinical endoscopy will be given the option to donate two – four (2-4) biopsies for this research. The research biopsies will be used for RNA and/or protein analysis. The immunological and histological analyses will generally be performed on the tissue normally taken for routine pathological evaluation.

Biopsy slide review- This study is multi-institutional and therefore biopsies will initially be reviewed by multiple different pathologists. We will ensure the integrity of the study and minimize variability in biopsy interpretation by having a Central Review Pathology Committee (CRPC), comprised of 3 pathologists who are experts in EoE, EG and EC biopsy interpretation. We will employ slide scanning and imaging software to achieve review by the CRPC.

Archived biopsy specimens upon which the subject's diagnosis was based prior to the start of this study will be scanned or sent to the Pathology Committee. If this is not possible because the original specimen was not obtained locally or is not available in the archive, a follow up biopsy with active disease will be scanned/sent for review by the Pathology Committee. If all subsequent diagnostic biopsies are inactive due to therapy then this will be noted in the subject file at DMCC and locally.

Biopsy specimens (original diagnostic and follow up collected during enrollment in study) will be scanned or sent to the Pathology Committee for review and agreement of the diagnosis will be confirmed with the local site. If the first endoscopy during the study is the diagnostic endoscopy and diagnostic criteria is not met, the subject will be withdrawn from the study.

For a subset of 30 subjects, the histology scoring tool will be tested for inter- and intra-observer reliability at baseline and end of treatment for acceptable variability.

Pathology Evaluation Forms- Esophageal, gastric and colonic pathology forms have been developed by Dr. Collins, and approved by Drs. Capocelli and Yang, with the goal of capturing features of eosinophilia relevant to the affected tissue and also other features indicative of mucosal health and

inflammation. All 3 pathology forms will be completed on-line by a pathologist. The final forms containing one set of scores for each biopsy will be submitted for data entry following resolution of differences among the pathologists. These numerical indices of eosinophil inflammation and associated pathology will be correlated to endoscopic and symptom metrics.

Specific Aim 2

Primary Endpoints

- Disease specific transcript profiles that distinguish EG from controls and EC from controls.
- Correlation of the pattern of gene expression in each disease with tissue eosinophil level for each disease.
- Correlation of dysregulated genes and other EoE-, EG-, and EC-related tissue features as described in Aim 1 (histology scoring tools).
- Resulting gene-of-interest (GOI) pool validated by qPCR and/or protein detection. We are planning on performing a genome wide expression (transcriptome) analysis of biopsy derived RNA. After we identify genes of interest, we will validate their expression changes by qPCR and/or protein detection (e.g. ELISA, Western Blot analysis, etc).
- Set of diagnostic genes embedded into a PCR-based high throughput fluid card. Similar to the EoE Diagnostic Panel, we will consider embedding the informative genes into a custom array, based on the ABI fluid card PCR system.

4.2.2 Secondary Endpoints

Specific Aim 1

- To determine whether COMs change during standard of care treatment
- To determine whether other histological features of eosinophilic inflammation are associated with peak eosinophil counts
- To define the basic clinical and laboratory features of EG and EC.

Specific Aim 2

- To determine if specific transcripts within the EoE, EG and EC DPs are reflective of disease activity as determined by COMS.
- To compare the transcriptomes to COMs to determine which genes best correlate with specific clinical outcomes
- To determine whether patient biopsy samples can be accurately classified according to diagnosis based on their specific gene expression signatures.

4.2.3 Exploratory Endpoints

The study subjects utilized for the exploratory analyses will be the same as those recruited in Aim 1, but the exploratory study will be limited to analysis of 20 samples from each subject group with active diseases and no therapy, as well as respective control samples (tissue from subjects that have normal histology and no history of any GI disorder).

Additionally, financial resources limit performance of gene expression profiles on all patients enrolled in this study. It is important to point out that all patients will have biopsy samples collected as part of SOC and will have archived FFPE specimens available. All subjects will be given the option to donate additional biopsies for RNA analysis. So, we will be in a position to subsequently analyze specific genes in follow up validation studies and/or to test specific hypotheses that may later develop such as those about particular patient phenotypes. If the subject subsequently undergoes successful therapy, we will also assess his/her second biopsy for changes in the mRNA

transcript profile following SOC treatment. EoE, EG, and EC as well as resolution of EoE, EG, and EC will be defined as in Aim 1. Patients who consent to the procurement of an extra biopsy sample, will have two research biopsies collected in RNALater and shipped to the Rothenberg lab. Since EG and EC are not yet well characterized, and based on our preliminary analysis that gastric tissue has minimal overlap with the EoE transcriptome, we will initially focus on taking a genome wide approach using commercial high density expression chips. As a control, we will analyze the tissue specific transcriptome for gastric and colonic biopsy tissue from subjects who underwent endoscopy for workup of EGID (EG and EC) who subsequently were found to not have EGID or other pathology and no history of prior gastrointestinal disease. The analyses will be controlled for age, gender, and other parameters. Samples will be analyzed from FFPE tissue and fresh RNA, similar to the samples from diseased tissue.

5 Study Population

5.1 Participant Inclusion Criteria

Three groups of patients will be recruited: 1) patients with an established diagnosis of EoE, EG or EC 2) patients who have a new diagnosis of EoE, EG or EC and 3) healthy controls that are having endoscopy and/or colonoscopy (this group is for the mechanistic microbiome sub-study). . . . Diagnosis of EoE is based on published criteria EG will be defined as consistent symptoms and ≥ 30 eos /HPF in 5 HPFs^{21, 23} , and EC will be defined as consistent symptoms and ≥ 65 eos/HPF²¹; in each case alternative causes of mucosal eosinophilia need to be ruled out. Inclusion and exclusion criteria and rationale are listed in Table 2. The enrollment of subjects who meet diagnostic criteria for EoE but who have not had a trial of high dose PPI will be allowed.

Following enrollment, if a subject is found to have eosinophilia in more than one site, the Pathology Committee will determine where the greatest degree of eosinophilic inflammation exists. Subjects will be categorized according to the site of greatest inflammation and the appropriate COMs for that location will be used to assess. The database will reflect that the subject has eosinophilia at multiple locations.

Table 2a- Summary of inclusion criteria for EoE, EG, EC.

<i>Inclusion criteria</i>	<i>Rationale</i>
Males or females ≥ 3 years of age;	Presence of available tools starting at this age
Presence of symptoms*#	Meets the requirement for symptoms to use PRO metrics
Mucosal eosinophilia (see below)	Essential diagnostic criterion for EoE, EG and EC
EoE- ≥ 15 eosinophils/HPF	Consensus recommendation ²
EG- ≥ 30 eosinophils/HPF in 5 HPFs	Minimum threshold ²³ and consistent with 2x Normal ²¹
EC ≥ 65 eosinophils/hpf	Minimum threshold as defined by 2x normal ²¹
<i>MicroBiome Sub-study Controls¹</i>	
No eosinophils on clinically indicated EGD	

5.2 Participant Exclusion Criteria

Table 2b- Summary of exclusion criteria for EoE, EG, EC.

Exclusion criteria	Rationale
History of intestinal surgery other than G tube placement	Can alter PRO metrics
Planned or recent enrollment in blinded investigational studies	COMs will be uninterpretable because of lack of knowledge of treatment
Esophageal stricture < 3mm	Prevents assessment of esophageal mucosa
Other identifiable causes for eosinophilia, infections, GI cancer, other GI inflammatory disease	Prevents assessment of pure subject population
Any physical, mental, or social condition, history or concurrent illness or laboratory abnormality that, in the investigator's judgment, might interfere with study procedures or the ability of the subject to adhere to and complete the study.	Could interfere with study procedures or impact subjects' ability to participate in the study.
Potential participants will be excluded if the investigator determines that the potential participant has diminished capacity and is not cognitively able to participate fully in the consenting process.	Not appropriate to enroll subjects with diminished capacity.
MicroBiome Sub-study Controls	
Use of antibiotic within 30 days of scheduled EGD	Can alter gut microbiome

*Symptoms include, but are not limited to: EoE-abdominal pain, vomiting, heartburn, feeding / eating problems, dysphagia, food impaction, EG- abdominal pain, vomiting, EC-bloody/non bloody diarrhea, tenesmus, abdominal pain

#Symptoms are not required for subjects with previously diagnosed EoE, EG, or EC. Symptoms required for new diagnoses

¹ Children and adults scheduled for a clinically indicated upper or lower GI endoscopy but not suspected to have an Eosinophilic Gastrointestinal Disorder

All diseases except for non EoE for microbiome controls will require clinical symptoms plus a minimal threshold for mucosal eosinophilia as defined in the listed references.

Participation of Women: Subjects will be recruited/enrolled without regard to sex or gender. This is a natural history study. There are no treatments involved which may pose a risk for pregnant mothers, unborn fetuses, or women who are breastfeeding. We will follow all subjects enrolled in this study. There are no requirements for birth control.

Participation of Minorities: Subjects will be recruited/enrolled without regard to racial/ethnic group.

Participation of Children: Children aged 3 years and older will be recruited/enrolled.

Co-enrollment Guidelines: If a subject enrolls in a therapeutic trial during the course of this study, COMs will not be collected during the time they are participating in the trial.

If a subject meeting criteria at the local study site does not have a confirmed diagnosis from the central review pathology committee, the subject will not continue in other OMEGA visits.

5.3 Clinical Evaluations

Subjects will be evaluated using the COMs as described in section 4.2.1. No additional clinic visits will be needed outside of standard-of-care.

5.4 Laboratory Evaluations

5.4.1 Clinical and Research Laboratory Evaluations and Specimen Collection

Pediatric and adult subjects aged ≥ 3 years with upcoming clinical endoscopy will be asked to allow the investigators access to the FFPE specimens obtained as part of SOC, as well as given the option to donate two additional biopsies for this research. Subjects will be asked to provide these specimens at each SOC endoscopy for the life of the study. The research biopsies will be used for RNA and/or protein analysis. The immunological and histological analyses will generally be performed on the tissue normally taken for routine pathological evaluation.

It is important to point out that all subjects will have archived FFPE specimens available. So, despite some subjects declining the additional research biopsies, we will still be in a position to subsequently analyze specific genes in follow up validation studies and/or to test specific hypotheses that may later develop such as those about particular patient phenotypes.

5.5 Substudies

For a subset of 30 subjects, the histology scoring tool will be tested for inter- and intra-observer reliability at baseline and end of treatment for acceptable variability.

Exploratory

Study subjects will be the same as those recruited in Aim 1 but the study will be limited to analysis of 20 samples from each subject group with active diseases and no therapy, as well as respective control samples (tissue from subjects that have normal histology and no history of any GI disorder). This number was selected based on our prior work, where we were clearly able to detect substantial and significant differences between EoE and EG vs. their respective controls with even fewer patients³⁴.

Additionally, financial resources limit performance of gene expression profiles on all patients enrolled in this study. It is important to point out that all patients will have biopsy samples collected for RNA analysis as well as archived FFPE specimens available. So, we will be in a position to subsequently analyze specific genes in follow up validation studies and/or to test specific hypotheses that may later develop such as those about particular patient phenotypes. If the subject subsequently undergoes successful therapy, we will also assess his/her second biopsy for changes in the mRNA transcript profile following SOC treatment. EoE, EG, and EC as well as resolution of EoE, EG, and EC will be defined as in Aim 1. Patients who consent to the procurement of an extra biopsy sample, will have 2 research biopsies collected in RNALater and shipped to the Rothenberg lab. Since EG and EC are

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not yet well characterized, and based on our preliminary analysis that gastric tissue has minimal overlap with the EoE transcriptome, we will initially focus on taking a genome wide approach using commercial high density expression chips, as reported^{34, 36}. As a control, we will analyze gastric and colonic biopsy tissue from subjects who have no histological abnormalities and no history of prior gastrointestinal disease, similar to the approach we have taken for the esophagus^{34, 36}.

Creation of Bio repository

Exploratory Objective- To isolate and study some of the proteins, RNA, and DNA (the material contained in genes) from the blood/saliva/stool such as the level of eosinophil attraction proteins (eotaxins) and eosinophil growth factors (such as interleukin 5). We will bank the DNA for future studies including genome wide association analyses, candidate gene analyses, microbiome analysis and sequencing. Results will be deposited into dbGAP if required by NIH

5.6 Mechanistic sub-study

Role of the Intestinal Microbiome in Eosinophilic Esophagitis (EoE), Eosinophilic Gastritis (EG), and Eosinophilic Colitis (EC)

For a subset of 144 to 150 subjects we will conduct a prospective, observational study that will quantitatively characterize the microbiome of pediatric and adult subjects with Eosinophilic Esophagitis (EoE), Eosinophilic Gastritis (EG), and Eosinophilic Colitis (EC) by characterizing alpha and beta diversity at the taxonomic level within and between these groups.

5.7 Background Information

5.7.1 Description of the Study

This protocol aims to study patients with Eosinophilic Esophagitis (EoE), Eosinophilic Gastritis (EG), and Eosinophilic Colitis (EC) and compare them with normal controls. We hypothesize that the GI tissue, serum and stool from EoE, EG, and EC subjects will have a distinct dysbiotic microbial pattern as compared with the GI tissue, serum and stool from healthy control subjects. We further hypothesize that EoE, EG, and EC will have both similar and unique patterns of dysbiosis from one another.

5.7.2 Summary of Epidemiological Data

Substantial research has provided evidence that the recent emergence of numerous chronic inflammatory autoimmune and allergic disorders can be explained by the hygiene hypothesis, which postulates that exposure to microorganisms directs immune development and response, particularly during early life³⁷⁻⁴⁰. Experimental and epidemiological studies have tested this hypothesis by exploring the role of endogenous commensal flora in the GI tract since dysbiosis of commensal organisms has been linked to non-infectious pathological immune responses in mice and humans⁴¹. For example, elegant studies of gnotobiotic and specific pathogen-free mice have found significant defects in the development of gut-associated immune tissues in germ-free mice [6,7]. These mice also develop significant autoimmunity upon pathogenic challenge. Additionally, dietary consumption of low-level antibiotics used for the treatment of infections leads to microbial patterns associated with allergy predisposition⁴⁰. These studies clearly suggest a complex relationship between endogenous bacterial populations and the patterning of immune regulation/dysregulation with regard to allergic and inflammatory diseases.

In the context of EoE and exposures, CEGIR Investigator Dr. Dellon has recently demonstrated that early-life antibiotic exposures increase EoE risk by 6 fold⁴⁴. Additionally, Dellon et al. have

shown that gastric *Helicobacter pylori* infection is inversely related to EoE risk, providing further support for the complex interactions of microorganism exposure, microbiome population, and esophageal and gastric immune responses⁴⁵. A collaboration of several CEGIR Investigators has recently shown that dizygotic twins have a 10-fold higher EoE concordance rate compared with non-twin siblings, providing strong evidence for a profound impact of early-life exposures in addition to known genetic underpinnings for susceptibility to EoE⁴⁶. Importantly, CEGIR Investigators Drs. Furuta and Fillon have provided provocative data about the esophageal microbiome that shows the presence of both overlapping and distinct microbial patterns from that seen in the oral and nasal mucosa⁴⁷. When combined, these pieces of evidence suggest that there may be specific gene-environment interactions in the pathoetiology of EoE, EG, and/or EC. This proposed Pilot/Demonstration Clinical Research Project will explore microbiome profiles in the rare diseases EoE, EG, and EC in order to further characterize disease etiology and progression.

It is feasible to measure the esophageal microbiome. Previous work from CEGIR Investigators Drs. Furuta and Fillon employed 16S ribosomal RNA gene sequencing to describe the microbiome of normal esophageal mucosal biopsies⁴⁷. These data were originally produced to provide proof of concept for the applicability of the esophageal string test (EST) and are presented here for further proof of feasibility of successfully completing this pilot proposal. Furthermore, differences in the microbiome in health and disease can be identified. Fillon et al. observed a significant enrichment of *Streptococcus* species in the healthy esophagus; in contrast, an assessment of the microbiome in untreated EoE subjects demonstrated a shift toward *Haemophilus* species. *Haemophilus* was significantly increased in untreated patients with EoE (n = 11; P = 0.047) compared to controls (n = 25)⁴⁸; no significant differences were observed between controls and treated patients with EoE (steroids and/or diet; n = 26;). Of note, 45% of patients with EoE and control subjects were treated with PPI therapy in an attempt to insure patient homogeneity, eliminate the influence of PPI-responsive esophageal eosinophilia, and control for the potential effect of PPI on the microbiome.

5.8 Rationale

We hypothesize that the GI tissue, serum and/or stool from EoE, EG, and EC subjects will have a distinct dysbiotic microbial pattern as compared with the GI tissue, serum and stool from healthy control subjects. We further hypothesize that EoE, EG, and EC will have both similar and unique patterns of dysbiosis from one another. Due to the similarity of the inflammatory composition in EoE, EG, and EC, dysbiosis in these diseases may follow a common mechanism. However, important and identifiable differences in the local mucosal environments between these diseases are likely to affect the resident microbial taxonomic composition. Understanding both the commonalities and disparities between the esophageal (neutral pH, squamous mucosa), gastric (acidic pH, enzymes, gastric mucosa), and colonic (neutral pH, high baseline bacterial load) dysbiosis will significantly advance our understanding and treatment of EoE, EG, and EC. Furthermore, an understanding of the microbiome may form the foundation for future intervention trials based on antibiotics, probiotics, and/or elemental formulas.

5.9 Primary Objective

We propose a study to quantitatively characterize the microbiome of subjects with EoE, EC, and EG by characterizing alpha and beta diversity at the taxonomic level within and between these groups. We will test the central hypothesis that there will be measurable microbiome differences between these three diseases, which will distinguish each disease from the others and from their disease-specific controls. Following microbiome analysis, a subtractive taxonomic analysis will be performed in order to evaluate and compare dysbiotic mechanisms in EoE vs. EG vs. EC.

5.10 Description of the Study Design

Patients with EoE and the corresponding control patients will be on PPI at the time of biopsy. Patients with EG or EC will not have this PPI requirement however PPI may have an effect on the lower gut microbiome as detected in stool⁴⁹⁻⁵¹. Normal subjects will be identified from patients that undergo endoscopy for clinical indications, including screening colonoscopies, and are found to have no pathology. Patients and controls will be matched by gender, race/ethnicity, and age (≥ 3 years of age). Individuals with recent antibiotic exposure will be excluded (use of antibiotics within the last month). The EC microbiome will be assessed using sigmoid or descending colon biopsies, EG microbiome will be assessed using antral or body biopsies, and EoE microbiome will be assessed using a distal esophageal biopsy, each from macroscopically affected tissue. A concurrent specimen from an adjacent area will be analyzed for the presence of active inflammation. Stool and serum samples will be collected from all subjects. DNA extraction will be performed as previously described⁴⁷ and amplified in triplicate with barcoded polymerase chain reaction (PCR) primers that include adaptors for the Illumina MiSeq sequencing platform. Negative PCR controls will be performed for each barcode, and PCR will be repeated for any sample in which the negative control was positive. Amplicons will be pooled after normalization of DNA concentration and sequenced using the Illumina MiSeq platform. Sequence data will be assigned to samples of origin using barcode sequences added during PCR and screened for basic quality defects (short sequences < 200 nucleotides in length, > 1 sequence ambiguity, best read with quality ≥ 20 over a 10-nucleotide moving window) using custom Python software as well as Explicet and Qiime⁵². Sequences identified as potential chimeras by UChime will be removed from datasets. The SINA Classifier software will be used to identify operational transcription units. We will construct sequence groups with identical taxonomic rank, which will be used for bacterial community analyses in order to identify specific bacteria that are differentially represented between disease states (EoE, EG, and EC) and between disease states and their controls.

5.11 Study Endpoints

5.11.1 Primary Endpoint

Identify significant differences in bacterial genera between EoE, EG and EC and their respective controls in both mucosal biopsies, serum and stool.

5.11.2 Secondary Endpoints

Identify different microbiome or specific bacterial patterns in EoE vs, EG, vs EC in both mucosal biopsies, serum and stool.

5.11.3 Exploratory Endpoints

Determine the impact of PPI treatment on the microbiota in non-inflamed controls and inflamed groups in comparison to the non-PPI treated subjects.

Compare the microbiome in each group between the different geographical locations.
Compare the mucosal biopsy to the stool microbiome in each group and determine if specific IgA and IgG coated bacteria are identified in the stool samples.

5.12 Laboratory Evaluations

5.12.1 Clinical and Research Laboratory Evaluations and Specimen Collection

Pediatric and adult subjects aged ≥ 3 years with upcoming clinical endoscopy will be asked to donate two biopsies, serum and a stool sample for this research. The research biopsies will be used for microbial 16S sequencing, culture, future RNA seq and staining. The serum will be used for future identification of peripheral biomarkers, including total Ig levels, as well as Ig recognizing specific bacteria identified in EGIDs. The stool sample will be used for 16S sequencing and microbiome identification. The samples for blood (5ml), stool, and biopsy will be collected in addition to those in the main study in those patients who consent to sample collection.

6 Potential Risks and Benefits

6.1.1 Potential Risks

Confidentiality - there are minimal risks related to confidentiality of the COMs.

Mucosal Biopsies - there are minimal risks related to obtaining mucosal biopsies. Additional endoscopy procedures will not be performed because of participation in this study. In some cases, we will be able to use the standard biopsy tissue for research purposes (such as histology and immunohistochemistry) since a typical biopsy can yield at least 5-10 sections for microscopy. In subjects who consent to the collection of research biopsies, 2 additional biopsy samples will be obtained from the organ on interest (EoE-esophagus, EG- stomach, EC-colon), and these may be associated with risks of: bleeding at the site of tissue (biopsy) collection, and a small chance of perforation (hole) of the colon, stomach, duodenum, or esophagus. Perforation is the most severe gastrointestinal complication, but generally is self-resolving and poses no life-threatening risk.

Blood collection- Risks associated with the collection of blood are bleeding, bruising, swelling, dizziness, fainting and infection at the site where the blood is drawn. In general, these procedures will be performed by individuals with expert skills in phlebotomy. To minimize the additional risks associated with phlebotomy, blood will be obtained during the standard placement of intravenous lines when possible. The amount of blood drawn will adhere to institutional policy.

Saliva collection- There is no known risk associated with saliva collection.

Potential risks of these studies will be fully disclosed at the time of the consent. Privacy and confidentiality will be maintained according to HIPAA guidelines. These and other risks are reviewed in the Informed Consent form which has been approved by the IRB.

6.1.2 Potential Benefits

Although there are no direct benefits to participating in this study, it is likely that participation will advance the understanding of these poorly understood diseases. Patients with EoE, EG and EC suffer from a variety of life-impairing problems including difficulty feeding, failure to thrive, vomiting, epigastric or chest pain, dysphagia, food impaction, diarrhea and bloody stools^{2, 53-56}. A better understanding of EoE, EG or EC should help in the formulation of a rational approach to diagnosis and therapeutic intervention and, hopefully, lead to a decrease in the morbidity and mortality associated with these rare diseases.

7 Research Use of Stored Human Samples, Specimens or Data

7.1 Use of Stored Samples/Data

If an endoscopy is to be scheduled, the endoscopic score will be assigned at the time of the procedure. If the endoscopy has been already completed, the score will be performed on the recorded video/pictures.

Archived tissue (biopsy slides) will be evaluated by immunohistochemical staining as described previously.

7.2 Disposition of Stored Samples/Data

The Data Management and Coordinating Center (DMCC) is being used for the storage and management of data for this study. Research samples will be stored at Cincinnati Children's Hospital Medical Center, Colorado Children's Hospital, and the Data Management and Coordinating Center.

8 Study Schedule

8.1 Screening

Subjects enrolled will have a confirmed diagnosis of EoE, EG or EC regardless of their treatment status as we are interested in the relationship between mucosal inflammation, whether currently present or not, with the COMs. Patients who are recruited will either have an established diagnosis of EoE, EG or EC and are undergoing SOC endoscopic analysis at the point of entry or will be patients who are undergoing endoscopy to establish a new diagnosis of EoE, EG or EC. *If EoE, EG, or EC diagnosis is new, enrollment and COM completion will occur within 4 weeks of the diagnostic endoscopy and prior to any treatments for their disease so that the impact of treatments will not impact any of the COMs.*

8.2 Enrollment/Baseline

Investigators and research assistants (RAs) will recruit subjects within their clinical programs, using flyers at the hospital and in outpatient settings, on their respective websites, at academic meetings, and on clinicaltrials.gov. We will notify our academic partners, the American Academy of Allergy, Asthma, and Immunology (AAAAI); the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN); the American Gastroenterology Association (AGA); and the International Eosinophil Society (IES) about this study so that a recruitment link can be advertised on their respective websites.

Three groups of patients will be recruited: 1) patients with an established diagnosis of EoE, EG or EC 2) patients who are undergoing endoscopy to establish a new diagnosis of EoE, EG or EC 3) Non EoE for controls that are having endoscopy and/or colonoscopy (this group is for the mechanistic microbiome study).. Diagnosis of EoE is based on published criteria, as described earlier in section 5.1. EG will be defined as consistent symptoms and >30 eos /HPF in 5 HPFs^{21, 23}, (25) (23), and EC will be defined as consistent symptoms and >64 eos/HPF²¹; (23); in each case alternative causes of mucosal eosinophilia need to be ruled out. Inclusion and exclusion criteria and rationale are listed in Table 2.

Once enrolled, the new diagnosis cohort of subjects becomes, by definition, part of a longitudinal cohort. Recruited patients will 1.) sign informed consent, 2.) undergo standard of care (SOC) evaluation to capture defined clinical data, 3.) complete PRO questionnaires, and 4.) if not completed, undergo SOC endoscopy and analysis of their esophageal, gastric, or colonic biopsy to measure peak eosinophil count and associated features. Please see section 4.2.1 for a description of the COMs.

8.3 Active Phase (Phase 1)

Subjects receive a complete SOC clinical evaluation that includes: 1) full history of symptoms, atopy, age, gender, 2) family, social and past medical history, 3) history and physical examination, 4) Upper and/or lower endoscopy with biopsy, 5) labs, 6) evaluation of biopsy specimens to determine the number of eosinophils per hpf at 400x light microscopy in the most intensely inflamed hpf (EoE and EC) or in the 5 most intensely inflamed hpf (EG) by the local pathologist. FFPE biopsy specimens obtained as part of SOC will be subsequently verified by the central review pathologists. Each of these will be recorded on standardized histology evaluation forms (included with submission) by the research assistant and logged into the central database. For those subjects who consent to donate research biopsies and blood, these will be collected at each standard of care endoscopy. For those subjects who consent to saliva collection, these samples will be obtained one time only at one of the standard of care endoscopies.

8.4 Follow-up (Phase 2)

In Phase 2 of this study, subjects enrolled in Phase 1 will continue to be followed and assessed at each of the following time points: 1.) annually, 2.) at the time of any change in SOC treatment and 3.) at the time of any endoscopic procedure (up to 4 endoscopies in one year). In scenarios 1, or 2, only PRO and QoL COMs will be completed and in scenario 3, all COMs and histological analysis will be analyzed. All patients will be undergoing SOC treatment during this time; SOC treatment will be the choice of the attending physician and may include systemic or topical steroids, diet, or immunosuppression. This is not an interventional study and thus *treatment will be monitored, but not prescribed* as a part of this protocol. We have taken this approach for several reasons including the capture of “real world” data such that study findings are broadly applicable to the EoE, EG, and EC populations at large, to allow entry of the largest cohort of subjects independent of therapeutic intervention, and to better understand patient adherence to a prescribed therapeutic regimen as it relates to disease course.

8.5 Early Termination Visit

If a subject withdraws from the study, we will assess their symptoms on the current treatment during their clinic visit.

9 Assessment of Safety

This is not an interventional study. As such, monitoring of AEs will be limited to those developing within 3 days after the research biopsies are collected (for those subjects who consent to the additional biopsies).

9.1 Adverse Event (AE)

This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly to the sponsor. Information in this section complies with International Conference on Harmonization (ICH) Guideline E2A: *Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and ICH Guideline E6: *Guideline for Good Clinical Practice*, and applies the standards set forth in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) (version 4.03 2010-06-14). These criteria have been reviewed by the study investigators and the sponsor and have been determined appropriate for this study population.

Reportable AEs for this observational study will be limited to those related to the additional biopsy's. The worsening of a participant's pre-existing medical condition will not be considered an AE unless

the worsening is related to the additional biopsy procedure. Reportable AEs will include, but are not limited to, the following:

Collection of additional biopsies at Endoscopy - Adverse events associated with the collection of additional biopsies include bleeding, infection and perforation. Each of these would be categorized as being significant enough to require a visit to a clinician to evaluate association with laboratory or testing abnormalities.

9.2 Serious Adverse Event (SAE)

Reportable SAEs for this observational study will be limited to those related to additional endoscopy biopsies. A Serious Adverse Event (SAE) is defined as an AE meeting one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life Threatening Event (defined as an event that places a participant at immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance
- Congenital anomaly or birth defect
- Persistent or significant disability/incapacity
- Any other condition that, in the judgment of the investigator, represents a significant hazard, such as an important medical event that does not result in one of the above outcomes, may be considered an SAE when the event is related to the additional endoscopy biopsy and jeopardizes the participant or requires medical or surgical intervention to prevent one of the outcomes listed above.

9.3 Methods and Timing for Assessing, Recording, and Analyzing Adverse Events

9.3.1 Methods and Timing for Assessment

Subjects who consent to collection of additional research biopsies during a SOC endoscopy procedure will have their electronic medical record accessed within 3 days after the procedure to evaluate whether the patient had any difficulties reported.

AE/SAE Grading and Relationship Assignment

Intensity (severity) Scale

Each AE, will be assessed using NCI-CTCAE grading criteria for severity and classified into one the categories below:

- **Grade 1 (Mild):** Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Event requires minimal or no treatment and does not interfere with the participant's daily activities.
- **Grade 2 (Moderate):** minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*. Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Grade 3 (Severe):** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**. Event interrupts a subject's usual daily activity or functioning and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Grade 4 (Life threatening):** Urgent intervention indicated. Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred.
- **Grade 5 (Death):** Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

9.3.1.1 Relationship Assessment

For the purpose of this observational study, only AEs related to the collection of additional endoscopy biopsy will be reportable and by definition will always be assessed as related. The degree of certainty about causality will be graded using the 2 categories below.

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs within a reasonable timeframe after study procedure(s) and cannot be explained by concurrent disease or other drugs or chemicals.
- **Possibly Related:** There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after study procedure(s)). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse event may be judged only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

9.3.1.2 Recording/Documentation

All reportable adverse events that are identified will be recorded on an appropriate case report form (CRF). AEs developing within 3 days after the SOC endoscopy in subjects who consented to collection of extra research biopsies during SOC endoscopy procedure will be considered. All reported adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

9.3.2 Reporting Pregnancy

A pregnancy will not be reported as an AE and is not an exclusionary condition for this observational study. However, pregnancy may prohibit participants from performing certain SOC procedures.

9.3.3 Analysis/Management

The incidence of AEs is expected to be low in this observational study; therefore, the primary analysis of AEs will occur by the medical monitor as described in Section 9.4.1 **Error! Reference source not found.** Additional management is not expected to be necessary.

9.4 Reporting Procedures

As this study is a low risk nonintervention study, safety data will not be reviewed by the National Institute of Allergy and Infectious Diseases (NIAID) Data and Safety Monitoring Board or the Safety Monitoring Committee. The safety oversight will be conducted by the principal investigator and the NIAID Medical Monitor.

9.4.1 NIAID Medical Monitor Review

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the, site PIs, the NIAID Medical Officer (MO), of any reported adverse events via email.

Serious adverse events will be reviewed by The NIAID Medical Officer (MO) within 2 business days after notification by the DMCC via AEDAMS. A back-up notification system is in place so that any delays in review by the MO beyond a specified period of time are forwarded to a secondary reviewer. Cumulative reports of AEs and SAEs will be reviewed at a minimum of every 6 to 12 months.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days).

The DMCC will post aggregate reports of all reported adverse events for review by NIAID Medical Officer and Project Manager

9.4.2 Notifying the Institutional Review Board

SAEs will be reported to regulatory authorities per applicable federal regulations and institutional policy by the site principal investigator. Adverse events will be reported, in summary form, at the time of continuing annual review to the IRB.

9.4.3 Reporting Timeline

Requirements for reporting SAEs for this observational study are as follows:

- Within 24 hours (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
 - Is considered life-threatening/disabling or results in death of subject
 - OR-
 - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within 5 working days (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within 20 working days of the notification of the event or of the site becoming aware of the event.
- Investigators will forward all safety reports and related communications to the IRB within 15 days of receipt.
- A summary of all adverse events will be reported to the IRB with a continuing review submission.

9.5 Type and Duration of the Follow-up of Participants after Adverse Events

Subjects experiencing adverse events **due to research-related activities will be followed until:**

- a) the AE resolves
- b) the participant is stable

9.6 Participant Discontinuation

Participants may choose to withdraw from the observational study at any time, during a SOC visit, or afterwards in person, by telephone, or in writing.

Participants may be prematurely terminated from the observational study for the following reasons:

1. The participant elects to withdraw consent from all future observational study activities.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to re-establish contact with the participant have failed).
3. The investigator no longer believes participation is in the best interest of the participant.

10 Protocol Deviations

10.1 Protocol Deviation Definitions

10.1.1. Protocol Deviation

The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

10.1.2. Major Protocol Deviation (Protocol Violation)

A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

10.1.3. Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

10.1.4. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation (major or minor) has occurred, the study staff will a) notify the site Principal Investigator, b) notify the DMCC and c) will complete a Protocol Deviation form. The Protocol Deviation form will document at a minimum the date PD occurred, the date PD identified, a description of event, whether the deviation resulted in SAE/AE, the signature of PI, report to central IRB, and documentation of a corrective action plan. The DMCC and DAIT/NIAID may request discussion with the PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will complete and sign the Protocol Deviation form and submit it to the DMCC and to the central IRB, per IRB regulations. Major protocol deviations will be reported to the SMC by the NIAID Medical Monitor at the Medical Monitor's discretion.

11 Clinical Monitoring Structure

11.1 Site Monitoring Plan

Clinical site monitoring will be conducted according to the Safety Monitoring Plan (SMP) to ensure that human subject protection, observational procedures, and laboratory and data collection processes are of high quality and meet sponsor, ICH, Good Clinical Practice, and regulatory guidelines. Representatives from the DMCC and/or NIAID will visit each clinical site or meet with each clinical site via telephone during a specified timeframe according to the Project 1 CMP. Key study personnel must be available to assist the visitors during these visits or attend the call if completed via telephone. Additional details regarding clinical site monitoring, including remote monitoring, are outlined in the Project 1 CMP and MOO.

12 Statistical Considerations

12.1 Description of the Analyses

Through the use of a large sample of prospective clinical data collected in a standardized way, this research aims to eventually develop novel COMs that will provide new paradigms to diagnose, treat, and monitor subjects with EoE, EG and EC. The current protocol focuses on examining the correlation of COMs, histology and molecular transcriptome as well as the longitudinal profile of these variables. The analysis plans discussed in this section should give us clues to narrow down COMs for developing composite scores for each disease entity in future studies. Analysis for Aim I will be conducted by senior statisticians, Drs. Eileen King and Zhaoxing Pan. Analysis for Aim II will be conducted by cytogenetic statisticians, Drs. Lisa Martin and Bruce Aronow.

12.2 Appropriate Methods and Timing for Analyzing Outcome Measures

This is an observational study. No interim analysis for early conclusion of the study is planned.

12.3 Addressing Study Objectives

This study can be appropriately deemed as an early phase study of developing innovative tools for diagnosis and monitoring clinical outcomes. The current protocol is more exploratory in nature as opposed to validation of the tool, focusing on correlation between phenotypes, histology and genetic biomarkers. Aim 1 is determine the correlation of EoE, EG, and EC clinical outcome measures (COMs) with mucosal eosinophilia, while Aim 2 is to determine the correlation of the molecular profile for EoE, EG, and EC with clinical outcome measures (COMs) and mucosal eosinophilia. With the analyses discussed in 11.6, we hope to identify a set of variables that can be used to develop a composite score for EoE, EG, and EC respectively for further validation.

12.4 Sample Size Consideration

We aim to enroll 600 EoE, 300 EG, and 150 EC patients in the trial between all participating sites. This sample size will provide at least 80% power to detect a true correlation of 0.26 in the EC patients, 0.17 in the EG patients, and 0.14 in the EoE patients (Table 3). This sample size will also provide at least 80% power to detect disease group effect sizes of at least 0.35 when comparing phenotypic and clinical outcomes among disease groups. Based on the sample size numbers provided from each, after taking attrition into account, we anticipate more than an adequate sample size to reach power of 80% with $\alpha=0.01$ when assuming an adjustment for 6 covariates within each disease (EoE, EG, EC), even if the correlation of eosinophil peak and clinical outcome measures is low. These ~1,000 patients will allow for some subgroup analyses to explore consistency of effects within subgroups (e.g. adults versus pediatrics, newly diagnosed versus previously). Patients will be enrolled so that they can be followed for a minimum of one year. Up to 1200 patients may be enrolled to reach the target number of 1050.

Table 3. Intradisease Correlations

Sample Size	Detectable Increase in R ²	Corresponding Correlation (R) (<i>adjustment for covariates</i>)	Correlation (<i>no adjustment</i>)
100	0.10	0.32	0.33
150	0.07	0.26	0.27
200	0.05	0.22	0.24
300	0.03	0.17	0.20
400	0.03	0.17	0.17
600	0.02	0.14	0.14

12.5 Final analysis plan

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number. Each enrolled subject becomes part of the longitudinal study.

AIM 1: We will perform intergroup and intragroup baseline analysis at study entry, longitudinal analysis, and supportive and exploratory analysis with the expertise of our statisticians Drs. Eileen King and Zhaoxing Pan. To describe the clinical profile for each disease entity, separate analysis will be performed at baseline for participants in the newly diagnosed cohort and the previously

diagnosed (ongoing cohort). Summary statistics, grouped by adults or children, will be calculated for the demographic and the outcome variables. Differences in these phenotypic variables between EoE, EG, and EC will be tested using ANOVA or chi square tests. To evaluate the correlation of peak eosinophils with COMs (e.g. PROs, EREFS, QoL metrics), multivariate regression analysis will be conducted separately for each COM, with the COM serving as the dependent variable and peak eosinophil counts as the independent variable. Covariates in the model will include, but are not limited to, sex, age group, atopic disease, race, disease duration, and medication usage. To assess the difference in the extent of this correlation between cohorts or age groups, an interaction term of peak eosinophil counts by the selected categorical variable will be introduced to and tested under the above model. Similarly, PROs will be correlated with the other endoscopy (EREFs, Lanza score, UCCIS) and histology (biopsy evaluation forms) measures using the same approach. The normality assumptions will be evaluated, and transformations will be used (e.g. square root, log, rank) as appropriate. The correlation analyses discussed above will be conducted separately for EoE, EG, and EC.

Longitudinal analysis will determine whether changes in COMs correlate with changes in peak eosinophil counts over time. To minimize the confounding effect of the change in therapies and in accompanying medical condition over time, the changes (or percent changes, as appropriate) at the clinic visit one year from enrollment will serve as the primary endpoint; therefore, patients will be enrolled in the longitudinal study for a minimum of one year. The treatment that participants received over this period will be determined and used to classify participants into treatment group. Multivariate regression analysis will be conducted separately for each COM with change in peak eosinophil count as the independent variable and the change in COM as the dependent variable. Model covariates will be the same as above; similar analysis of endoscopic and histological scores will be performed. As a supportive and exploratory analysis, the change in COMs will be compared between treatment groups while adjusting for imbalanced demographic variables. Should there be significant differences among treatment groups, the change in peak eosinophil count or histological variables will be introduced into the model to see whether the change in COMs is mediated through the change in peak eosinophil counts or the histological condition. Change in the significance of the coefficient for the group difference is in support of the mediator effect. In addition, a linear or non-linear mixed effects model, as appropriate, will be fit to the serial data of each outcome to describe the course of the disease. The normality assumptions will be evaluated, and transformations will be used (e.g. square root, log, rank) as appropriate. These analyses will be conducted separately for each of the three diseases (EoE, EG, EC).

AIM 2: Data analysis and statistical methods for EG and EC will be accomplished as previously reported for EoE^{26, 36}. In brief, individual array quality analyses, normalization, referencing, detection of differences at the exon level, and hierarchical and other clustering approaches are carried out using BioConductor, R, and GeneSpring version v7.3.1 (Agilent, Santa Clara, CA) software as detailed previously²⁶. To determine whether GOIs are also associated with tissue eosinophilia and COMs, we will use either Pearson or Spearman correlations for quantitative variables depending on the variable's distributional properties. Dichotomous outcomes t-tests or Mann-Whitney U will be used for comparisons. To determine the appropriate number of individuals (power), we used MD Anderson's Sample Size for Microarray Experiments. Assuming 30,000 genes and that some genes analyzed will not reach minimal expression levels, a sample size of 20 per group will yield 80% power to detect at least 2-fold differences with no more than 10 false positives. Given the exploratory nature of this Aim, we feel that this sample size appropriately balances statistical power with the currently available resources. Of note, depending upon the findings, results will be validated with qPCR and replication could be performed using additional resources available to CEGIR.

13 Quality Control and Quality Assurance

Training of observational study staff will be conducted by representatives of the DMCC and NIAID prior to beginning recruitment. All staff members will be required to complete certification and quality control in all applicable procedures as outlined in the MOO. The site principal investigators and coordinator(s) will be responsible for ensuring that all procedures are performed according to the protocol. Periodic reviews of procedures will be conducted by the coordinator or other trained personnel according to an individual schedule for each staff member, which is based on the activities he/she is responsible for conducting. Details of the quality control plan, including certifications and quality control of study procedures, are provided in the MOO.

14 Ethics/Protection of Human Subjects

14.1 The Belmont Report

In accordance with the National Institutes of Health's federal wide assurance ([FWA00005897](#)): "This institution assures that all of its activities related to human subject research, regardless of funding source, will be guided by the ethical principles of The Belmont Report." Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of The Belmont Report, 45 CFR 46 and all of its subparts (A, B, C and D).

14.2 Institutional Review Board

A copy of the protocol, informed consent forms, assent form, any advertising/recruitment materials and other information to be completed by participants, such as survey instruments or questionnaires, will be submitted to the IRB for written approval.

The investigator must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

The investigator will notify the IRB of serious adverse events and protocol violations.

14.3 Informed Consent Process

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The research study will be explained in lay terms to each potential research participant. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

Once the informed consent has been signed, the participant is considered enrolled in the study.

14.3.1 Assent or Informed Consent Process (in Case of a Minor)

All participants will receive a full oral explanation of the study, and informed consent will be obtained prior to participation. The consent form that is used for this study will be approved by a central IRB. Informed consent will be obtained by one of the investigators or his/her authorized delegate. All study staff receive training in the informed consent process. Prospective subjects are given time to read over the entire consent form in order to make their decision and are also given

an opportunity to ask any questions concerning the study. They may request what they would like done with their material and clinical information when the study is completed, or if they should happen to withdraw from the study. They are then asked to check boxes indicating the components of the study to which they will consent; for example, some individuals can participate in database entry but not consent for tissue procurement. If the child is over the age of assent (varies by institution), they are also asked to sign an assent form. A copy of the consent/assent form is given to the family so that they have the information regarding the study. Another copy is placed in their medical chart, and the third copy is kept in the research office.

14.4 Participant Confidentiality

All of the practices will adhere to institutional and national policies regarding the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

Following Health Insurance Portability and Accountability Act guidelines, a participant's privacy and confidentiality will be respected throughout the observational study. Each participant will be assigned a sequential identification number, and this number rather than a name will be used to collect, store, and report participant information. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way.

Participant confidentiality will be strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biologic samples in addition to the clinical information relating to participating subjects.

Research samples will be labeled with code numbers so that direct identifiers will not be visible. All information concerning patient identification will be kept in protected storage areas, including password-protected computer files and locked files and/or offices. The Principal Investigator, primary researchers, and clinical research coordinator(s) will have access to the patient identifiers

Some samples that are collected during an endoscopy and/or colonoscopy for research purposes may be frozen and shipped to other hospitals, institutions, and testing companies for analysis. Data may also be shared. The data and/or samples will be coded with indirect identifiers per HIPAA and have no PHI associated with them. The data and/or samples will be used in a collaborative relationship between institutions, or testing company receiving the data and/or samples. All of these samples will be shared under an MTA, or other applicable agreement.

14.5 Transfer of Data to DMCC and Federal data repository

The clinical information collected for this study will be stored at the Data Management and Coordinating Center at the University of South Florida in Tampa, FL and also sent to a Federal data repository.

14.6 Registration on ClinicalTrials.gov

A description of this study will be available on <http://www.ClinicalTrials.gov>.

14.7 Study Discontinuation

This study will not have study discontinuation rules as it is an observational study. The NIH and local IRBs (at their local site) have the authority to stop or suspend this trial at any time.

15 Data Handling and Record Keeping

15.1 Data Management Responsibilities

All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity. Data retained by DMCC will be returned at study completion to the CEGIR.

15.2 Data Capture Methods

Clinical features of subjects will be determined during SOC evaluation as well as a REGID (Registry for Eosinophilic GI Diseases) Intake Form. The REGID Intake Form includes self-reported demographic, race/ethnicity, exposure and clinical. In order to effectively store and integrate all of the collected data, we will utilize the REGID infrastructure located at the Biomedical Informatics Division at CCHMC. The division has locally implemented the i2b2 (Informatics for Integrating Biology and the Bedside; Research Data Warehouse) for use by the research community. i2b2 is an NIH-supported National Center for Biomedical Computing which represents a scalable informatics framework designed for translational research that is based upon Research Patient Data Registry developed at Massachusetts General Hospital (1). It is designed primarily for cohort identification from a deep data repository derived from all of clinical data in a hospital electronic medical record, and provides researchers with summaries of de-identified data, as based upon *ad hoc* user queries. i2b2 provides the underlying data warehouse for REGID which currently provides for a web-based research registry. Standard templates within REGID have been developed so that valid and reliable clinical data are captured for each clinical encounter for deposit into the i2b2 data warehouse inclusive of the pediatric PROs (Pediatric Eosinophilic Esophagitis Symptom Score, PEES v2.0, PedsQL EoE Module). We can utilize REGID to incorporate new data sets (e.g. Eosinophilic Esophagitis Activity Index, EEsAI, Clinical Trial Identifier: NCT00939263).

15.2.1 Registration

Registration of participants in this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant.

15.2.2 Data Entry

Data will be collected either electronically (i.e. via tablet/computer) or on paper CRFs at each site and entered into online electronic case report forms maintained by the DMCC. Participants will be given the option to complete questionnaires at home. The first recording of any information

captured for the registry will be considered the source document, which may be, but is not limited to, a medical record, a laboratory or clinical report, a paper CRF, or an eCRF.

15.3 Types of Data

Clinical, demographic, laboratory, tissue and AE data will be collected for this observational study.

15.3.1 Source documents and Access to Source Data/Documents

The clinical sites participating in this observational study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants. Medical and research records will be maintained at each clinical site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the clinical site must permit authorized representatives of the sponsor to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the registry safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to individuals. The clinical site will normally be notified before auditing visits occur.

15.4 Timing/Reports

Data will be monitored by staff at the DMCC. Status reports on the progress of the observational study and data collection will be generated regularly. Reports will be sent to the NIAID project manager and medical officer on a regular basis.

15.5 Study Records Retention

Observational study documents must be maintained at the clinical site or a local storage facility for at least 5 years following the completion of the registry. Study documents that must be retained include all hard copies of CRFs, IRB approval documentation and related correspondence, and signed informed consent forms.

16 Publication Policy

Presentations and publications will follow the publication committee policy that has been established under the administrative core of the U54.

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Appendix B: Schedule of Procedures/Evaluations

Assessment or Procedure	Time Point or Visit			
	Recruitment	SOC endoscopy	Change in SOC treatment	Annual follow-up
Core procedures				
Signing of informed consent and assent forms	X			
Collection of research biopsies (may be repeated if subject enrolls in longitudinal portion and follow-up endoscopies are ordered)		X		
Collection of Microbiome Stool Sample (one time only)		X ²		
Collection of DNA sample from blood (5 ml)/saliva (one time only) ³		X		
Collection of blood (5 ml) for serum sample		X		
Collection of blood for Microbiome (5 ml) serum sample		X		
Contact information questionnaire	X	+/-	+/-	+/-
Demographic questionnaire	X			
Patient Intake Questionnaire	X			X
Medical history	X	+/-	+/-	+/-
Physical Examination	X	+/-	+/-	+/-
PROs—Symptoms and QOL				
PEESSv2.0 Child/Teen Report	X	X	X	X
PEESSv2.0 Parent Report	X	X	X	X
PedsQL™ – Parents of Toddlers 2 – 4 years	X	X	X	X
PedsQL™ – Young Children 5 – 7 years	X	X	X	X
PedsQL™ – Parents of Young Children 5 – 7 years	X	X	X	X
PedsQL™ – Children 8 – 12 years	X	X	X	X
PedsQL™ – Parents of Children 8 – 12 years	X	X	X	X

² Stool sample can be collected up to one week before or one week after the SOC endoscopy

³ Blood for DNA is collected each time the subject has an SOC endoscopy; Saliva is collected once is the subject declines the collection of blood for DNA but consents to the collection of saliva

Assessment or Procedure	Time Point or Visit			
	Recruitment	SOC endoscopy	Change in SOC treatment	Annual follow-up
PedsQL™ –Adolescents 13 – 18 years	X	X	X	X
PedsQL™ – Parents of Adolescents 13 – 18 years	X	X	X	X
EEsAI	X	X	X	X
EoE-QoL-A	X	X	X	X
Likert Dyspepsia Scale	X	X	X	X
SODA	X	X	X	X
PUCAI	X	X	X	X
SCCAI	X	X	X	X
PROMIS Peds Profile 25	X	X	X	X
PROMIS Adult Profile 29	X	X	X	X
ENDOSCOPY				
EREFS	X	X		
Lanza score	X	X		
UCCIS	X	X		
Histological COMs				
EoE Biopsy Evaluation	X	X		
EG Biopsy Evaluation	X	X		
EC Biopsy Evaluation	X	X		

Appendix C: Lab Processing Flow Sheet/Template for Specimen Collection

All subjects will have biopsies (FFPE blocks or cut sections) scanned into Aperio and sent to CCHMC or slides will be shipped directly to CCHMC for review by the central pathologists. Subjects age ≥ 3 years who consent to the procurement of additional biopsies will have 2 research biopsies collected in RNALater and shipped to the Rothenberg lab. These will be collected at each SOC endoscopy for the life of the study. Subjects who consent to DNA analysis of their blood/saliva sample will have the sample collected one time only, in conjunction with a SOC endoscopy. Storage and shipping instructions can be found in the Manual of Operations.

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