Six Food vs One Food Eosinophilic Esophagitis Elimination Diet (SOFEED) Followed by Swallowed Glucocorticoid Trial

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Rare Diseases Clinical Research Network

Six Food vs One Food Eosinophilic Esophagitis Elimination Diet (SOFEED) followed by Swallowed Glucocorticoid Trial

Consortium for Eosinophilic Gastrointestinal Disease Researchers

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Study Sponsors: National Center for Advancing Translational Sciences (NCATS) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: *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, please upload the signed document to the DMCC E-regulatory binder, IoRA cubby:* Christina 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	
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 *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. 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. **Confidentiality Statement** This document contains confidential information of the Sponsor. This information is to be disclosed only to the recipient study staff and the Institutional Review Board or Board of Ethics Committee reviewing this protocol. This information can be used for no other purpose than evaluation or conduct of this study without prior written consent from the Sponsor _____ **Site Principal Investigator (Print)** _____ **Site Principal Investigator (Signature)** **Date**	

PROTOCOL SYNOPSIS

Protocol Title:	Six Food vs One Food Eosinophilic Esophagitis Elimination Diet (SOFEED) followed by Swallowed Glucocorticoid Trial
Short Title	SOFEED
Clinical Phase	Phase II/III
Consortium:	Consortium for Eosinophilic Gastrointestinal Disease Researchers (CEGIR)
IND Sponsor/Number	Marc Rothenberg, MD, PhD / IND # 76820
Study Objectives	Primary Objective: • Determine the rate of remission following 1FED vs. 6FED and evaluate the relative efficacy of these dietary therapies Secondary Objectives: • To extend Phase 1 of this study with a prospective non-blinded trial that determines the rate of remission following SGC in the 6FED diet non-responders (Phase 2). • To extend Phase 1 of this study with a prospective non-blinded trial that determines the rate of remission following 6FED in the 1FED non-responders (Phase 2). • To evaluate the effect of each therapy on histological remission defined by a variety of changes in eosinophils, including (a) pre- and post-therapy peak eosinophil counts; (b) partial remission (2-14 peak eosinophils/hpf); and (c) complete histological remission (≤ 1 peak eosinophils/hpf). • To evaluate the effect of each therapy by utilizing the histology scoring system (HSS) created to express the severity and extent of other abnormalities in the gastrointestinal (GI) tract that often accompany eosinophilic inflammation. • To evaluate endoscopic outcomes as assessed by the endoscopy scoring system (EREFS). • To determine the impact of each therapeutic intervention on biomarkers using the EoE Diagnostic Panel (EDP). • To evaluate the clinical and psychosocial effect of each therapy utilizing surveys to assess EoE symptoms and problems/feelings related to eating.

	• To determine if any clinical parameters predict response to therapeutic intervention. • To determine if any biomarkers including serum food specific IgE, CRD, and IgG4 predict response to therapeutic intervention. • To determine if skin testing, in the form of prick and patch testing, predicts response to therapeutic intervention. • To determine if milk-driven T cells in the blood positively correlate with milk atopy patch test results and effectiveness of dietary therapies. • To bank DNA so that we can later elucidate genetic components of EoE
Study Design	Prospective, non-blinded clinical trial of individuals ages 18-60 years with EoE
Primary Endpoint(s)	The primary endpoint for this study will be the percent of participants who achieve histologic remission (<15 peak eosinophils/HPF) post therapy assessed via endoscopy with biopsy after the end of the 6 week Phase 1.
Secondary Endpoint(s)	• Histology and endoscopy measures a. Division of patients into complete (peak eosinophils≤1/HPF) or partial (≤6 or ≤10) remission b. Histology Scoring system c. Endoscopic scoring system (EREFS) • Biomarkers pre- and post-treatment a. Assessment of EDP Score • Patient Reported Outcome (PRO) metrics pre- and post-treatment a. EoE Adult Symptom Score Activity Index, and Adult EoE Quality of Life Score b. PROMIS General Health Questionnaire • Clinical parameters and biomarkers to predict responsiveness to diet and SGC therapy a. Correlation of clinical characteristics to response to therapy b. Assessment of predictive value of skin prick and patch allergy testing c. Correlation between presence of positive milk component allergy testing to response to 1FED d. Correlation of level of circulating milk-specific T cells with patch testing and response to 1FED e. Predictive biomarker-analysis of EoE transcriptome from EDP analysis on baseline biopsies in correlation to response to treatment.

Sample Size:	136
Study Duration	Minimum 18 weeks to maximum 27 weeks
Inclusion Criteria	1. Subject must be able to understand and provide informed consent 2. Have diagnosis of EoE (based on consensus criteria)[1]. 3. Are aged 18 to 60 years 4. Have histologically confirmed active disease ≥15 eosinophils/hpf in the esophagus within 12 weeks of screening visit 5. Symptomatic (have experienced symptoms within the last month prior to enrollment) 6. PPI confirmation. (As a part of the diagnosis of active EoE, it must be demonstrated that acid reflux disease is not the primary cause of the participant's symptoms by documentation that the participant has been on a high dose of PPI (at least one dose, once daily) for at least 8 weeks prior to a diagnostic endoscopy of EoE without histologic resolution (i.e., ≥ 15 eosinophils/hpf). (Due to the variety of doses and various PPIs available, the dose will be confirmed adequate at the discretion of the PI).) 7. Have a negative urine pregnancy test at screening if of childbearing potential. Females of childbearing potential must have a negative urine pregnancy test (β-hCG) prior to enrollment into the study (i.e., at screening). These participants must agree to use adequate birth control measures (e.g., condom, oral/injectable/subcutaneous contraceptives, intrauterine device, or sexual abstinence) during the study and for at least one month after the last dose of study drug which will be documented in the source documents.
Exclusion Criteria	1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol 2. Have been treated with topical swallowed steroids within the last 2 months or systemic steroids within the last 3 months 3. Have pathological eosinophilia in segments of the GI tract other than the esophagus determined by local review 4. Have been diagnosed with a GI malabsorption disorder (i.e., Inflammatory bowel disease, Crohn's disease) or Celiac disease 5. Are currently on dietary therapy strictly avoiding milk or on a 6FED 6. Have concurrent *H. pylori* gastritis or parasitic infection 7. Have history of anaphylaxis to milk (with current avoidance of milk) 8. Unable to complete study procedures, including EGD. 9. Have previously failed strict dietary therapy clearly documented with one of these regimens or topical steroid treatment (i.e. have not achieved histological remission of <15 eos/hpf after having

	been on 2 mg of budesonide per day or 1760 mcg of fluticasone per day for 8 weeks prior to the EGD) . 10. Use of investigational drugs within 4 weeks (one month) prior to enrollment 11. Are concurrently receiving any of the prohibited medications listed in section 8.3 12. Not fluent in English 13. On immunotherapy for pollen (if not on maintenance therapy) or IgE-mediated food allergy. 14. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
Statistical Considerations (sample size and analysis plan):	Conduct a prospective non-blinded randomized trial that compares a 1-food elimination diet (1FED) versus a 6-food elimination diet (6FED) for 6 weeks (Phase 1). Subjects who fail 6FED (i.e. dietary non-responders) in Phase 1 may choose to continue into Phase 2, where they will receive topical swallowed steroid therapy (swallowed fluticasone at a dose of 880 mcg twice a day) for 6 weeks, while on an unrestricted diet. Subjects who fail 1FED will also continue into Phase 2, but they will maintain 6FED for 6 weeks instead of receiving SGC. Participants receiving SGC will return to an unrestricted diet prior to initiating SGC therapy. Empiric dietary therapy interventions are as follows: 1-food elimination diet (1FED)—avoidance of milk vs. 6-food elimination diet (6FED)—avoidance of milk, egg, wheat, soy, fish/shellfish, and peanut/tree nuts, for 6 weeks.

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Glossary of Abbreviations

CCHMC	Cincinnati Children's Hospital Medical Center
CI	Confidence level
CFR	Code of Federal Regulations
CHCO	Children's Hospital of Colorado
CRF	Case Report Form
CRPC	Central Review Pathology Committee
Ct	Threshold Cycle
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DMCC	Data Management and Coordinating Center
DSMB	Data Safety Monitoring Board
DSQ	Dysphagia Symptom Questionnaire
EDP	EoE Diagnostic Panel
EGD	Esophagogastroduodenoscopy
EoE	Eosinophilic Esophagitis
EOT	End of treatment
FDA	Food and Drug Administration
1FED	1-food elimination diet
6FED	6-food elimination diet
FP	fluticasone propionate
GCP	Good Clinical Practice
GI	Gastrointestinal
HPF	high power field
HSS	Histology Scoring System
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases

NIH	National Institutes of Health
NW	Northwestern University
PHI	Protected health information
PI	[Site] Principal Investigator
PPI	Proton pump inhibitor
PRO	Patient Reported Outcomes
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SGC	Swallowed Glucocorticoid
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

1. INTRODUCTION

The primary objective of this proposal is to conduct a prospective, non-blinded randomized trial comparing rates of remission of novel empiric elimination dietary therapies in eosinophilic esophagitis (EoE) in order to assess the therapeutic viability of empiric elimination diets. Moreover, we aim to assess response to topical swallowed steroids in non-responders to empiric six food dietary therapy regimens and the response to 6FED in 1FED non-responders. Participants aged 18 to 60 years with active EoE will be enrolled and the primary efficacy outcome will be the rate of histologic remission assessed using esophageal biopsies obtained following a six week randomized trial of one of two empiric diets. During the screening process, active EoE will be confirmed by histologic evaluation of esophageal biopsies obtained during an esophagogastroduodenoscopy (EGD) in subjects with a history consistent with EoE.

This trial is comprised of a screening (up to 12 weeks in duration) and 2 phases, with each phase lasting 6 weeks. Phase 2 is optional, and eligible participants may choose whether or not to continue into Phase 2. In the first phase, all participants will be randomized 1:1 to either 1 food elimination (milk elimination alone, 1FED) or 6 food elimination (milk, egg, wheat, soy, fish/shellfish, peanut/tree nuts elimination, 6FED) therapeutic diet. At the end of this phase, an EGD will be performed to assess remission. Attainment of remission (esophageal eosinophil counts <15 eosinophils/high powered field) after phase 1 will result in study discontinuation and maintenance of the "successful" dietary therapy. Dietary therapy non-responders who were on 6FED during Phase 1 and choose to continue into Phase 2 will receive topical swallowed steroids for 6 weeks (Phase 2) while on an unrestricted diet, followed by EGD with esophageal biopsies. Dietary therapy non-responders who were on 1FED during Phase 1 and choose to continue into Phase 2 will proceed to 6FED for six weeks (Phase 2) followed by EGD with esophageal biopsies.

The primary efficacy endpoint will assess and compare the percentage of patients achieving remission by following the two diets. Pre- and post-therapy peak eosinophil counts per hpf and percentages of patients that attain histologic remission (<15 eosinophils/hpf) will be reported. The peak eosinophil level refers to the peak eosinophil count from the esophagus as a whole, although peak eosinophil levels are recorded by the esophageal segment. During Phase 1 screening, the peak eosinophil count obtained from local pathology review will be used to assess subject eligibility for study participation. Eligibility for entry into Phase 2 of the study will be determined by the peak count obtained from the central pathology committee's review of the slides. A variety of secondary endpoints, including more detailed histologic and endoscopic scores, novel biomarkers, and several Patient Reported Outcome (PRO) measures will also be assessed. In addition, we will assess the possible value of a variety of clinical characteristics and biomarkers in terms of predicting response to therapy.

This study will provide significant novel information. The use of 6FED after 1FED failure will provide practical information for clinical recommendations. This will be a prospective dietary therapy trial using these empiric elimination diets. As part of our secondary aims, we will also be utilizing a number of novel potential biomarkers to assess their ability in predicting and assessing response to therapy as well as a number of PRO metrics.

2. BACKGROUND AND RATIONALE

3.1 Background and Scientific Rationale

Eosinophilic Esophagitis

EoE represents a complex clinical entity often requiring coordinated care within the fields of both allergy and gastroenterology. EoE is diagnostically defined by revised consensus criteria, that in brief require esophageal-specific inflammation of ≥ 15 eosinophils per high power field (hpf) as obtained from two to four biopsies from the esophagus, and that is not mitigated by the use of proton pump inhibitor (PPI) therapy[1]. Symptomatically, patients with EoE often complain of dysphagia, vomiting, reflux, food aversion, and food impaction[1-3]. These patients commonly have diagnoses associated with atopy and other allergic disorders such as IgE-mediated food allergy, asthma, allergic rhinoconjunctivitis, and eczema. Current treatment modalities focus on the use of the six food elimination diet (6FED), which consists of the empiric avoidance of the top 6 most common food allergens in the U.S. (milk, egg, wheat, soy, peanut/tree nuts, and fish/shellfish); implementation of a variety of directed elimination diets that rely on the use of allergy test results; the elimination of all antigenic proteins via the use of elemental formulas; or the use of swallowed steroid therapies (topical swallowed preparations of fluticasone or budesonide or oral systemic steroids) [4-13]. While all of these treatments are efficacious, they require continuous use or the disease will recrudesce in the majority of patients. Due to concerns about the risk of chronic topical swallowed steroid, patients and/or their families frequently opt to initially implement dietary therapy. This study is designed in keeping with this preference; therefore one of two empiric dietary therapies will be implemented initially prior to the use of topical swallowed steroids in participants who do not respond to the dietary treatment. The use of dietary restrictions as a treatment modality requires family and/or personal commitment to eliminating food, which may lead to significant nutritional deficiencies and is often life-altering.

3.2 Rationale for Selection of Investigational Product or Intervention

Current treatment modalities focus on the use of the six food elimination diet (6FED), which consists of the empiric avoidance of the top 6 most common food allergens in the U.S. (milk, egg, wheat, soy, peanut/tree nuts, and fish/shellfish); implementation of a variety of directed elimination diets that rely on the use of allergy test results; the elimination of all antigenic proteins via the use of elemental formulas; or the use of swallowed steroid therapies (topical swallowed preparations of fluticasone or budesonide or oral systemic steroids) [4-13]. While all of these treatments are efficacious, they require continuous use or the disease will recrudesce in the majority of patients.

Strictly adhering to any of these elimination diets is challenging for most patients, and so comparing response rates to a minimally restrictive therapy (1FED) against a more rigorous therapy (6FED) would be informative and potentially valuable.

It is considered standard of care for adults to undergo 6 weeks of dietary therapy, and studies have demonstrated that a 6-week trial of dietary therapy or even shorter for swallowed glucocorticoids is adequate for detecting change in disease status.[58, 59, 31, 21] Compliance is expected to be greater with a shorter therapy period as opposed to a longer one. It is also noted that the standard of care for children is to undergo 12 weeks of dietary therapy, at least in part because esophageal biopsies every 6 weeks may be a higher risk to children than biopsies every 12 weeks.

3.3 Preclinical Experience

3.3.1 Previous Research on the Use of Dietary Therapy in Eosinophilic Esophagitis

EoE is a chronic esophageal inflammatory disease typically triggered by exposure to food antigens. Given this, dietary elimination of these antigens has been tested as a therapeutic option. Previous work has shown that the exclusive use of elemental formula resulted in a response rate from 88-100% when histology and symptoms are considered, but difficulties with compliance, cost, and formula palatability have led to attempts to find alternative dietary approaches[9, 13, 14]. Use of allergy testing to direct dietary elimination has been advocated, and in studies response rates from 53-77% have been reported. However, in practice the utility of allergy testing is very dependent on local expertise, and the response rate has been lower than hoped[8, 13, 15]. Given this, there has been an attempt to use an empiric 6FED, which removes milk, soy, wheat, egg, peanuts/tree nuts, and fish/shellfish from the diet. This intervention has shown response rates ranging from 53-81% and is attractive due to the fact that no allergy testing is required. In all of these currently prescribed dietary therapies, the goal is to reintroduce foods systematically with subsequent endoscopy to try to identify specific causative antigens. A single small study of 17 children showed a partial or complete response rate of 65% using milk elimination alone[16]. In published reports where causative antigens have been identified, milk, wheat, and eggs have consistently been reported to be the top 3 offending food antigens[8, 13, 17]. Strictly adhering to any of these elimination diets is challenging for most patients, and so comparing response rates to a minimally restrictive therapy (1FED) against a more rigorous therapy (6FED) would be informative and potentially valuable.

3.4 Clinical Studies

In a randomized controlled trial conducted by our group we assessed the efficacy of high dose fluticasone. Forty-two participants were enrolled in this study (28 FP, 14 placebo). After 3 months, 65% of FP-treated and 0% of placebo-treated participants had complete remission (P = 0.0001); 12% of FP-treated and 8% of placebo-treated

participants had partial remission. Of the FP-treated participants in complete remission, 73% continued in complete remission, and 20% were in partial remission after halving the daily FP dosage.[53] Vital signs and lab results were stable throughout the study. No participant was reported to exhibit clinical signs of adrenal insufficiency or glucocorticoid toxicity.[53]

3. STUDY HYPOTHESES/OBJECTIVES

3.1 Hypotheses

We hypothesize that 6FED will be superior to 1FED, although we expect that a substantial (meaningful) percentage of patients will respond to 1FED.

We hypothesize that some patients who fail to respond to 1FED will respond to 6FED.

We hypothesize that some patients who fail to respond to 6FED will respond to SGC.

We hypothesize that HSS will more accurately quantify histological changes associated with EoE and be reversible following each therapeutic intervention. Furthermore, we hypothesize that some patients may show partial responses, where a subset of the HSS may be improved earlier or in dissociation with eosinophil levels, for example.

We hypothesize that molecular biomarkers will change (improve) with each treatment phase, at least in part. Furthermore, we hypothesize that biomarkers may reveal (a) novel insight into therapeutic resistance and/or relapse; (b) reveal patient sub-groups not identified by histology; and (c) ultimately prove to be readily usable biomarkers in clinical settings and possess personalized medicine value. If our hypotheses are proven to be correct, they have potential to transform monitoring of patients during therapeutic intervention, by identifying molecular markers that have therapeutic significance, and also by reducing the need for biopsies, particularly after therapy is initiated.

We hypothesize that the therapeutic interventions utilized in this study will improve clinical symptoms as measured by PRO metrics.

We hypothesize that certain patient phenotypes will correlate with response to each intervention in the trial.

We hypothesize that skin test results (prick testing to the 6 test foods and atopy patch testing to milk) will provide significant predictive value for outcomes in the diet trial phases.

3.2 Primary Objectives

To perform a prospective, randomized, non-blinded trial that determines the rate of remission following 1FED (milk elimination alone) vs. 6FED (milk, egg, wheat, soy, fish/shellfish, and peanut/tree nuts avoidance) and evaluates the relative efficacy of these dietary therapies (Phase 1).

The primary endpoint for this study will be the percent of participants who achieve histologic remission (<15 peak eosinophils/HPF) post therapy assessed after the end of the six week Phase 1.

3.3 Secondary Objective(s)

- To extend Phase 1 of this study with a prospective non-blinded trial that determines the rate of remission following SGC in the 6FED non-responders and the rate of remission following 6FED in the 1FED non-responders (Phase 2).
- To extend Phase 1 of this study with a prospective non-blinded trial that determines the rate of remission following 6FED in the 1FED non-responders (Phase 2).
- To evaluate the effect of each therapy on histological remission defined by a variety of changes in eosinophils, including (a) pre- and post-therapy peak eosinophil counts; (b) partial remission (2-14 peak eosinophils/hpf); and (c) complete histological remission (≤ 1 peak eosinophils/hpf).
- To evaluate the effect of each therapy by utilizing the histology scoring system (HSS) created to express the severity and extent of other abnormalities in the gastrointestinal (GI) tract that often accompany eosinophilic inflammation.
- To evaluate endoscopic outcomes as assessed by the endoscopy scoring system (EREFS).
- To determine the impact of each therapeutic intervention on biomarkers using the EoE Diagnostic Panel (EDP).
- To evaluate the clinical and psychosocial effect of each therapy utilizing surveys to assess EoE symptoms and problems/feelings related to eating.
- To determine if any clinical parameters predict response to therapeutic intervention.
- To determine if any biomarkers including serum food specific IgE, CRD, and IgG4 predict response to therapeutic intervention.
- To determine if skin testing, in the form of prick and patch testing, predicts response to therapeutic intervention.
- To determine if milk-driven T cells in the blood positively correlate with milk atopy patch test results and effectiveness of dietary therapies.
- To bank DNA so that we can later elucidate genetic components of EoE

4. STUDY DESIGN

4.1 Description of Study Design

We will be recruiting a total of 136 participants, between the ages of 18-60. Participants will be enrolled in this study based on the presence of active eosinophilic esophagitis and adherence to the inclusion and exclusion criteria.

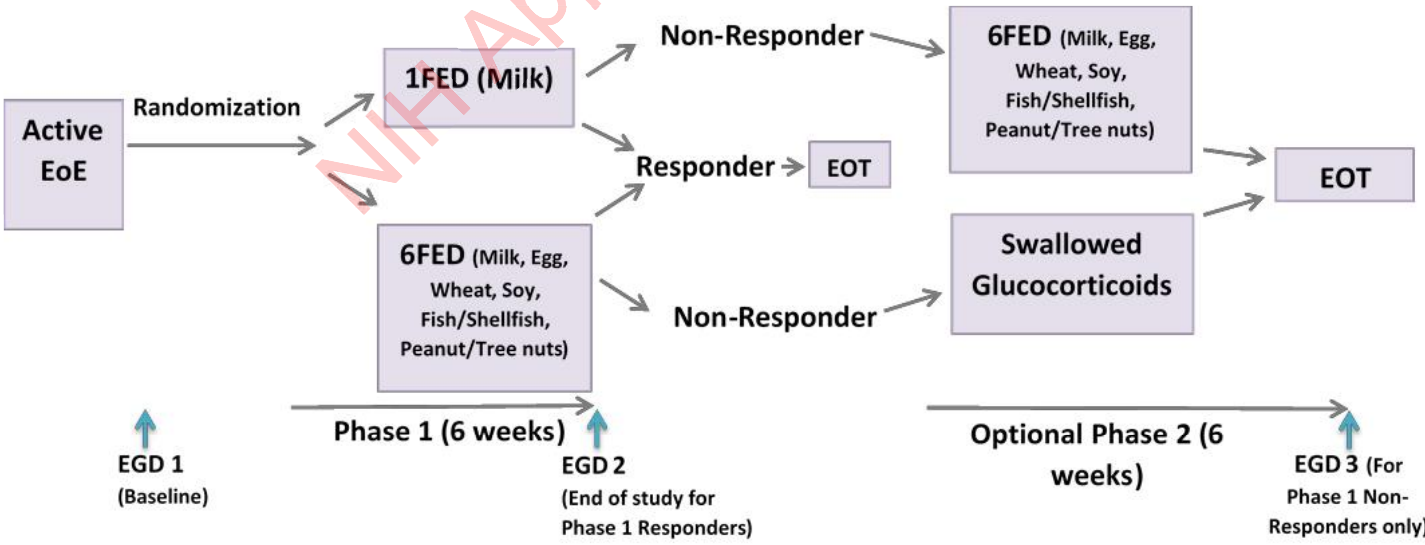
We intend to conduct a prospective non-blinded randomized trial that compares a 1-food elimination diet (1FED) versus a 6-food elimination diet (6FED) for six weeks (Phase 1). Subsequently, non-responders to 1FED dietary elimination therapy will be treated with 6FED and non-responders to 6FED will be treated with SGC (swallowed fluticasone at a dose of 880 mcg twice a day) for six weeks (Phase 2) while on an unrestricted diet, if the participant chooses to continue in to Phase 2. The empiric dietary therapy interventions are as follows: 1-food elimination diet (1FED)—avoidance of milk vs. 6-food elimination diet (6FED)—avoidance of milk, egg, wheat, soy, fish/shellfish, and peanut/tree nuts, for six weeks. Milk elimination for both 1FED and 6FED includes all mammal milk (i.e. goat, sheep, and cow's milk must all be eliminated). The study design is shown schematically in Figure 1 Study Design Flowchart.

In Phase 1, all participants will be randomized 1:1 to either 1 food elimination (milk elimination alone, 1FED) or 6 food elimination (milk, egg, wheat, soy, fish/shellfish, peanut/tree nuts elimination, 6FED) therapeutic diet. At the end of this phase, an EGD will be performed to assess remission. Attainment of remission (esophageal eosinophil counts <15 eosinophils/high powered field) after Phase 1 will result in study discontinuation and maintenance of the "successful" dietary therapy. Treatment non-responders may choose whether or not to continue into Phase 2. Dietary therapy non-responders who were on 6FED in Phase 1 and continue in to Phase 2 will receive topical swallowed steroids for six weeks (Phase 2) followed by EGD with esophageal biopsies. These participants will return to an unrestricted diet (i.e. stop 6FED) prior to beginning topical swallowed steroid therapy. Dietary non-responders in Phase 1 who were on 1FED and continue in to Phase 2 will continue to a 6FED therapeutic diet (Phase 2) for six weeks followed by EGD with esophageal biopsies to assess remission.

The instructions for the 1FED and 6FED therapeutic diets will be standardized across sites by providing consensus documents produced by a central Registered Dietitian who is an expert in dietary elimination in EoE. There are currently no validated dietary questionnaires for measuring food allergen intake in adults in the US. Dietary intake and *consumption* of the allergens will be measured at the start of the trial and adherence to the dietary elimination (i.e. *avoidance* of the allergens) during the trial will be measured using modified versions of the NIC's usual dietary intake/diet history questionnaires and food diaries from published pediatric EGID studies (http://cancercontrol.cancer.gov) and assisted by metabolomics. Stool samples may be collected from subjects who consent to stool collection to evaluate the effect of diet on the microbiome.

Similarly, standardized instructions for using SGC will be provided, based on our prior studies. We have chosen to use fluticasone propionate (FP) rather than budesonide in this study, given our prior work in this area, and our recent finding that high-dose fluticasone is well tolerated and highly effective, and our preliminary findings concerning esophageal transcripts that predict responsiveness to FP therapy. The study design is shown schematically in Figure 1 Study Design Flowchart.

Figure 1 Study Design Flowchart



4.2 Primary Endpoints

AIM 1: To determine the efficacy of a 1FED vs. 6FED in EoE.

a. Primary end-point The primary efficacy endpoint will be determined at the end of Phase 1 at which time all participants will undergo EGD with biopsy and the percentage of patients who have <15 peak eosinophils/hpf will be determined. The slides will be reviewed by local pathologists for clinical purposes, who will be blinded to the treatment group. The Central Review Pathology Committee (CRPC), who will also be blind to the treatment group, will also review the slides, and the peak eosinophil count from this CRPC review will be used to determine whether or not participants are eligible to continue into Phase 2 of the study. Participants with ≥15 peak eosinophils/hpf who were on 6FED during Phase 1 and choose to continue into Phase 2 will receive SGC for six weeks while on an unrestricted diet. Participants with ≥15 peak eosinophils/hpf who were on 1FED during Phase 1 and choose to continue into Phase 2 will maintain the 6FED therapeutic diet for 6 weeks. The CRPC will subsequently review the esophageal biopsy slides to determine other parameters (such as the Histology Scoring System (HSS)). CRPC review will be completed, whenever possible, within 2 weeks of a subject's endoscopy. Participants who achieve histologic remission at the end of Phase 1 (as determined by CRPC review) or who complete Phase 1 and opt out of Phase 2 will have completed the study and will resume follow-up care with their primary gastroenterologist or allergist.

4.3 Secondary Endpoints

Secondary end-point analysis: histology and endoscopy. Change in peak eosinophil count will be measured and we will also aim to determine the percentage of patients who achieve remission by more stringent requirements than the primary end-point, defined as both complete remission (≤1 peak eosinophils/HPF) and partial remission (<10 and <6 peak eosinophils/HPF) as reported[6]. This will allow us to compare with other studies that have used

6FED[14,17,23,24,]. Furthermore, we aim to more broadly examine the impact of each therapeutic intervention on other histological parameters associated with EoE using a quantitative histology scoring system (HSS) that has been developed by our expert pathologist, Dr. Margaret Collins[26]. The HSS evaluates esophageal biopsies for various features (ex: basal layer hyperplasia, dilated intercellular spaces, surface epithelial alteration, apoptotic epithelial cells), eosinophilic inflammation (peak count, eosinophil abscess, eosinophil surface layering), and lamina propria (fibrosis). For central review, slides of relevant esophageal biopsies obtained during the course of the study will be scanned using an Aperio Imaging scanner and viewed by the CRPC using Aperio Imaging Software. Each member of the CRPC has access to an Aperio scanner in his or her pathology department and to the Aperio Imaging Software. Slides may be scanned into Aperio at the local site, or they may be sent to Cincinnati Children's Hospital Medical Center (CCHMC) to be scanned into Aperio (if the local site does not have an Aperio scanner). These slides will be returned to the local site once they have been scanned in. A password-protected website housing the scanned images will be created at CCHMC, and the CRPC will have access to that site. A secure image repository may also be housed at the Data Management Coordinating Center of the Rare Diseases Clinical Research Network at the University of South Florida. The CRPC will establish interobserver and intraobserver variability in their evaluation of eosinophils and other pathologic features: The central pathologists will re-count eosinophils in the scanned images of biopsies and compare the peak eosinophil counts that they obtained by viewing the scanned images to evaluate the variability in the counts. The CRPC pathologists will compare their results amongst themselves and resolve differences via teleconferences in which the scanned images are displayed (meetings hosted by GoToMeeting or equivalent sites). Ten percent intraobserver and interobserver variability will be considered optimum. Each review pathologist will review study slides from subjects enrolled at their own institution (Dr. Collins, CCHMC; Dr. Capocelli, CHCO; Dr. Yang, NW). Relevant biopsy slides from the other participating sites will also be reviewed by the CRPC. The workload will be distributed as equally as possible among the CRPC pathologists with the assistance of the DMCC. Gastric and/or duodenal biopsies will only be reviewed by local clinical pathologists and will not be sent to the CRPC for review, except for cases where a participant's diagnosis is unclear (for example, when it is unclear whether a potential participant does or does not have eosinophilic gastritis or colitis). In such cases, the CRPC will review gastric and/or duodenal slides to confirm the appropriate diagnosis. Subjects may be included, without stomach or duodenal biopsies, if there is no clinical history of involvement of stomach or duodenum. We hypothesize that HSS will more accurately quantify changes associated with EoE and be reversible following each therapeutic intervention. Furthermore, we hypothesize that some patients may show partial responses, where a subset of the HSS may be improved earlier or in dissociation with eosinophil levels, for example. Finally, we will aim to determine the impact of the various therapeutic interventions on gross endoscopic changes, as determined by a recently developed endoscopy scoring system (EREFS) developed by Dr. Hirano and colleagues[27].

4.4 Exploratory Endpoint(s)/Outcome(s)

Secondary end-point analysis: biomarkers. In this sub-aim, we hypothesize that molecular biomarkers will change (improve) with each treatment phase, at least in part. Furthermore, we hypothesize that biomarkers may reveal (a) novel insight into therapeutic resistance and/or relapse; (b) reveal patient sub-groups not identified by histology; and (c) ultimately prove to be readily usable biomarkers in clinical settings and possess personalized medicine value. We will focus on molecular profiling of esophageal genes with the EoE Diagnostic Panel (EDP), a set of 94 mRNA transcripts that have been established to differentiate EoE from controls (e.g. GERD and normal

individuals), and to identify exposure and transcriptional signaling to SGCs[28]. The EDP is typically performed on an extra biopsy taken from the distal esophagus[28]. If our hypotheses are proven to be correct, they have potential to transform monitoring of patients during therapeutic intervention, by identifying molecular markers that have therapeutic significance, and also by reducing the need for biopsies, particularly after therapy is initiated. Essentially, the associated EDP algorithm renders the raw Ct. values of each embedded gene after real-time PCR in a way such that the upregulated genes and downregulated genes are summed up individually. A quantitative "EoE score" is derived from the ΔCt. (normalized to GAPDH) summation to reflect disease severity and for statistical analysis. Utilizing different gene sets, EDP is also able to predict steroid exposure and remission status based on the same algorithm [28]. In this case, the EoE score will be calculated at baseline endoscopy and in the posttreatment biopsies to assess change with treatment.

Secondary end-point analysis: Patient Reported Outcome (PRO) metrics. In this sub-aim, we hypothesize that therapeutic interventions will improve clinical symptoms as measured by PRO metrics. This is an important issue to resolve as only a few prior studies have shown the clinical efficacy of therapeutic interventions. In fact, several studies have shown dissociation between histology (eosinophil levels) and clinical improvement[31,32], but these studies have not used validated metrics for measuring symptoms. The measurement of pre/post diet symptoms using tools that have undergone at least some validation is a novel aspect of our study. While we have made substantial progress with the development and validation of a number of PRO measurements, there is still not agreement around the best measurements, related in part by [1]the uncertainty of whether these instruments truly measure meaningful and accurate parameters. Agreement about these measurements has significant bearing on the eventual FDA approval of therapeutic agents[33]. As such, we will take several approaches in this sub-aim. First, we will employ EoE specific measurement tools, including the adult EoE dysphagia questionnaire, EoE Adult Symptom Score Activity Index (EESAI), and the adult EoE quality of life score (EoE-QOL-A[36,37,38,39]. In addition, we will also employ generic Health Related QOL metrics, such as PROMIS General Health Questionnaire, and eventually compare the changes in disease specific versus general health questionnaires. These PROs will be assessed at the screening visit and again at the end of each treatment phase to assess the effects of treatment.

AIM 2: To determine clinical parameters and biomarkers that predict responsiveness to therapies.

2a. Clinical phenotypes. In this sub-aim, we hypothesize that certain patient phenotypes will correlate with response to each intervention in the trial. We will assess the relationship between response to 1FED, 6FED, and SGC on age, height, weight, BMI Z-score, race, ethnicity, allergic status, participant compliance (defined by diet and SGC usage diaries), and screening eosinophil levels as readouts.

2b. Skin testing. In this sub-aim, we hypothesize that skin test results (prick testing to the 6 foods in the 6FED and atopy patch testing to milk) will provide significant predictive value for outcomes in the diet trial phases. While a substantial body of work has already been done concerning skin testing, there still remains

debate about its practice in managing EoE. As such, in this sub-aim, we will take several different approaches compared with prior work. In particular, we will be conducting a multi-site trial and have the advantage that we can standardize the skin testing procedure, by using agreed upon techniques, including food extracts, skin test reagents, and measurement parameters. We will thus be in a good position to dissect discrepant results between centers. Furthermore, our trial is unique in that half of the patients will undergo a milk elimination diet as part of the first phase. Thus, we will be in a position to focus on the value of skin testing for milk, which is particularly attractive as milk is the number one food trigger of EoE [19]. It is important to point out that although skin testing (including patch testing) is now readily performed in EoE, there have been no studies that have assessed the local response. For example, it has not yet even been determined if positive responses involve immunological reactions, so basic cytokine and histological analyses are likely to be fundamentally informative.

2c. Serum food specific IgE, CRD, and IgG4. The level of food specific IgE, CRD, and IgG4 will be determined by a solid phase immunoabsorbent assay (ELISA) focusing on the 6FED foods. Serum will be banked and processed in batches. Component testing for at least each of the 6 foods will be performed as feasible. The Investigators reserve the right to perform these analyses using commercially available platforms including UniCAP and the Immuno Solid-phase Allergen Chip (ISAC, Thermo Scientific).

2d. Assessment of milk-driven T cells. In this sub-aim, we will perform exploratory studies designed to test the presence and significance of circulating milk-specific T cells in EoE patients. We will test the hypothesis that milk-specific T cells will be primarily CD4+, IL-5+, IL-13+, consistent with prior work.[47-49] Furthermore, we will test the sub-hypothesis that the level of milk-specific T cells will positively correlate with positive atopy patch tests to milk and the response rate to milk elimination (1FED and secondarily 6FED, which also contains milk). The frequency of Th2 cells will be determined by FACS analysis, and milk-specificity will be estimated by milk protein stimulation in vitro, particularly looking at CD154 induction, a marker of T cell activation. These exploratory studies, will be performed with shipped blood samples, by Dr. Jonathan Spergel, as published.[47,48,50,51] The study will be limited to a pilot analysis of patients who only enter the 1FED and we will compare patients with positive and negative skin tests (prick and patch).

2e. Predictive Biomarker-analysis of EoE transcriptome. In this sub-aim, we aim to identify biomarkers that may predict responsiveness to any or all of the treatment interventions associated with this trial. Determining such a biomarker would have a profound effect on disease management as patients could be quickly triaged into different therapies, e.g. diet versus SGC; thus saving time, reducing the number of endoscopies and potentially improving quality of life issues. As such, in this sub-aim, we will perform the EDP analysis on baseline biopsies, focused on identifying transcript levels that differentiate patients that respond to each of the interventions. In preliminary studies, we have conducted this type of analysis on patients that recently participated in the randomized controlled trial of high dose fluticasone (1760 mcg per day) for the treatment of EoE. Forty-two participants were enrolled in this study (28 FP, 14 placebo). After 3 months, 65% of FP-treated and 0% of placebo-treated participants had complete remission (P = 0.0001); 12% of FP-treated and 8% of placebo-treated participants had partial remission. Of the FP-treated participants in complete remission, 73% continued in complete remission, and 20% were in partial remission after halving the daily FP dosage.[53]

Baseline esophageal gene expression was compared between FP responders and non-responder patients by statistically screening two cohorts of samples before the FP exposure, namely the FP responders and FP-non-responders. A total of 15 genes (Figure 4 in the reference, $p < 0.05$, fold change > 2.0) on the EDP were identified with a tendency to predict subsequent FP efficacy with CR criteria (≤1 eosinophil/HPF in the distal esophagus). It is interesting to note that some of these transcripts include cardinal EoE transcriptome genes such as CCL26 (eotaxin-3) and periostin and CLC (Charcot-Leyden crystals, a marker of eosinophils) suggesting that elevated production was a positive prognostic factor for FP responsiveness; these findings are consistent with a recent report suggesting that more severe tissue inflammation including eosinophil levels was associated with FP responsiveness.[52] This preliminary result highlights the potential ability of baseline molecular transcripts to differentiate patients but also importance of repeating this analysis with an independent cohort of FP treated patient samples, as preliminarily identified genes have not yet passed the false discovery rate (FDR) correction due to a relatively small n. It is expected that a replication cohort will bring in a higher power to pass the FDR filter. Meanwhile, multiple false-correction methods will be applied to the collective data set, such as the West-Young and Bonferroni methods.

2f. Elucidation of genetic components of EoE. In this sub-aim, we seek to elucidate the genetic components of EoE through the collection of blood and/or saliva for genetic analysis. Blood and/or saliva specimens will be obtained at one of the participants' study visits and will be used for DNA isolation. Blood will be drawn by a certified phlebotomist or an RN, LPN, DO, or MD. A standard protocol for collection of blood derived DNA will be utilized, and approximately 2 teaspoons (10 milliliters) of blood will be collected. If blood cannot be obtained or the subject elects to limit DNA collection to non-blood sources, an oral DNA specimen (saliva) will be obtained instead. Whether the sample collected is blood or saliva, samples will be deidentified, processed, and shipped to CCHMC for storage. DNA will remain available for future genetic testing.

4.5 End of therapy (EOT) and Follow-up

The primary efficacy endpoint (e.g. achievement of histologic remission of less than 15 eosinophils/HPF) will be determined at the end of phase 1 (week 6) at which time all participants will undergo EGD with biopsy. Participants who achieve histologic remission at the end of phase 1 will have completed the study and will resume follow-up care with their primary GI or Allergy physician. Participants whose disease remains active after being on 1FED and choose to continue into Phase 2 will continue on to 6FED in Phase 2. Participants whose disease remains active after being on 6FED in Phase 1 and choose to continue into Phase 2 will receive 6 weeks of SGC in Phase 2.

4.6 Stratification, Randomization, and Blinding/Masking

All eligible participants will be randomized 1:1 to either the 1FED or 6FED. Following this six week phase 1, all patients will undergo EGD with biopsy, and those whose disease remains active may choose to move on to Phase 2 (to either 6FED or treatment with swallowed SGC). The sponsor, investigator, study staff, and participant will all have knowledge of the treatment being received.

5. SELECTION OF PARTICIPANTS AND CLINICAL SITES/LABORATORIES

5.1 Rationale for Study Population

Participants of any gender or race who are between the ages of 18-60 years old are eligible for entry into this study. We have chosen to limit our study to adult (ages 18-60) subjects due to evidence from previous studies that there may be different rates of response to given antigens between adult and pediatric populations (for instance, pediatric trials of dietary intervention have consistently shown that milk is the most common causative antigen in this age group, while studies in adult populations have suggested that other antigens including wheat are more likely to drive eosinophilia in adults.)[23, 24]. Given this apparent difference in response, we will limit the study population to ensure that we can accurately identify responses to the different dietary regimens.

5.2 Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

1. Subject must be able to understand and provide informed consent
2. Have diagnosis of EoE (based on consensus criteria)[1]
3. Are aged 18 to 60 years
4. Have histologically confirmed active disease ≥15 eosinophils/hpf in the esophagus within 12 weeks of screening visit
5. Symptomatic (have experienced symptoms within the last month prior to enrollment)
6. PPI confirmation. (As a part of the diagnosis of active EoE, it must be demonstrated that acid reflux disease is not the primary cause of the participant's symptoms by documentation that the participant has been on a high dose of PPI (at least one dose, once daily) for at least 8 weeks prior to a diagnostic endoscopy of EoE without histologic resolution (i.e., ≥ 15 eosinophils/hpf). (Due to the variety of doses and various PPIs available, the dose will be confirmed adequate at the discretion of the PI).)
7. Have a negative urine pregnancy test at screening if of childbearing potential. Females of childbearing potential must have a negative urine pregnancy test (β-hCG) prior to enrollment into the study (i.e., at screening). These participants must agree to use adequate birth control measures (e.g., condom, oral/injectable/subcutaneous contraceptives, intrauterine device, or sexual abstinence) during the study and for at least one month after the last dose of study drug which will be documented in the source documents.

5.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
2. Have been treated with topical swallowed steroids within the last 2 months or systemic steroids within the last 3 months
3. Have pathological eosinophilia in segments of the GI tract other than the esophagus determined by local review
4. Have been diagnosed with a GI malabsorption disorder (i.e., Inflammatory bowel disease, Crohn's disease) or Celiac disease
5. Are currently on dietary therapy avoiding milk or on a 6FED.
6. Have concurrent *H. pylori* gastritis or parasitic infection

7. Have history of anaphylaxis to milk (with current avoidance of milk)
8. Unable to complete study procedures, including EGD.
9. Have previously failed strict dietary therapy clearly documented with one of these regimens or topical steroid treatment (i.e. have not achieved histological remission of <15 eos/hpf after having been on 2 mg of budesonide per day or 1760 mcg of fluticasone per day for 8 weeks prior to the EGD).
10. Use of investigational drugs within one month prior to enrollment
11. Are concurrently receiving any of the prohibited medications listed in section 8.3
12. Not fluent in English
13. On immunotherapy for pollen (if not on maintenance therapy) or IgE-mediated food allergy.
14. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

6. KNOWN AND POTENTIAL RISKS AND BENEFITS TO PARTICIPANTS

6.1 Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

Treatment with topical swallowed steroids

In the short term (such as the Phase 2 six week period steroid study interval), the primary risk is the development of oral thrush, which is a known side effect of topical swallowed steroid use[6]. Other reported concerns include behavior changes and difficulty sleeping. Such side effects are reversible with medication reduction or discontinuation. Risks from longer term use of topical swallowed corticosteroid are less well understood, but might include bone demineralization, trouble with elevated blood sugar or hypertension, and/or eye changes such as glaucoma or cataracts. However, these have not been commonly seen with use of inhaled steroids for other diseases such as asthma and have not yet been seen in any of the controlled studies with topical steroids for EoE. There is also a concern about the potential for adrenal suppression with chronic steroid use, and we will monitor for this with morning cortisol levels at the beginning and end of Phase 2. There is also a theoretical risk for immunosuppression due to the chronic use of high-dose swallowed inhaled steroids, although this has never been shown to occur in previous studies or in the collective experience of the centers involved in the trial.

6.2 Risks of Investigational Product or Intervention cited in Medical Literature

N/A

6.3 Risks of Other Protocol Specified Medications

N/A

6.4 Risk of Study Procedures

6.4.1 Treatment with Dietary Therapy

Minimal risk exists related to implementation of short-term dietary therapies in EoE. Dietary instruction regarding

tenets of each dietary therapy and nutrition-related recommendations will be provided by a Registered Dietitian,

who will show participants how to implement and make appropriate substitutions for foods eliminated to ensure nutritional adequacy. A central dietitian will develop standardized instructional materials for use at all of the participating sites.

6.4.2 Histology Scoring System (HSS)

The histology scoring system is a method to evaluate esophageal biopsies containing eosinophils for various features (ex: basal layer hyperplasia, dilated intercellular spaces, surface epithelial alteration, apoptotic epithelial cells), eosinophilic inflammation (peak count, eosinophil abscess, eosinophil surface layering), and lamina propria (fibrosis). There is no risk associated with the utilization of the HSS.

6.4.3 Study Survey

Each participant between the ages of 18 to 60 years old will be asked to complete several surveys, including PRO measures regarding both Health Related Quality of Life as well as disease-specific symptom scores and QOL measures in order to address their EoE symptoms and problems/ feelings related to eating. The data gathered from these questionnaires will be correlated with several biomarkers of interest that have been associated with EoE. These surveys will be conducted at baseline and the end of each study phase. There are no foreseeable physical discomforts or significant risks related to answering the PROs or study questionnaires. However, some questions may be difficult or uncomfortable for participants to answer. Participants may refuse to answer any questions that they are uncomfortable with. The participants may also feel inconvenienced by completing the questionnaires. The participant questionnaires typically take approximately 15 to 20 minutes to complete. All participants will be given ample time to complete the questionnaires. Please reference the table below for guidelines regarding study questionnaire completion.

6.4.4 Esophageal Biopsies

The risks associated with collecting additional esophageal biopsies (up to a total of four obtained from the proximal and/or distal esophagus) for research at the time of the endoscopy include: bleeding at the site of tissue (biopsy) collection, and a small chance of perforation (hole) of the stomach, duodenum, or esophagus. Perforation is the most severe gastrointestinal complication, but generally it is self-resolving and poses no life-threatening risk. Transient bacteremia as a result of diagnostic UGI endoscopy has been reported at rates as high as 8%, but the frequency of infectious endocarditis and other clinical sequelae is extremely low, such that current American Heart Association and American Society for Gastrointestinal Endoscopy guidelines do not recommend antibiotic prophylaxis with diagnostic UGI endoscopy solely to prevent infectious endocarditis.[54-57] The risk of aspiration during endoscopy is miniscule. To minimize the risks of collecting additional biopsies during endoscopy, the procedure will be performed or supervised by a skilled endoscopist, and additional biopsies will only be collected if the endoscopist feels it is appropriate to do so. Some samples that are collected during an endoscopy 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 de-identified per HIPPA and have no PHI associated with them. The data and/or samples will be used in a collaborative relationship between institutions, or testing companies receiving the data and/or samples. All of these samples will be shared under a MTA, or other applicable agreement.

6.4.5 Blood Draws

Risks associated with the collection of blood are bleeding, bruising, and 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.

6.4.6 Skin Testing For Allergies—Prick Testing

Skin testing for allergies may cause mild discomfort when the skin is pricked. Participants may experience symptoms such as itching, stuffy nose, red watery eyes, or a skin rash if they are allergic to the substance being tested. A rare but serious whole-body allergic reaction, known as anaphylaxis, may occur. Anaphylaxis can be life threatening, but this usually only occurs with intradermal testing, and the health care provider performing the skin testing will be prepared to treat this serious reaction.

6.4.7 Skin Testing For Allergies—Patch Testing

Skin patch testing for allergies may cause mild discomfort at the local site including rash, itching and skin redness.

6.4.8 Swallowed Glucocorticoids

Swallowed glucocorticoids may cause oral or esophageal candidiasis; if this is noted, it will be treated with topical or systemic anti-fungal therapy, as clinically indicated. Swallowed glucocorticoids may cause adrenal suppression which will be monitored by serum AM cortisol determination before and after the study protocol. If a depressed value of AM cortisol is found at study completion, this information will be provided to the primary doctor and patient.

6.5 Standard of Care Procedures Associated with This Study Esophagogastroduodenoscopy (EGD)

Endoscopy with biopsy is a well-established procedure. Few patients have unexpected or serious complications. Since it is considered standard of care for adult patients with EoE to have an EGD with biopsies within a six week time frame to monitor the disease when trialing new therapies (foods or medications), the EGDs with biopsies that are associated with this study are not study procedures. Endoscopies will be performed regardless of study participation as patients' standard of care, and additional endoscopic procedures will not be performed because of participation in this study. It is considered standard of care for biopsies to be collected from the esophagus, stomach, and duodenum during endoscopy, and biopsies from all of these sites will be collected during endoscopic procedures at all participating sites whenever possible. Biopsies collected from the stomach and duodenum are evaluated to determine study eligibility. Subjects may be included, without stomach or duodenal biopsies, if there is no clinical history of involvement of stomach or duodenum.

6.6 Potential Benefits

Standard therapies for EoE consist of allergy test-directed and empiric elimination dietary therapies, complete elimination of all dietary antigens requiring the exclusive use of elemental formula, or the chronic use of topical swallowed steroids. These therapies are all associated with risks and benefits. Comparing a minimally restrictive dietary elimination regimen to a more restrictive dietary regimen to control esophageal inflammation may permit earlier identification of a diet that controls symptoms and achieves remission. In addition, by limiting the number of potential reintroductions, this less restrictive diet has the potential to limit the need for endoscopies and attendant sedation and thus reduce risk to patients and costs to the health care system.

There are no guaranteed benefits for any study participant. Potential benefits to the individual participant would include the use of a less restrictive diet compared to the standard 6FED, as well as a reduction in the time to identification of a minimally restrictive effective diet

7. INVESTIGATIONAL AGENT /DEVICE/INTERVENTION

7.1 Investigational Agents/Devices/Interventions

Investigational Agent #1

Flovent HFA Inhalation aerosol: swallowed fluticasone propionate 1760mcg daily (in Phase 2), manufactured by GlaxoSmithKline.

7.1.1 Formulation, Packaging, and Labeling

Page 10 of Flovent Investigator's Brochure:

FP HFA (GSK Lab Code GR106642X) inhalation aerosol is a pressurized MDI for oral inhalation. Each inhaler consists of a white to off-white suspension of FP (micronized) in a liquefied HFA propellant (GR106642X) which is contained in an aluminium can sealed with a metering valve. The canister is presented in a plastic actuator fitted with a dust cap.

Ingredients include FP (micronized), and GR106642X (1,1,1,2-Tetrafluoroethane). No other excipients are included in the formulation. Stability is at most 18 months when stored under labeled storage conditions of 25°C. As further real-time stability data become available, the shelf life will be extended.

A placebo inhaler containing GR106642X propellant 75mg per actuation alone is also available.

7.1.2 Dosage, Preparation, and Administration

Page 14 of Flovent Investigator's Brochure:
For all products, storage should be at a controlled room temperature between 25-30°C in a dry place and not above 30°C.

7.1.3 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

7.1.4 Assessment of Participant Compliance with Investigational Agent

Compliance with the dietary therapies will be assessed via completion of a food diary that will be reviewed by a registered dietitian at regular intervals throughout the study.

Compliance with Flovent will be determined by the counter that is on the Flovent inhaler. Greater than 80% compliance will be deemed "good" compliance for the study; subjects with less than 80% compliance will be encouraged by study staff to adhere to study dosages. All non-compliance issues will be documented, and compliance that is <70% or >120% will be reported to the study sponsor.

7.1.5 Toxicity Prevention and Management

In order to measure potential systemic toxicity of FP we have chosen to measure AM serum cortisol levels as a measure of adrenal suppression. Cortisol will be collected at the Treatment Phase 2 and the EOT Phase 2 visits. If the AM cortisol is low at the EOT Phase 2 visit, then study staff will communicate this information to the subject's primary care physician for appropriate follow-up care.

7.1.6 Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

1. The PI feels that the study treatment is no longer in the best interest of the participant.
2. The participant experiences an AE that the PI feels warrants discontinuation of the study therapy.

8. OTHER MEDICATIONS

8.1 Concomitant Medications

8.1.1 Protocol-mandated

N/A

8.1.2 Other permitted concomitant medications

Throughout the study duration, all participants are expected to maintain medications such as a PPI, oral or nasal allergy medications such as antihistamines and any asthma-related medications that were prescribed prior to study entry.

8.2 Prophylactic Medications

N/A

8.3 Prohibited Medications

N/A

Medication	Washout Time Prior to Screening Visit (i.e., Study Entry)
Anti-immunoglobulin E [IgE] mAb	6 months
Methotrexate, cyclosporine, interferon-α	3 months
Anti-tumor necrosis factor [TNF] mAb)	6 months
Other systemic immunosuppressive or immunomodulation agents	3 months
Oral or intravenous systemic steroids	3 months
Topical swallowed steroids	2 months
Other investigative biologic	1 month
Anti-IL 5 agents	3 months
Anti-IL 13 agents	6 months
Other investigative drugs or device	1 month

8.4 Rescue Medications

If a subject has a worsening of EoE symptoms, any treatment that the subject would take for EoE, besides the study drug, would then exclude the subject from the study. As such, there are no treatments that will be provided on study for "rescue therapy".

9. STUDY PROCEDURES

9.1 Screening/Baseline Visit (Weeks -12 to -1)

The screening visit should occur at a maximum of twelve weeks and a minimum of 1 week prior to the enrollment visit. A research staff member will perform the informed consent process. After obtaining informed consent, the following study procedures will be performed and documented:

- Assess eligibility (inclusion and exclusion criteria)
- Collect Medical, surgical and diet history
- Vitals (height, weight, blood pressure, heart rate, respiratory rate, temperature)
- Demographics (age, gender, race)
- Physical exam performed by a study clinician or qualified staff member
- Collect stool sample (if possible) from subjects who have consented to stool collection

- Obtain up to 4 biopsies from the distal and proximal esophagus for research purposes (taken during standard of care EGD)
    - One of these esophageal biopsies will be used for EDP
- Research lab samples
    - Blood (6-10 mL) or saliva (1-2 mL for future genetic testing may be collected at this visit
    - Blood for metabolomics (3-5 mL)
    - CBC with differential (2-3 mL)- if a CBC with differential was obtained clinically as part of standard of care within the screening period, obtain the results from the standard of care CBC
- Assessment of concomitant medications and therapies
- Pregnancy Test (urine)

Participants may qualify for rescreening if they are ineligible due to any of the reversible illnesses listed in the exclusion criteria if their condition improves while recruitment is ongoing and they still fall within the screening window.

Esophageal biopsies for research may be taken during a participant's standard of care EGD (SOC EGD). If the participant has had an EGD and biopsies performed prior to the screening visit, the results of the EGD may be used to determine eligibility if it falls within the four-week window prior to enrollment. EGDs performed at an outside institution may be used as baseline if the corresponding pathology report and slides are available and the eosinophil counts meet the inclusion criterion of ≥15 eosinophils/hpf. In cases where the local pathologist has concerns regarding the eosinophil count, slides may be sent to the CRPC for rapid review in order to verify eligibility.

The amount of blood drawn (clinical plus research) will follow the NIH clinical center guidelines "Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center" (May 26 2012).

Stool samples may be collected from participants who have consented to stool collection at the Screening/Baseline visit. If it is difficult to obtain the stool sample at this visit, it may alternatively be collected at the Enrollment visit. Stool will only be collected at some, but not all, participating centers.

9.2 Enrollment (Week 0)

All eligible participants will be enrolled into the study at Week 0 if they meet all entry criteria and have completed the screening procedures. Study procedures will not be repeated if the enrollment visit takes place within 5-10 business days of the screening visit. At this visit, staff members will:

- Assess eligibility (inclusion and exclusion criteria)
- Measure vitals (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Obtain stool sample (if possible and not collected at Screening/Baseline) from subjects who have consented to stool collection
- Administer patient reported outcome questionnaires, which will include dietary questionnaires to measure *intake* of the food allergens and a food diary to assess baseline diet
- Completion of DSQ
- Assess concomitant medications and/or therapies

- Skin testing for allergies including prick and patch testing
- Randomize eligible participants to the 1FED or 6FED diet
- Provide standardized dietary instructions for 1FED or 6FED
- Research lab samples
    - Blood serum (~1-2 tsp, or 5-10 mL) for food IgE, CRD, and IgG4
    - Blood serum (~3-6 tsp, or 15-30 mL) for T cell studies

The participants will be encouraged to call the PI and/or study coordinator if they have any questions, concerns, adverse events, or changes in medication.

Stool samples may be collected from participants who have consented to stool collection at the Enrollment visit if they were unable to be obtained at the Screening/Baseline visit. Stool will only be collected at some, but not all, participating centers.

9.3 Phone Visits, Phase 1

A study staff member will conduct three phone visits (at weeks 1, 3, and 5) during Phase 1 in order to monitor participants' progress throughout the trial. The following procedures will occur during these phone visits:

- Assess con-med and/or con-therapy compliance
- Assess AEs
- Determine compliance with treatment
    - A dietary questionnaire to measure *intake* of the food allergens will be completed by the participants during weeks 1 and 5
    - A food diary will be completed by participants during weeks 1 and 5 to measure adherence
- Daily completion of the DSQ within the two weeks prior to the Phase 1 EOT EGD (Weeks 4 and 5)

9.4 End of 1st Treatment phase or Early Withdrawal (Week 6)

For participants who have completed the 6 week Phase 1 or have withdrawn early from the study, the following procedures will occur:

- Measure vitals (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Physical exam performed by a study clinician or qualified staff member
- Collect stool sample (if possible) from subjects who have consented to stool collection
- Administer patient reported outcome questionnaires
- Assess AEs
- Obtain up to 4 biopsies from the esophagus for research purposes (taken during SOC EGD)
    - One of these esophageal biopsies will be used for EDP
- Research lab samples
    - Blood serum (~1-2 tsp, or 5-10 mL) for food IgE, CRD, and IgG4
    - Blood serum (~3-6 tsp, or 15-30 mL) for T cell studies
    - Blood (6-10 mL) or saliva (1-2 mL) for future genetic testing may be collected at this visit (if not collected previously)
    - Blood for metabolomics (1 tsp, or 3-5 mL)
    - CBC with differential - if a CBC with differential was obtained clinically as part of standard of

care, obtain the results from the standard of care CBC

- Assess participant compliance with diet therapy and concomitant medications
- Pregnancy Test (urine)

Stool samples may be collected from participants who have consented to stool collection. Stool will only be collected at some, but not all, participating centers.

If a participant withdraws from the study before completing 6 weeks of dietary therapy, the end of treatment/early withdrawal visit should be conducted within one week of the participant's stopping dietary therapy. Participants should stay on their dietary therapy until results of biopsies are communicated to them.

Those participants who have resolution of their inflammation on endoscopy at the end of Phase 1 will complete the study at this time. Subjects with continued active inflammation will move on to phase 2, 6FED non –responders will start treatment with swallowed SGC (880mcg BID fluticasone propionate) and unrestricted diet and 1FED non- responders will start treatment of 6FED.

9.5 Phase 2 Visit 1 (Week 10)

Those participants who have completed the phase 1 diet protocol but have continued active inflammation may choose to continue into Phase 2 and will return to clinic once their biopsies have been read. This visit should happen within 4 weeks of their EGD. At this visit, the following procedures will occur:

- Measure vitals (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Assess AEs
- Assess participant compliance with concomitant medications
- Provide standardized instructions for administration of the fluticasone (for participants who will be on fluticasone)
- Provide standardized instructions for 6FED for participants who will be on 6FED
- Provide diet or medication diary/log to participants
- Administer dietary questionnaire to measure *intake* of the 6FED food allergens (for those who will be on 6FED)
- Complete DSQ
- Dispense fluticasone to participant (for those moving on to SGC)
- Measure morning cortisol (serum, ~1 tsp or 5 mL) (for those moving on to SGC in Phase 2)

9.6 Phone Visits, Phase 2

A study staff member will conduct two phone visits (at weeks 11 and 15) during Phase 2 in order to monitor participants' progress throughout the trial. The following procedures will occur during these phone visits:

- Assess con-med and/or con-therapy compliance
- Assess AEs
- Determine compliance with dietary treatment

    - A dietary questionnaire to measure *intake* of the food allergens will be completed by participants who are on 6FED during weeks 11 and 15
- A food diary will be completed by participants who are on 6FED during weeks 11 and 15 to measure adherence
- Daily completion of the DSQ within the two weeks prior to the Phase 2 EOT EGD (Weeks 14 and 15)

9.7 End of 2nd treatment phase or Early Withdrawal (Week 16)

For those participants who have completed the phase 2 therapy or have withdrawn early from the study, the following procedures will occur:

- Measure vitals (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Physical exam performed by a study clinician or qualified staff member
- Administer patient-reported outcome questionnaires
- Assess AEs
- Obtain up to 4 biopsies from the esophagus for research purposes (taken during SOC EGD)
    - One of these esophageal biopsies will be used for EDP
- Assess participant compliance with therapy (SGC) and concomitant medications
- Pregnancy Test (urine)
- Measure morning cortisol (serum, ~1 tsp, or 5 mL)
- Collect fluticasone from participant (for those on SGC)
- Collect participant compliance logs; dietary log or SGC log
- Research lab samples
    - Blood (6-10 mL) or saliva (1-2 mL) for future genetic testing may be collected at this visit (if not collected previously)
    - Obtain CBC with differential (2-3 mL)- IF a CBC with differential was obtained clinically as part of standard of care within the screening period, obtain the results from the standard of care CBC

If a participant withdraws from the study before completing 6 weeks of SGC or 6FED therapy in Phase 2, the end of treatment/early withdrawal visit should be conducted within one week of the participant's stopping therapy. Participants should stay on SGC or 6FED until results of biopsies are communicated to them.
Following the receipt of their biopsy results, the participant will return to standard clinical care.

Should pregnancy occur during either phase of the study, dietary therapy and/or dosing of study medication will be discontinued and the participant will be withdrawn from the study. The pregnancy will be followed clinically to term.

9.8 Unscheduled Visits

Unscheduled visits will take place for any complication or AE/SAE that requires an extra visit. The procedures to be performed at the unscheduled visits will be at the discretion of the PI. Procedures that may be performed at an unscheduled visit include:

- Vital signs (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Physical exam
- Assess AEs

- Lab tests
- Assess/address problems with dietary adherence

These visits will be documented in the unscheduled visit case report.

9.9 Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated on the Table 1 Schedule of Assessments.

Each visit window (for visits 2-10) is ± 3 days.

10. MECHANISTIC ASSAYS

10.1 EDP

We will focus on molecular profiling of esophageal genes with the EoE Diagnostic Panel (EDP), a set of 94 mRNA transcripts that have been established to differentiate EoE from controls (e.g. GERD and normal individuals), and to identify exposure and transcriptional signaling to SGCs[28]. The EDP is typically performed on an extra biopsy taken from the distal esophagus[28]. If our hypotheses are proven to be correct, they have potential to transform monitoring of patients during therapeutic intervention, by identifying molecular markers that have therapeutic significance, and also by reducing the need for biopsies, particularly after therapy is initiated. Essentially, the associated EDP algorithm renders the raw Ct. values of each embedded gene after real-time PCR in a way such that the upregulated genes and downregulated genes are summed up individually. A quantitative "EoE score" is derived from the ΔCt. (normalized to GAPDH) summation to reflect disease severity and for statistical analysis. Utilizing different gene sets, EDP is also able to predict steroid exposure and remission status based on the same algorithm [28]. In this case, the EoE score will be calculated at baseline endoscopy and in the posttreatment biopsies to assess change with treatment.

10.2 T Cell Studies

We will test the hypothesis that milk-specific T cells will be primarily CD4+, IL-5+, IL-13+, consistent with prior work.[47-49] Furthermore, we will test the sub-hypothesis that the level of milk-specific T cells will positively correlate with positive atopy patch tests to milk and the response rate to milk elimination (1FED and secondarily 6FED, which also contains milk). The frequency of Th2 cells will be determined by FACS analysis, and milk-specificity will be estimated by milk protein stimulation in vitro, particularly looking at CD154 induction, a marker of T cell activation. These exploratory studies, will be performed with shipped blood samples, by Dr. Jonathan Spergel, as published.[47,48,50,51] The study will be limited to a pilot analysis of patients who only enter the 1FED and we will compare patients with positive and negative skin tests (prick and patch).

10.3 Biospecimen Storage

Blood, saliva, and/or esophageal biopsies may be collected and stored for future analysis. Specimens will be obtained at one of the participants' study visits and will be used for DNA isolation. Blood will be drawn by a certified phlebotomist or an RN, LPN, DO, or MD. A standard protocol for collection of blood derived DNA will be utilized, and approximately 2 teaspoons (10 mL) of blood will be collected. If blood cannot be obtained or the subject elects to limit DNA collection to non-blood sources, an oral DNA specimen (saliva) will be obtained

instead. Whether the sample collected is blood, tissue, or saliva, samples will be deidentified, processed, and shipped to CCHMC for storage. DNA will remain available for future genetic testing.

11. CRITERIA FOR PARTICIPANT AND STUDY COMPLETION AND PREMATURE STUDY TERMINATION

11.1 Participant Completion

Participant completion is defined by completion of the EGD at the end of Phase 1 and Phase 2 (for those who choose to continue into Phase 2); there are two levels of completion, those that complete Phase 1 and those that complete Phase 2.

11.2 Participant Stopping Rules and Withdrawal Criteria

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

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. Participant is unable to comply with the dietary restrictions.
3. Participant develops severe complications from their EoE (for instance, esophageal strictures)
4. Participant becomes pregnant.
5. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
6. The participant develops an SAE related to therapy in Phase 1 or 2 of the study.
7. The participant dies.
8. The Investigator no longer believes participation is in the best interest of the participant.

11.3 Participant Replacement

Enrolled participants who start on the initial dietary protocol and prematurely discontinue/withdraw from the study will be replaced if they do not reach Week 6 of the study including the EGD. Additional participants will be enrolled in the same manner as all other participants. Participant numbers will not be re-used. Any enrolled participant will be included into any intent to treat analysis.

11.4 Follow-up after Early Study Withdrawal

Refer to section 9.7.

11.5 Study Stopping Rules

The study can be terminated or stopped at the site for reasons including but not limited to:

1. Investigator or sponsor request to withdraw from study participation.
2. Serious and/or persistent noncompliance by the investigator with the protocol or other local applicable regulatory guidelines in conducting the study.
3. IRB decision to terminate or suspend approval for the investigation or the investigator.

12.SAFETY MONITORING AND REPORTING

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of the FDA must be reported promptly (per Section 12.5.1, *Reporting of Serious Adverse Events and Adverse Events* to *DAIT/NIAID).* Appropriate notifications will also be made to site principal investigators and the Institutional Review Board (IRB).

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version *[enter 4.0 or correct version applicable to trial]* : http://ctep.cancer.gov/reporting/ctc.html.

Safety will be assessed through documentation of AEs, adverse drug reactions, physical examination findings and vital signs, and pre- and post-treatment morning cortisol for those patients on SGC in Phase 2.

12.2 Definitions

12.2.1 Adverse Event (AE)

An Adverse Event is any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

Study therapy regimen: Topical swallowed steroids

- Development of oral thrush
- Behavior change
- Difficulty sleeping
- Bone demineralization
- Elevated blood sugar
- Hypertension
- Eye changes such as glaucoma or cataracts
- Potential for adrenal suppression
- Potential risk of immunosuppression

12.2.2. Study mandated procedures:

Esophagogastroduodenoscopy (EGD) Biopsies

- Bleeding at the site of biopsy collection
- Perforation of the stomach, duodenum or esophagus

Allergen Scratch Skin Testing

- Prolonged (>24 hours) itching at test site
- Swelling (> 10 cm) at site of test lasting more than 24 hours
- Nasal allergic symptoms within 30 minutes of the procedure
- Fainting/Vasovagal event within 30 minutes of the procedure
- Anaphylaxis

Blood Draws

- Fainting/Vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 30 minutes
- Swelling at puncture site larger than 2 cm

12.2.3 Suspected Adverse Reaction (SAR)

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the investigational study therapy regimen caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.4 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the GlaxoSmithKline *package insert* or is not listed at the specificity, severity or rate of occurrence that has been observed; or is not consistent with the risk information described in the protocol.
"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a)).

12.2.5 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)).

Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.

A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.

Inpatient hospitalization or prolongation of existing hospitalization.

Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

Congenital anomaly or birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) *[Version 4.03, June 14, 2010]* This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the Protocol Chair and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild adverse event.
Grade 2 = moderate adverse event.
Grade 3 = severe and undesirable adverse event.
Grade 4 = life-threatening or disabling adverse event.
Grade 5 = death.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE case report form. Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

Adverse events (including SAEs) will be collected from the time of consent until a subject completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.1 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

Observing the participant.

Interviewing the participant

Receiving an unsolicited complaint from the participant.

In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, Grading and Attribution of Adverse Events.

12.4.2 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, *Definitions)* on the appropriate AE/SAE CRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to Sponsor DAIT/NIAID

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via SAE CRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators will report to the DMCC (USF) and DAIT/NIAID all serious adverse events (see Section 12.2.4, Serious Adverse Event*)*, regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE CRF will be updated and submitted. AE/SAE reports should be reviewed by the investigator.

Upon entry of a SAE, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the Medical Review Officer, and any additional agencies (if applicable- industry sponsor, CTEP, etc.) of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO [and, if applicable, sponsor] may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

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 events will be presented in tabular form and given to the MRO and RDCRN DSMB on an [observational- "annual" / interventional "bi-annual"] basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.
The DMCC will post aggregate reports of all reported adverse events for site investigators and IRBs.

12.5.2 Reporting to the FDA

The study PI, Dr. Marc E. Rothenberg, MD, PhD, holds the IND for this study. Each site will report SAEs in the DMCC system as defined above in section 12. The DMCC adverse event notification system will notify the IND sponsor. SAEs will be reported to regulatory authorities per 21 CFR 312.32 and institutional policy. All AEs will tabulated by the DMCC and available to the IND sponsor on a monthly basis. Adverse events will be reported by the IND holder, in summary form, at the time of continuing annual review to the IRB, FDA, DSMB and the NIAID.

12.5.3 Annual Reporting

Dr. Marc Rothenberg (the IND holder) will include in the annual study report all adverse events classified as:

Serious, expected, suspected adverse reactions (see Section 12.2.2, *Suspected Adverse Reaction,* and Section

12.2.3, *Unexpected Adverse Event*).

Serious and not a suspected adverse reaction (see Section 12.2.2, *Suspected Adverse Reaction*).

Pregnancies not reported as serious adverse events.

Note that all adverse events (not just those requiring 24-hour reporting) will be reported annually.

12.5.4 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction [SUSAR]** (see Section 12.2.2, *Suspected Adverse Reaction* and Section 12.2.3, *Unexpected Adverse Event* and 21 CFR 312.32(c)(1)i).
The sponsor must report any suspected adverse reaction that is both serious and unexpected. The DAIT/NIAID shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);

One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);

An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Category 2: Any findings from studies that suggests a significant human risk

The sponsor must report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

Dr. Marc Rothenberg (the IND holder) shall notify the appropriate health authorities, the FDA, central IRB and all participating investigators of *expedited Safety Reports* within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.5 Reporting of Adverse Events to IRBs/IECs

Adverse events, including expedited reports, will be reported by CCHMC, in a timely fashion to the central IRB in accordance with applicable regulations and guidelines.

12.5.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject. A pregnant subject shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the Data Management and Coordination Center (DMCC*)* and DAIT/NIAID all pregnancies within 1 business day of becoming aware of the event using the CRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy CRF shall be updated and submitted to the Data Management and Coordination Center (DMCC*)* when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the Data Management and Coordination Center (DMCC*)* and DAIT/NIAID using the SAE reporting procedures described above.

12.5.7 Reporting of Other Safety Information

An investigator shall promptly notify the study sponsor (Dr. Rothenberg) as well as the DMCC and DAIT/NIAID when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event. The DMCC will notify the central IRB.

12.5.8 Review of Safety Information

12.5.9 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive monthly reports from the DMCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate CRFs.

In addition, the Medical Monitor shall review and make decisions on the causality and relatedness of the SAE and pregnancy reports received by the DMCC (See Sections 12.5.1, Reporting of Serious Adverse Events to DAIT/NIAID and 12.5.6, Pregnancy Reporting).

12.5.10 DSMB Review

The DMCC will provide the DSMB with listings of all SAEs on an ongoing basis, including quarterly reports of all SAEs. Furthermore, the DSMB will be informed of expedited reports of SAEs.

12.5.10.1 Planned DSMB Reviews

The NIAID Data Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported

AEs and SAEs.

The DSMB will be informed of an Expedited Safety Report in a timely manner. An SAE which the Medical Monitor determines to be an unexpected safety risk will be sent to the DSMB immediately.

12.5.10.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, any occurrence of meeting one of the study stopping rules as described in Section 11.5 will trigger an ad hoc comprehensive DSMB Safety Review. After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.5.10.3 Temporary Suspension of Enrollment for ad hoc DSMB Safety Review

A temporary halt in enrollment will be implemented if an ad hoc DSMB safety review is required.

13.STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

13.1 Overview

The primary objective of this proposal is to conduct a prospective, non-blinded randomized trial comparing rates of remission of novel empiric elimination dietary therapies in eosinophilic esophagitis (EoE) in order to assess the therapeutic viability of empiric elimination diets. Moreover, we aim to assess response to topical swallowed steroids in non-responders to empiric dietary therapy regimens. Participants aged 18 to 60 years with active EoE will be enrolled and the primary efficacy outcome will be the rate of histologic remission assessed using esophageal biopsies obtained following a six week randomized trial of one of two empiric diets. During the screening process, active EoE will be confirmed by histologic evaluation of esophageal biopsies obtained during an esophagogastroduodenoscopy (EGD) in subjects with a history consistent with EoE.

13.2 Endpoints/Outcomes

The primary efficacy endpoint will assess and compare the percentage of patients achieving remission by following the two diets. Pre- and post-therapy peak eosinophil counts per hpf and percentages of patients that attain histologic remission (<15 eosinophils/hpf) will be reported.

A variety of secondary endpoints, including more detailed histologic and endoscopic scores, novel biomarkers, and several Patient Reported Outcome (PRO) measures will also be assessed. In addition, we will assess the possible value of a variety of clinical characteristics and biomarkers in terms of predicting response to therapy. This study will provide significant novel information. This will be a prospective dietary therapy trial using these empiric elimination diets. As part of our secondary aims, we will also be utilizing a number of novel potential biomarkers to assess their ability in predicting and assessing response to therapy as well as a number of PRO metrics.

13.3 Measures to Minimize Bias

Patients will be randomized using a 1:1 scheme to 1FED or 6FED within each investigative site. Study staff will remain blinded to the randomization plan so they do not know the upcoming treatment assignment will be for a patient until after the patient has been assigned. Study personnel who will perform the histologic assessments will be blinded to treatment assignment for the slides they are evaluating. The CRPC will not be told which diet a

participant has been assigned to, and they will not receive any information or documentation indicative of dietary assignment. The slides and/or images of slides provided to the CRPC will not contain any information that reveals which diet the participant is on. Knowledge of dietary assignment will be limited to study staff who need to know which diet a participant has been assigned to (i.e. dietitians).

13.4 Analysis Plan

13.4.1 Analysis Populations

The analysis population for the primary and key secondary endpoints will be the intent-to-treat population. All patients that have been randomized will be included in this analysis. Patients for which the primary endpoint of histological remission cannot be determined will be imputed as treatment failures.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

The primary endpoint for this study will be histologic remission (<15 eosinophils/hpf) determined at the end of Phase 1 and will be compared between the two treatment groups. The formal statistical hypothesis for the primary endpoint is:

H_0: $p_{1FED} = p_{6FED}$
H_1: $p_{1FED} \neq p_{6FED}$
where

p_{1FED}=percentage of patients in histologic remission (<15 eosinophils/hpf) on the 1FED diet at end of Phase 1

p_{6FED}=percentage of patients in histologic remission(<15 eosinophils/hpf) on the 6FED diet at end of Phase 1

This hypothesis will be tested using the generalized linear mixed effects model with the logit link (for binary outcome) that accounts for the clustering within sites. Important covariates to be included in this model are sex, presence of atopy and duration of disease. All tests will be conducted at α=0.05.

The mixed effects model is planned in order to include the site as a random effect in the model. This approach assumes the sites included in this trial are representing a sample of sites about which we wish to make inferences and therefore will include site as a random effect instead of a fixed effect that implies we are only making inferences about those specific sites in the study.

13.4.3 Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Sensitivity analyses will be conducted to assess the robustness of the results. A chi-square test without covariate adjustment will be performed to compare the histologic remission rates at the end of Phase 1 between the two groups. As a second sensitivity analysis, additional covariates will be included in the generalized linear mixed effects model described above including PPI use at baseline, race and/or ethnicity and anthropomorphic measures. Forest plots displaying differences in remission rates along with two-sided 95% confidence intervals for subgroups defined by categorical covariates will be provided to visually examine the consistency of results among subgroups.

As an additional sensitivity analysis, multiple imputation (MI) for patients who are missing the primary outcome measure will be performed. The missing data mechanism will be investigated to determine if the data are missing

at random. In addition, a complete cases analysis will be conducted to investigate consistency of the conclusions from those who complete the study in comparison to conclusions from the intent to treat population with imputed values using the "set to failure" approach and also the MI approach.

All sensitivity analyses will be conducted at the nominal two-sided α=0.05.

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Following are the secondary objectives of the study:

1. To extend Phase 1 of this study with a prospective non-blinded trial that determines the rate of remission following SGC in the 6FED non-responders and the rate of remission following 6FED in the 1FED non-responders (Phase 2).
2. To evaluate the effect of each therapy on histological remission defined by a variety of changes in eosinophils, including (a) pre- and post-therapy peak eosinophil counts; (b) partial remission (2-14 peak eosinophils/hpf); and (c) complete histological remission ($\leq$ 1 peak eosinophils/hpf).
3. To evaluate the effect of each therapy by utilizing the histology scoring system (HSS) created to express the severity and extent of other abnormalities in the gastrointestinal (GI) tract that often accompany eosinophilic inflammation.
4. To evaluate endoscopic outcomes as assessed by the endoscopy scoring system (EREFS).
5. To determine the impact of each therapeutic intervention on biomarkers using the EoE Diagnostic Panel (EDP).

For secondary objective 1, the remission rate for non-responders who proceed to Phase 2 will be summarized separately depending on the treatment patients received during Phase 1. The 95% two-sided confidence interval for the rate will be provided. In addition, forest plots that display the estimated remission rate along with two-sided 95% confidence interval will be presented for the subgroups as defined for the primary endpoint. This will provide a visual representation to assess consistency of rates across subgroups. If the remission rate is missing for patients in Phase 2, it will be imputed as a treatment failure.

The formal statistical hypothesis for each of the key secondary endpoint is:

H_0: $\theta_{1FED} = \theta_{6FED}$

H_1: $\theta_{1FED} \neq \theta_{6FED}$

where

θ_{1FED}=population parameter (e.g. mean, proportion) for patients in histologic remission (<15 eosinophils/hpf) on the 1FED diet at end of Phase 1

θ_{6FED}=population parameter (e.g. mean, proportion) for patients in histologic remission(<15 eosinophils/hpf) on the 6FED diet at end of Phase 1.

To test these hypotheses for each of the secondary objectives 2-5, treatment group comparisons at the end of Phase 1 will be performed using the generalized linear mixed effects model using the link that is appropriate for the endpoint (i.e. logit for binary, cumulative logit for ordered multinomial categories, identity for continuous). These models will account for the clustering within sites and will include gender, presence of atopy and duration of disease as covariates. For continuous data, appropriate transformations will be considered (e.g. log, square root,

rank) to satisfy the assumptions of the analyses. If the remission status for the categorical outcomes is missing, it will be imputed as a treatment failure. Missing data for continuous outcomes will be imputed using multiple imputation techniques. Covariates for imputation will include investigative site, gender, presence of atopy and duration of disease. Each of the secondary outcomes 2-5 will be tested at the two-sided α=0.01 to account for multiple testing.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

Exploratory objectives are detailed below:

1. To evaluate the clinical and psychosocial effect of each therapy utilizing surveys to assess EoE symptoms and problems/feelings related to eating.
2. To determine if any clinical parameters predict response to therapeutic intervention.
3. To determine if any biomarkers including serum food specific IgE, CRD, and IgG4 predict response to therapeutic intervention.
4. To determine if skin testing, in the form of prick and patch testing, predicts response to therapeutic intervention.
5. To determine if milk-driven T cells in the blood positively correlate with milk atopy patch test results and effectiveness of dietary therapies.

At the start of the trial intake of the allergens will be determined and descriptive statistics will be used to describe how often/how much of the allergens were consumed as part of the usual diet. During the trial any accidental exposure will be documented and we can use descriptive statistics to describe how often/how much of the allergens were consumed accidentally.

To compare treatment groups at the end of Phase 1 for EoE symptoms and problems/ feelings related to eating, the generalized linear mixed effects model described for the secondary endpoints will be used with the link function appropriate for the endpoint. Imputation for missing data will follow that described for the secondary endpoints.

To evaluate the ability of biomarkers to predict response to therapeutic intervention, multivariable logistic regression analysis will be conducted with remission/no remission as the dependent variable and biomarker levels as the independent variables. Multi-collinearity of the independent variables will be assessed. The area under the receiver operating characteristic curve (AUROC) will be estimated along with 95% confidence interval. This analysis will be conducted based on the remission status at the end of Phase 1. This analytic approach will be used to determine if skin testing predicts response to therapeutic intervention and to determine if milk-driven T cells predict response to intervention and to determine if T cells correlate with milk atopy patch test results. All exploratory analyses will be conducted at the nominal two-sided α=0.05.

13.4.6 Descriptive Analyses

Demographic, patient characteristics, medication use and study completion status will be summarized for the two treatment groups using mean ± standard deviation or median [interquartile range] for continuous variables and frequency and percentages for categorical variables. Treatment comparisons will be performed using the two-sample t-test or Wilcoxon Rank sum test for continuous variables and the Fisher's Exact test for categorical variables. All tests will be conducted at α=0.05

Additionally, we may perform some exploratory analyses when the two groups in Phase 2 become substantially imbalanced in their demographics and disease characteristics. Also, wewill perform comparisons the groups who choose to continue on Phase 2 and those who opt out, in both arms. Any apparent difference will be noted.

13.5 Interim Analyses

No interim for efficacy nor futility analyses are planned for this study.

Interim data summaries will be provided to the DSMB in accordance with the DSMB charter. Data presentation by study arm will be provided in a blinded fashion with no indication of actual treatment assignment, unless specifically requested by the DSMB to facilitate their review.

13.5.1 Interim Analysis of Efficacy Data

N/A

13.5.2 Interim Analysis of Safety Data

N/A

13.5.3 Futility Analysis

N/A

13.5.4 Statistical Hypotheses

See above

13.6 Sample Size Considerations

The sample size was based on the primary endpoint of the percent of patients in histologic remission (<15 eosinophils/hpf) at the end of Phase 1. Based on preliminary data, it is assumed that the remission rate for the 1FED population is 45%.[16] We assume that the remission rate in the 6FED population will be increased to 70%. A sample size of 60 patients per group (120 total patients) will provide at least 80% power to detect a difference in rates between groups of 25% at the two-sided α=0.05. This calculation is based on a likelihood ratio test statistic for comparing proportions. To allow for a potential approximate 15% drop-out, the sample size will be increased to 68 patients per group (136 total patients). This calculation was performed using the PASS vers. 12 software. The following table displays the robustness of the sample size under various response rate and drop-out rate scenarios.

Sample Size per group	Difference to detect with 80% power if 1FED rate is 45%	Difference to detect with 80% power if 1FED rate is 50%	Difference to detect with 80% power if 1FED rate is 55%
56	26%	24%	24%
60	25%	23.5%	23.5%
64	24%	23%	23%
68	24%	23%	22%

14.IDENTIFICATION AND ACCESS TO SOURCE DATA

14.1 Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2 Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, the DMCC and their representatives, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals.

15.PROTOCOL DEVIATIONS

15.1 Protocol Deviation Definitions

15.1.1 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.

15.1.2 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.

15.1.3 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.

15.1.4 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 DSMB by the NIAID Medical Monitor at the Medical Monitor's discretion.

16. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

16.1 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the Cincinnati Children's Hospital Medical Center IRB (CCHMC IRB), which will serve as the central IRB for the study. Any amendments to the protocol or to the consent materials will also be approved by the CCHMC IRB before they are implemented.

16.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the delegation log will review the consent and answer questions. The consent designee must be listed on the delegation log, have knowledge of the study and received training (from the local IRB, PI, or study coordinator) in the consent process The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

This study will be conducted in compliance with ICH E6 GCP: Consolidated Guidelines pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, participants will give their written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. If the participant is unable to provide written informed consent, the participant's legally acceptable representative may provide written consent as approved by institutional specific guidelines. The informed consent document must be signed and dated by the participant, or the participant's legally authorized representative, prior to study participation. A copy of the informed consent document must be provided to the participant or the participant's legally authorized representative. Signed consent forms must remain in the participant's study file and be available for verification by the monitor, IRB, and/or regulatory authorities at any time. If participant's legally acceptable representative provides written consent, participants will also give their written assent to participate in the study as approved by institutional specific obtaining assent.

The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

17.INVESTIGATOR REQUIREMENTS

17.1 Protocol Adherence

The investigator must adhere to the protocol as detailed in this document and agree that the Sponsor and the IRB must approve any change to the protocol. The investigator will be responsible for enrolling only those participants who have met the protocol screening and study entry criteria.

17.2 Case Report Forms

The CRFs will be used for the recording of all information and study data as specified by this protocol. The CRFs must be completed by the research personnel. The principal investigator is responsible for ensuring that accurate CRFs are completed in a timely manner. Collected data will be entered into online electronic case report forms. Electronic case report forms will be developed in collaboration with the Data Management and Coordinating Center that contain the requisite data fields.

17.3 Source Document Maintenance

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, study progress notes, e-mail correspondence, computer printouts, clinical laboratory data, and recorded data from automated instruments. All source documents produced in this study will be maintained by the investigator and made available for inspection by regulatory authorities. The original signed dated informed consent form for each participating participant shall be filed with records kept by the investigator and a copy given to the participant. The patient outcome questionnaires will be recorded directly on the paper forms and will be considered as source data.

17.4 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

17.5 Registration

Registration of participants on 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. In this fashion, no personal identifiers would be accessible to the DMCC.

17.6 Laboratory Data Flow

The DMCC will provide laboratories with on-line forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. Online forms exist to verify specimen receipt, report specimen issues and submit test results for specimens. The preferred method to exchange data electronically is through the Specimen Management System Web Service. The Web Service allows laboratories to obtain specimen shipment information, receive individual specimens or specimen shipments, report specimen issues and communicate specimen aliquots in a secure manner (test result submission is planned). The DMCC will also support uploading of files electronically. All transactions are logged and validated for both methods.

17.7 Study Completion

Before a study can be considered completed or terminated, the investigator must have the following data and materials:

- Clinical laboratory findings, clinical data, and all special test results from screening through the EOT visit (to 30 days after the last dose of study agent).
- CRFs properly completed by appropriate study personnel and reviewed and approved by the investigator.
- Copies of protocol amendment(s) and IRB approval/notification if appropriate.
- A summary of the study prepared by the principal investigator (an IRB/IEC summary letter is acceptable).

17.8 Audits and Inspections

The principal investigator will permit study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to CRFs and source data/documents.

17.9 Institutional Review Board Approval

This protocol, the informed consent document, and all relevant supporting data must be submitted to the IRB for approval. IRB approval of the protocol, informed consent document, study materials and any advertisement (if applicable) used to recruit study participants must be obtained before the study may be initiated. This study will utilize a centralized IRB, and the IRB at Cincinnati Children's Hospital Medical Center will serve as the central IRB for the study.

The principal investigator (PI) is responsible for keeping the IRB advised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The PI is also responsible for notifying the IRB of any unanticipated AEs that occur during the study in accordance with local

IRB policies. Recent guidance from the USA FDA suggests that the following AEs should be reported to the IRB/IEC as "unanticipated problems:"

- Any AE that, even without detailed analysis, represents a serious unexpected AE that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- A series of AEs that, on analysis, is both unanticipated and a problem for the study. There would be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and were significant to the rights and welfare of participants.
- An AE that is described or addressed in the IB/package insert, protocol, or informed consent documents, or is expected to occur in study participants at an anticipated rate (e.g., expected progression of disease, occurrence of events consistent with background rate in participant population), but occurs at a greater frequency or at greater severity than expected.
- Any other AE that would cause the sponsor to modify the investigator brochure, study protocol, or informed consent form, or would prompt other action by the IRB to assure protection of human participants.

It will be the responsibility of the investigator to assure that the essential documents are available at the investigator site. Any or all of these documents may be subject to, and should be available for, audit by CCHMC or Sponsor auditor and inspection by the regulatory authorities as defined in the monitoring plan.

18.ETHICS

18.1 Ethics Review

The investigator will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable country and local-specific regulatory requirements prior to initiating the study.

18.2 Ethical Conduct of the Study

This study will be conducted in compliance with the ethical principles of the Declaration of Helsinki, and any additional national or IRB-required procedures.

The investigator is responsible for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential participants/parents or legal guardians (e.g. advertisements or information that supports or supplements the informed consent) are reviewed and approved by the IRB. The investigator agrees to allow the IRB direct access to all relevant documents. The IRB must be constituted in accordance with all applicable regulatory requirements. The investigator will provide the IRB with relevant document(s)/data that are needed for approval of the study.

18.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

19.DATA HANDLING AND RECORDKEEPING

19.1 Inspection of Records

Data generated by this study must be available for inspection by any regulatory authorities and the IRB as appropriate. At a participant's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Participant medical information obtained during the course of this study is confidential and disclosure to third parties other than those noted above is prohibited.

19.2 Retention of Records

The investigator shall retain records required to be maintained under this part for a period of 2 years.

20.PUBLICATION POLICY

The CEGIR policy on the publication of study results will apply to this trial.

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Schedule of Assessment

Phase	Pre	Treatment Phase 1					Optional Treatment Phase 2			
Visit Number	1	2	3	4	5	6	7	8	9	10
Visit Description	Screening	Enrollment/ Baseline	Phone Visit #1	Phone Visit #2	Phone Visit #3	EOT or Early Withdrawal	Phase 2 Visit 1	Phone Visit #4	Phone Visit #5	EOT or Early Withdrawal
Week	-12 to -1	0	1	3	5	6	10	11	15	16
Perform Informed Consent	X									
Assess Eligibility (inclusion/exclusion criteria)	X	X								
Obtain medical & dietary history	X									
Assess con-meds and/or con-therapies	X	X	X	X	X	X	X	X	X	X
Vitals (height, weight, BP, heart rate, respiratory rate, temperature)	X	X				X	X			X
Physical Exam	X					X				X
Research Biopsies (taken during SOC EGD)	X[a]					X				X[b]
Study Questionnaires		X				X				X
DSQ		X			X[e]		X		X[e]	
Dietary Questionnaires		X	X		X		X	X	X	
Skin testing for allergies (prick and patch)		X								
Research Lab Samples	EDP, other[c]	Serum food IgE, CRD, & IgG4; T cells				EDP, serum food IgE, CRD, & IgG4, T cells, other[c]				other[c]
Morning cortisol (serum)							X			X
CBC with differential	X					X				X
Stool sample	X[d]	X[d]				X				
Pregnancy test (urine)	X					X				X
Assess AEs			X	X	X	X	X	X	X	X
Provide food diary	X		X		X			X	X	
Provide instructions for diet or medication		X					X			
Determine compliance with treatment			X		X			X (diet)	X (diet)	X (SGC)
Dispense SGC							X			
Collect SGC										X
Collect compliance logs										X

[a] First SOC EGD used to determine eligibility may be conducted within 4 weeks prior to enrollment visit.

[b] This EOT SOC EGD pertains only to Phase 1 Non-Responders.

[c] Other refers to collection of blood or saliva for future genetic testing. These samples will only be collected once, but may be collected at any of the visits indicated.

[d] Stool samples may be collected at some participating centers from subjects who consent to stool collection. Stool may be obtained either at Screening/Baseline OR Enrollment, as well as at EOT Phase 1.

[e] The DSQ should be completed daily in the 2 weeks prior to each endoscopy.

Each visit window (for visits 2-10) is ±3 days.