PROTOCOL TITLE: Eosinophilic Esophagitis (EoE) Intervention Trial-Randomized 1 Food Elimination vs. 4 Food Elimination Diet followed by Swallowed Glucocorticoids

PROTOCOL NUMBER: 7805

STUDY DESIGN: Prospective, non-blinded clinical trial

AMENDMENT NUMBER: Not applicable

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Good Clinical Practice

This study was conducted under Good Clinical Practices, in accordance with the Declaration of Helsinki, in compliance with the International Conference on Harmonisation (ICH) guidelines.

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.

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1 ABBREVIATIONS AND DEFINITIONS

Abbreviation	Term
EoE	Eosinophilic Esophagitis
hpf	High Power Field
6FED	6-food elimination diet
4FED	4-food elimination diet
3FED	3-food elimination diet
1FED	1-food elimination diet
IRB	Institutional Review Board
CI	Confidence level
EOT	End of treatment
EGD	Esophagogastroduodenoscopy
GI	Gastroenterology
HSS	Histology Scoring System
PI	Principal Investigator
PHI	Protected health information
CCHMC	Cincinnati Children's Hospital Medical Center
GI	Gastrointestinal
PPI	Proton pump inhibitor
AE	Adverse Event
SAE	Serious Adverse Event

CHOP	Children's Hospital of Philadelphia
UNC	University of North Carolina
NW	Northwestern University
RHC	Riley Hospital for Children
UCSD	University of California at San Diego
EDP	Eosinophil Diagnostic Panel
PRO	Patient Related Outcomes

2 INTRODUCTION

The primary objective of this proposal is to conduct a prospective, non-blinded randomized trial comparing patient reported outcomes scores in novel empiric elimination dietary therapies in eosinophilic esophagitis (EoE) in order to assess the therapeutic viability of minimally restrictive empiric elimination diets as related to measures of patient's symptoms, quality of life, and pathology. Moreover, we aim to assess the response to topical swallowed steroids in those who were non-responders to empiric dietary therapy regimens used during the initial phases of this study. Participants aged 6 to 17 years with active EoE will be enrolled and the primary efficacy outcome will be the change in the standardized and validated Pediatric Eosinophilic Esophagitis Symptom Score v2 (PEESS)[1, 2] obtained following a 3 month randomized trial of one of two minimally restrictive 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 2 phases with each phase lasting 3 months. In the first phase, all participants will be randomized 1:1 to either 1 food elimination (milk elimination alone, 1FED) or 4 food elimination (milk, egg, wheat, and soy elimination, 4FED) therapeutic diet. At the end of this phase, an EGD will be performed to assess histologic remission, which represents one of the primary current tools used in evaluating EoE disease activity during clinical trials and daily EoE care. Attainment of remission (esophageal eosinophil counts <15 eosinophils/high powered field) after Phase I will result in study discontinuation and maintenance of the "successful" dietary therapy. Dietary therapy non-responders who were on 4FED during Phase I will receive topical swallowed steroids for 3 months while on an unrestricted diet (Phase 2) followed by EGD with esophageal biopsies. Participants receiving SGC will return to an unrestricted diet (i.e. stop 4FED) prior to initiating SGC therapy. Dietary therapy non-responders who were on 1FED during Phase 1 will proceed to 4FED for 3 months (Phase 2) followed by EGD with esophageal biopsies. Unlike previous studies, the primary efficacy endpoint reported will be the change in the PEESS score following each dietary regimen. A variety of secondary endpoints, including pre- and post-therapy peak eosinophil counts per hpf and percentages of patients that attain histologic remission (<15 eosinophils/hpf), along with detailed histologic and endoscopic scores, novel biomarkers, and patient related outcome (PRO) measures. 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 the first prospective dietary therapy trial using empiric elimination diets in pediatrics, and will be the first trial to assess these particular dietary

interventions prospectively, whilst monitoring dietary adherence and adequacy. This represents the first patient driven study of EoE, in which PROs are utilized as the primary outcome measure for the trial. Further, this study will contrast the current gold standard of EoE disease activity with the PROs. This will also be the first dietary study to determine whether diet failures can still be rescued with the use of topical steroids. 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.

3 BACKGROUND AND RATIONALE

3.1 Eosinophilic Esophagitis

EoE represents a complex clinical entity often requiring coordinated care within the fields of both allergy and gastroenterology (GI). 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 either the proximal or distal esophagus that is not mitigated by the use of proton pump inhibitor (PPI) therapy[3]. Symptomatically, patients with EoE often complain of dysphagia, vomiting, reflux, food aversion, and food impaction[3-5]. 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) [6-15]. 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 family frequently opt to initially implement dietary therapy. This study is designed in keeping with this preference; therefore one of two less-restrictive empiric dietary therapies will be implemented initially prior to the use of topical swallowed steroids in participants who do not respond to the 4FED 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 Previous Research On The Use Of Dietary Therapy In Eosinophilic Esophagitis

Eosinophilic esophagitis 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 in compliance, cost, and formula palatability have led to attempts

to find alternative dietary approaches[8, 15, 16]. 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[14, 15, 17]. 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[18]. In published reports where causative antigens have been identified, milk, wheat, eggs and soy have consistently been reported to be the top 4 offending food antigens, and these four foods are therefore the basis of our less restrictive diet regimens[14, 15, 19]. Strictly adhering to any of these elimination diets is challenging for most children and families, and so any dietary therapy which achieves a similar response while minimizing the number of foods eliminated would represent a major advance in therapy.

3.3 Population To Be Studied
Participants aged 6 to 17 years with active EoE are eligible for entry into the study.

3.4 Statement Of Compliance
This study will be conducted in compliance with the protocol, Good Clinical Practices (GCP), and applicable local regulatory and Institutional Review Board (IRB) requirements.

4 POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS

4.1 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. 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.

4.2 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 on 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.

4.3 Treatment With Topical Swallowed Steroids

In the short term (such as the Phase II three month period steroid study interval), the primary risk is the development of oral thrush, which is a known side effect of topical swallowed steroid use[11]. 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 II.

4.4 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, we obtain blood during the standard placement of intravenous lines, when possible. The amount of blood drawn will adhere to the institutional policy.

4.5 Skin Testing For Allergies

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.

4.6 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.

4.7 Study Survey

Each participant between the ages of 6 to 17 years old will be asked to complete several age appropriate surveys, including Patient Related Outcome 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 parent or legal guardian of participants will also be asked to complete questionnaires. 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 patient or parent-proxy reported outcomes or study questionnaires. However, some questions may be difficult to answer and may make the participants uncomfortable. The participants may also feel inconvenienced to complete the questionnaires. Participants may refuse to answer any questions asked on the survey. The participant questionnaires typically take approximately 15 to 20 minutes to complete. All participants will be given ample time to complete the survey and additional efforts will be made to have a complete survey of all questions, as this represents our primary outcome to be monitored. Please reference Table 1 for the guidelines on when the study questionnaires are completed.

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 company receiving the data and/or samples. All of these samples will be shared under a MTA, or other applicable agreement.

4.8 Privacy And Confidentiality

Although the study staff will do its best to adhere to strict privacy guidelines, the possible risk of a breach of confidentiality does exist. Every effort will be made to maintain the confidentiality of the participant's medical and research information. The Investigator will take all necessary measures to keep all of the participants' personal information private and confidential. Electronic study records will be protected with electronic safeguards (e.g., computer passwords, restricted access privileges). Paper-based private health information will be secured in locked cabinets. Access to study records will be limited only to research staff. For this study, the

research staff will collect and utilize data obtained from the participant's medical and research records and observations. This information, along with the study survey forms, will be used by the Investigator as a part of the study data. The participants will be assigned and identified by a participant study identification number. The link to the participants' study ID and their names and identifiers will be kept by the PI and/or the research staff in a secure location. Because the participant's protected health information (PHI) will be collected and used for the study, the research staff will obtain a signed HIPAA authorization which is located in the informed consent form from the participant and/or legal guardian. The research staff will perform informed consent and all study procedures in a private setting away from the public. In addition, the research staff will only collect the minimum amount of personal information about the participant necessary for the research study. Storage and management of electronic data will be centralized at the Data Management and Coordinating Center (DMCC) at the University of South Florida (USF) Health Informatics Institute (HII) in Tampa, Florida, which supports clinical research trials from around the world. The USF HII is also the DMCC for the RDCRN, which includes the newly formed Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR, NIH U54 Grant U54AI117804-01, PI Marc Rothenberg) that is designed to delve into the entire spectrum of eosinophilic gastrointestinal disorders. Dr. Jeffrey P. Krischer is the principal investigator responsible for the DMCC, and has supported not only research work from the NIH, but is also involved in several studies supported by PCORI.

This study will be a multi-site study in which CCHMC is the lead site. Each site will have access to the data collected at their site. Data from each site may be shared with the other sites. The data will be de-identified when possible, but PHI may also be disclosed to each study site if necessary for the research activities. The disclosure of PHI is outlined in the consent form. Each site will perform on-going quality checks to verify requirements of data collected and report outcomes to the CCHMC PI.

The Sponsor, PI, members of the study's research team, DMCC staff, CCHMC medical staff, CCHMC IRB and Office of Research Compliance and Regulatory Affairs may have access to the participant's medical and research records related to this study. The data from the study may be published: however, the participant will not be identified by name.

4.9 Standard of Care Procedures Associated with This Study

Esophagogastroduodenoscopy (EGD)

Endoscopy is a well-established procedure. Few patients have unexpected or serious complications. Since it is considered standard of care for patients with EoE to have an EGD at most every three months to monitor the disease when patients are trialing new therapies (foods or medications), the EGDs 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.

There may be unknown or unforeseen risks associated with study participation.

4.10 Potential Benefits

As stated above, standard therapies for eosinophilic esophagitis 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. Utilizing minimally restrictive dietary elimination regimens to control esophageal inflammation may permit earlier identification of a diet that controls symptoms and achieves remission compared with the standard strategy of starting with a more restrictive diet then reintroducing potentially causative antigens one-at-a time. In addition, by limiting the number of potential reintroductions, these less restrictive diets have the potential to limit the need for endoscopies and attendant sedation and thus reduce risk to patients and costs to the health care system. This study would have the additional benefit of explicitly assessing the response to the studied therapies in terms of their effect on symptoms and quality of life, which has generally not been the case to date.

There are no guaranteed benefits for any study participant. Potential benefits to the individual patient 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. In addition, the use of an effective less restrictive diet than the standard could potentially improve quality of life.

4.11 Risk/Benefit Analysis

This study poses minimal risk. The use of 6FED is currently a standard therapy used to treat EoE, with an expected histologic response rate of approximately 75%. The use of less restrictive diets may therefore increase the risk for treatment failure. This risk is mitigated by the short trial period and the fact that the patients will undergo follow-up endoscopy to assess their response, as well as telephone monitoring to make sure they are not experiencing excessive symptoms.

5 STUDY OBJECTIVES

The purpose of this study is to evaluate the following objectives:

5.1 Primary Objective

To perform a prospective, randomized, non-blinded trial that determines patient reported outcomes scores following 1FED (milk elimination alone) vs. 4FED (milk, egg, wheat, and soy avoidance) and their relative efficacy (Phase I).

- The primary endpoint for this study will be the change in the PEESS from baseline to post therapy assessed after the end of the 3 month Phase I.

5.2 Secondary Objectives

- To extend Phase I of this study with a prospective non-blinded trial that determines the rate of remission after treatment with 4FED (in participants failing 1FED in Phase I) or SGC (in participants failing 4FED in Phase I) (Phase II).
- To evaluate the effect of each therapy on histological remission (defined as a post therapy eosinophil count of <15 eosinophils/HPF) and 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 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 using the Peds Quality of Life Inventory Eosinophilic Esophagitis Module (PedsQL EoE) 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 component-resolved diagnostics (CRD) [serum IgE component testing] predict response to therapeutic intervention.
- To determine if skin testing, in the form of prick and patch testing, predicts response to therapeutic intervention.

6 INVESTIGATIONAL PLAN / OVERALL STUDY AND PLAN

6.1 Number Of Participants

We will be recruiting a total of 292 participants, approximately 50 participants between the ages of 6-7 years of age will be enrolled, approximately 120 participants between the ages of 8-12 years of age will be enrolled, and approximately120 participants between the ages of 13-17 years of age will be enrolled, to enter this study based

on the presence of active eosinophilic esophagitis and adherence to the inclusion and exclusion criteria. The DMCC at the University of South Florida Health Informatics Institute will serve as the central data management center, with CCHMC serving as the primary site and the central IRB. Additional sites include: Children's Hospital of Colorado, Riley Hospital for Children, Children's Hospital of Philadelphia, University of California at San Diego/Rady Children's Hospital, Northwestern University/Lurie Children's Hospital, Tufts Medical Center, Mt. Sinai Kravis Children's Hospital, Arkansas Children's Hospital, and the University of North Carolina.

6.2 Study Design

We intend to conduct a prospective non-blinded randomized trial that compares a 1-food elimination diet (1FED) versus a 4-food elimination diet (4FED) for three months (Phase I) in a 1:1 ratio, stratified by two age groups, 6-12 and 13-17 years of age. Subsequently, non-responders to 1FED dietary elimination therapy will be treated with 4FED and non-responders to 4FED will be treated with SGC (swallowed fluticasone at a dose of 880 mcg twice a day) for 3 months (Phase II). Empiric dietary therapy interventions are as follows: 1-food elimination diet (1FED)—avoidance of milk vs. 4-food elimination diet (4FED)—avoidance of milk, egg, soy, and wheat for three months. Milk elimination for both 1FED and 4FED includes all mammal milk (i.e. goat, sheep, and cow's milk must all be eliminated). The study design is shown schematically in Figure 2.

The instructions for dietary phases will be standardized across sites by providing consensus documents that contain diet information, produced by a research Registered Dietitian who is an expert in dietary elimination in EoE. Similarly, standardized instructions for using SGC will be provided, based on our prior studies. We have chosen to use fluticasone proprionate (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 clinical trial information will be entered into a databank on www.clinicaltrials.gov maintained by the U.S. National Library of Medicine (NLM) at the National Institutes of Health (NIH).

6.3 Screening Criteria

Participants will be recruited at participating sites from the GI and Allergy clinics, or other clinics in which patients with EoE are often seen. In addition, potential participants will be identified through the EGID database (CCHMC IRB studies #2008-0090 and 2008-0098). The research information will be entered into a databank on www.clinicaltrials.gov, maintained by the U.S National Library of Medicine (NLM) at the National Institutes of Health (NIH), and may be advertised on other websites (CCHMC web pages, such as the Cincinnati Center for

Eosinophilic Disorders website, the RDCRN webpage, etc.).
During screening (within twelve weeks of enrollment), a diagnosis of active EoE will be confirmed. Participants will be initially screened for the following:

1. Age between 6 and 17 years
2. An EGD with biopsies performed within twelve weeks prior to enrollment documenting active EoE (≥ 15 eosinophils/hpf)
3. PPI confirmed EoE. 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 6 - 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 proton pump inhibitors available, the dose and adherence will be confirmed adequate at the discretion of the principal investigator).

Participants who meet the screening entry criteria of EoE will be enrolled in the study. An EGD and biopsy results will be accepted as baseline if done within twelve weeks prior to the enrollment visit and there have been no changes in either dietary or pharmaceutical treatment during that pre-screening interval. If the participant is on a current dose of acid reflux therapy (e.g., PPIs or histamine H_2 receptor antagonists), the participant will continue his/her acid reflux therapy as long as the dose remains the same throughout the study. Screening exclusion criteria for patients entering the study include the presence of hypereosinophilic syndrome, parasitic infection, inflammatory bowel disease, Celiac disease, *Helicobacter pylori* infection, and patients who are currently on dietary therapy avoiding milk (i.e. on a 1FED) or milk, egg, soy, and wheat (i.e. on a 4FED). Ideally we would like to exclude patients with a history of diet and/or SGC therapy as knowing the results could bias selection of patients into the trial. However, in clinical practice most patients have already tried diet and/or steroid therapy by the time they are seen in EoE specialty clinics. In these situations, true compliance and efficacy of treatment are typically not clear. Narrowing the study to newly diagnosed patients is not practical. As such, we will exclude patients with known definitive response to diet and/or SGC, such as participation in prior clinical trials. In addition, patients cannot have been on topical swallowed steroids within the last 2 months or systemic steroids within the last 3 months prior to enrollment. Participants may continue any inhaled steroids needed for asthma control as appropriate. We have selected the 6-year lower age range to minimize concern about compliance with and safety of the SGC in Phase II. The 17-year old upper age range was selected to align with the age ranges supported by our instruments measuring quality of life and patient symptom scores.

All protocol-required study procedures, including efficacy and safety assessments are summarized in Table 1 in the Study Visits and Assessments Section.

6.4 Safety

Safety will be monitored by physical exam findings at the screening visit, at the visit on the day of the endoscopy at the end of Phase I (12 weeks), and again at the end of Phase II (week 25) for those who fail to respond to the dietary therapy. In addition, for those who receive SGC during Phase II, morning cortisol will be checked at the beginning and end of Phase II to monitor for adrenal suppression. No additional endoscopies beyond the standard of care will be required for participation in this study, and no more than 4 additional biopsies will be obtained for research during endoscopy. Blood draws will occur whenever possible with the start of the IV for endoscopy. In addition, adverse events will be monitored from when informed consent is signed, at each visit, and throughout the study.

The PI at each performance site will review all unexpected and study-related events and forward their assessments to the CCHMC PI and study sponsor for review. Finally, privacy and confidentiality will be maintained according to HIPAA guidelines. These and other risks are reviewed in the Informed Consent and approved by the central IRB.

6.5 Efficacy

6.5.1 AIM 1: To Determine The Efficacy Of A 1FED Vs. 4FED In EoE

6.5.1.1 Primary End-Point

The primary efficacy endpoint will be the change in PEESS v2 as measured prior to initiation of the study diet and at the end of Phases I & II[1, 20]. The PEESS v2, a validated PRO metric, measures clinical symptoms in EoE that broadly fall into four categories: dysphagia, gastrointestinal reflux disease, nausea/vomiting, and pain. We hypothesize that therapeutic interventions will improve clinical symptoms as measured by PRO metrics and 4FED will be more effective than a 1FED with this measurement. This is an important issue to resolve as only a few prior studies have shown the clinical efficacy of therapeutic interventions, and these have been mainly in adults. In fact, several studies have shown dissociation between histology (eosinophil levels) and clinical improvement[20, 21], but these studies have not used validated metrics for measuring symptoms. We will therefore employ validated EoE specific measurement tools (Pediatric EoE Symptom Score (PEESS Version 2.0) (used as the primary endpoint) and Pediatric EoE Quality of Life (Ped EoE QL)) metrics using parent reported outcomes by proxy for patients[1, 20, 22, 23] to measure the clinical efficacy of our therapeutic interventions and to correlate symptoms with histology. The measurement of pre/post diet symptoms using tools that have

undergone at least some validation in pediatric patients is a novel aspect of our study. Agreement about these measurement tools has significant bearing on the eventual FDA approval of therapeutic agents[24]

6.5.1.2 Secondary End-Point Analysis: Histology And Endoscopy

At the end of each phase, we will employ generic Health Related QOL metrics, such as PROMIS General Health Questionnaire and Peds-QL 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. 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 and patient study number. The local pathology review will be used to determine whether or not participants continue into Phase II of the study. Participants with ≥15 peak eosinophils/hpf who were on 4FED during Phase I will continue into Phase II to receive SGC for three months while on an unrestricted diet. Participants with ≥15 peak eosinophils/hpf who were on 1FED during Phase I will also continue into Phase II, but will maintain the 4FED therapeutic diet for 3 months. The Central Review Pathology Committee (CRPC), who will also be blind to the treatment group, 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 I (as determined by the local pathology review) will have completed the study and will resume follow-up care with their primary gastroenterologist or allergist. Peak eosinophil change will be measured and we will also aim to determine the percentage of patients who achieve remission, defined as both complete remission (≤1 peak eosinophils/HPF) and partial remission (<10 and <6 peak eosinophils/HPF) as reported[11]; this will allow us to compare with other studies that have used 6FED[16, 19, 25, 26]. 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[27]. 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 central review pathologists 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 that they previously reviewed at their microscope 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. Cappocelli, 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[28].

6.5.1.3 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 Eosinophil 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[29]. The EDP is typically performed on an extra

biopsy taken from the distal esophagus[29]. If our hypotheses are proven to be correct, this has 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 that the upregulated genes and down-regulated 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[29]. In this case, the EoE score will be calculated at baseline endoscopy and in the post-treatment biopsies to assess change with treatment.

6.5.2 AIM 2: Clinical Parameters & Biomarkers That Predict Therapeutic Response

6.5.2.1 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, 4FED, 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.

6.5.2.2 Skin Testing

In this sub-aim, we hypothesize that skin test results (prick testing and atopy patch testing to the four food groups) 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[30]. 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.

6.5.2.3 Component Testing

Increased levels of food-specific IgE are present in patients with EoE yet their predictive value in guiding food

elimination diets for disease treatment is limited[31]. Recently, it has been proposed that the inability of food specific IgE tests to predict clinical reactivity may related in part to the use of allergen extract reagents that do not contain the full set of relevant allergens. This diagnostic limitation has spurred the development of molecular diagnostic tests, referred to as component-resolved diagnostics (CRD), which utilize purified native or recombinant allergens to detect IgE sensitivity to individual allergen molecules; this also has the advantage as interactions between IgE sensitization and clinical reactivity can be examined, such as food-pollen cross-reactivity[32-35]. One of the goals of the present study is to examine the potential value of CRD in predicting diet outcomes in EoE. To address this goal, we will subject a baseline serum sample to CRD ImmunoCap (ThermoFischer Scientific); includes 3 egg components (Gal d 1 [ovomucoid], Gal d 2 [ovalbumin], and Gal d 3 [ovotransferrin]), and 4 milk components (Bos d 4 [alpha lactoglobulin], Bos d5 [beta lactoglobulin], Bos d8 [casein] and Bos d lactoferrin) as well as other foods and pollens when indicated. We will initially focus on the correlation between the presence of positive milk component (>0.1 kUA/L) and responsiveness in the group doing 1FED (milk elimination diet) because (a) this will provide proof-of-principle for other foods; (b) milk is the most common food that shows positive IgE in EoE patients[36]; and (c) all patients in this group will undergo sole milk elimination and the results should be definitive concerning whether milk is indeed driving disease induction. With the wider range of CRD data, we will perform principal component analysis (PCA) to dissect the key allergens that are contributing to most of the variation. Similar to the way we process the EDP dataset, we also plan to derive personal heat-diagrams and present the CRD data in reviewable format by dimensionality reduction, such as multi-dimensional scaling (MDS) analysis. These approaches are expected to prioritize the key drivers in the component results and multiple statistical analyses can be subsequently investigated. For cost savings, serum samples from all cohorts will be analyzed in batches so we can determine if any trends are present before we analyze all samples.

6.5.2.4 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[37].

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 1, $p < 0.05$, 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[38]. 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.

6.6 FIGURE 1: Esophageal Transcriptome Analysis

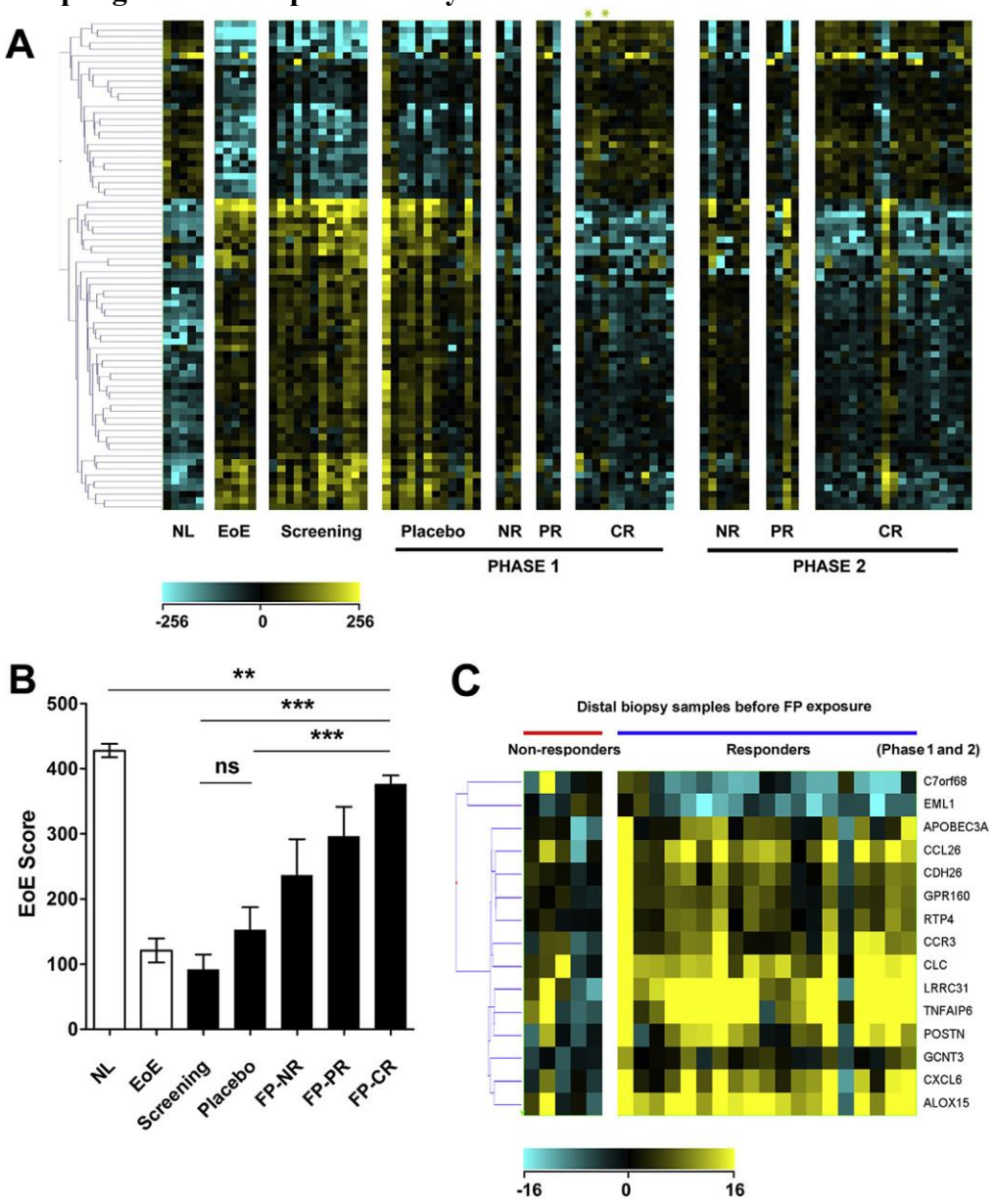

Figure 1. Esophageal Transcriptome Analysis: Total RNA from separately acquired biopsy specimens was subjected to EDP signature analysis. (A) The heat diagram depicts the changes after FP exposure in the bidirectional gene dysregulation in each group with reference historical EoE and normal (NL) cohorts (n = 15 and 14, respectively), and the samples at screening (no FP exposure) on the *left*. At the end of phases 1 and 2, expression signatures from FP non-responders (NRs), FP responders with partial remission (PRs), and FP responders with complete remission (CRs) are shown in the heat diagram (yellow, increased expression; blue, decreased expression). (B) On the basis of the expression levels of the 77 diagnostic genes and a dimensionality reduction algorithm, an EoE score reflecting the EoE disease activity was calculated at the end of phase 1 (**$P < .001$, ***$P < .0001$, 2-tailed Student t-test). (C) To assess the prediction value of the EDP on FP responsiveness, samples at screening (i.e., pre-FP exposure) and the placebo samples at the end of phase 1 (i.e., pre-FP exposure) were screened by bioinformatics in the context of their subsequent FP responsiveness on the basis of the CR criteria of fewer than 2 eosinophils/HPF in the distal esophagus, where the biopsy specimens were acquired. The 15-gene cluster listed shows a potential for FP efficacy prediction with a P value of less than .05 and fold change greater than 2.0.

6.7 End Of Therapy (EOT) And Follow-Up

The primary efficacy endpoint (e.g. PEESS v2 scoring) will be determined at the end of Phase I & II (weeks 12 & 25) at which time all participants will undergo EGD with biopsy. Histologic scoring of biopsy samples obtained during the end of Phase I endoscopy, will be used to determine the treatment pathway to be followed.

Participants who achieve histologic remission at the end of Phase I will have completed the study and will resume follow-up care with their primary GI and/or Allergy physician(s). Those whose disease remains active in terms of histologic disease activity (eosinophil count ≥15 per HPF) will go on to Phase II (twelve week treatment with 4FED for Phase I 1FED non-responders **OR** SGC for Phase I 4FED non-responders). This was modified from the original protocol on the basis of discussion with the PAGs, and their strong interest in potentially pursuing a more extensive dietary restriction as a means of achieving symptomatic and histologic improvement in disease activity. The study design is shown schematically in Figure 2 below.

6.8 FIGURE 2: STUDY DESIGN FLOWCHART

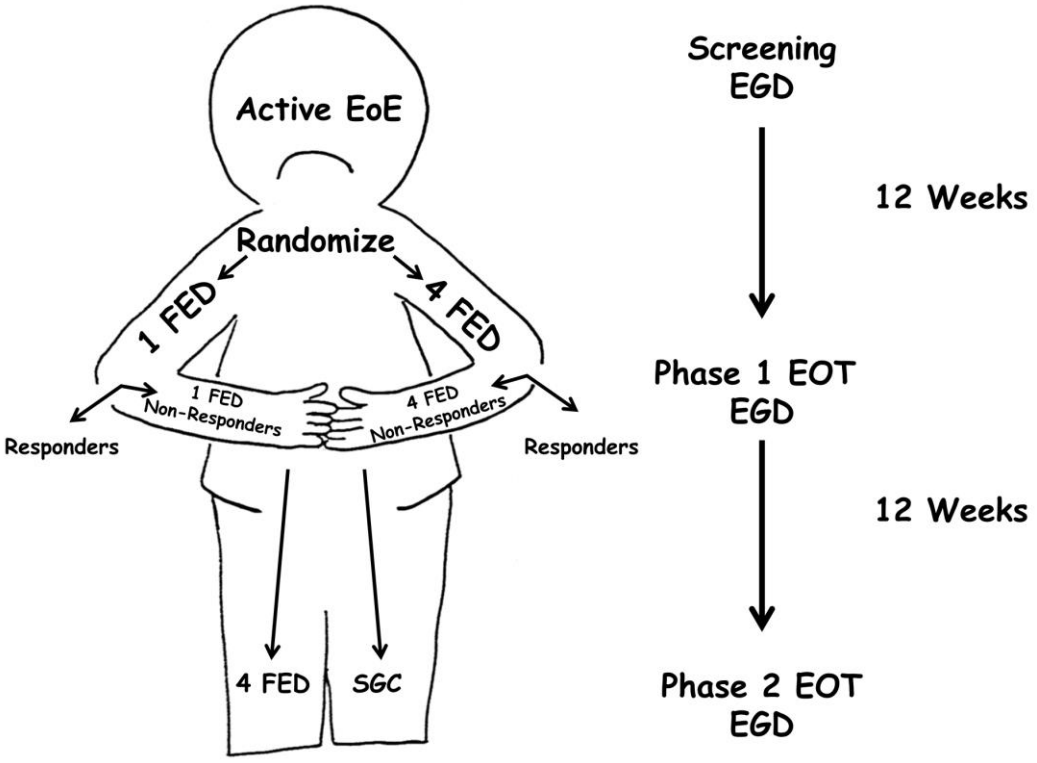

Figure 2. Study Design Flowchart: Diagram demonstrating the flow of subjects through the study. Following completion of the screening process and EGD #1, subjects undergo randomization into one of the two dietary arms of Phase I. Those subjects who respond histologically following either of the initial dietary therapies will exit the study, and the PEESS v2 will be used to score for the presence of symptomatic relief as the primary outcome. Those subjects, who histologically fail dietary therapy, will enter Phase II. Phase I 1FED non-responders will go onto 4FED in Phase II. Phase 1 4FED non-responders will be treated with swallowed glucocorticoids. Upon completion of Phase II, EGD #3 would be performed and scoring of symptoms with PEESS, and histologic scoring would be performed as seen for those subjects who responded at the end of Phase I.

6.9 Minimization Of Bias And Method Of Treatment Assignment

All eligible participants will be randomized 1:1 to either the 1FED or 4FED. Randomization will be stratified by parameters as determined by our statistician. Following this 3 month phase I, all patients will undergo EGD with biopsy and all those whose disease remains active will move on to Phase II. The sponsor, investigator, select study staff, and participant will all have knowledge of the treatment being received.

6.10 Expected Duration Of Study And Participant Participation

Each participant will participate in the study for a maximum of 36 weeks. This includes a maximum twelve week screening period, followed by 2 sequential 12-week treatment periods. At the end of each 12-week treatment phase there will be a clinic visit and EGD to assess treatment response. Participants whose disease goes into remission will complete the trial at the end of Phase I, and the remainder will move on to the next treatment phase with more extensive dietary restriction or SGC. Participants will have an initial screening visit, followed by clinic visit and EGD at week 12, and (if disease remains active at week 12), a visit to initiate Phase II at around week 13, followed by another clinic visit and EGD 12 weeks later at approximately week 25. It is estimated that the duration of the entire study—including enrollment, performance of study procedures, data collection, analysis, and report writing—will last approximately 2-3 years.

7 STUDY VISITS AND ASSESSMENTS

7.1 TABLE 1. STUDY DESIGN AND SCHEDULE OF ASSESSMENTS

Phase	Pre-treatment	Treatment Phase 1					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	2	4	11	12	13	14	24	25
Perform Informed Consent	X									
Assess Eligibility (inclusion/exclusion criteria)	X	X								
Obtain medical & dietary history	X									
Assess con-meds and/or con-therapies	X									
Vitals (height, w eight, 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
Dietary Questionnaires		X	X		X		X	X	X	
Skin testing for allergies (prick and patch)		X								
Research Lab Samples	EDP, other[c]	Component Testing				EDP, component testing, other[c]				EDP, other[c]
Morning cortisol (serum)							X			X
CBC w ith differential	X					X				
Pregnancy test (urine)	X					X				X
Assess AEs			X	X	X	X	X	X	X	X
Assess diet and/or con-med compliance		X	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 w ith treatment			X		X			X	X	X (SGC)
Dispense SGC							X			

[a] First SOC EGD used to determine eligibility may be conducted within 12 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

Each visit window (for visits 2-10) is ±7 days.

7.2 Screening (Weeks -12 To -1)

Participants will be screened as above in Table 1. The screening visit should occur at a maximum of twelve weeks prior to the enrollment visit. The research staff member will perform the informed consent process after obtaining informed consent, the following study procedures should be performed and documented up to twelve

weeks prior to the enrollment (Week 0) visit:

- 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
- Obtain CBC with differential
- 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 or saliva) for future genetic testing may be collected at this visit
- Assessment of concomitant medications and therapies
- Provide food diary
- Pregnancy Test (urine)

Esophageal biopsies for research may be taken during a participant's standard of care EGD (SOC EGD). If the participant has had a SOC EGD and biopsies performed at a participating site or at an outside institution prior to the screening visit (and consent), the results of the EGD may be used to determine eligibility if it falls within the twelve-week window prior to enrollment. In these cases, research biopsies and EREFS evaluation will not be required at baseline. 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. These participants will have all other screening procedures completed at the in-office, screening visit.

CBC results will be obtained from a participant's standard of care lab. The amount of blood drawn (clinical plus research) will adhere to institutional policy limits.

7.3 Phase I: Enrollment & Dietary Treatment (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. If a participant is screened and enrolled within a 5 day time frame study procedures that are repeated will only be performed once. At this visit, staff members will:

- Assess eligibility (inclusion and exclusion criteria)
- Measure vitals (blood pressure, heart rate, respiratory rate, temperature), height and weight
- Administer patient and parent-reported outcome questionnaires, which will include a dietary questionnaire to measure intake of the food allergens
- Review food diary (from screening) with subject
- Assess concomitant medications and/or therapies
- Serum collection for component-resolved diagnostics (CRD) [serum IgE component testing], IgG4
- Skin testing for allergies including prick (milk, egg, wheat and soy) and patch testing (milk only).
- Randomize patients to the 1FED or 4FED diet
- Provide standardized dietary instructions for 1FED or 4FED

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.

7.4 Phone Visits, Phase I

A study staff member will conduct three phone visits (at weeks 2, 4, and 11) during Phase I 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 2 and 11 for therapy compliance assessment
    - A food diary will be completed by participants during weeks 2 and 11 to measure adherence

7.5 End Of Treatment Phase I Or Early Withdrawal (Week 12)

For participants who have completed the 12 week Phase I 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 study clinician or qualified staff member
- Administer patient and parent-reported outcome questionnaires
- Assess AEs
- Obtain up to 4 biopsies from the distal and proximal esophagus for research purposes (taken during standard of care EGD)Research lab samples:
    - Esophageal biopsy for EDP
    - Serum (~2 tsp, or 10 mL) for serum IgE/CRD/IgG4
    - Blood or saliva for future genetic testing may be collected at this visit
- Obtain CBC with differential (SOC)
- Assess participant compliance with diet and concomitant medications
- Pregnancy Test (urine)

If a participant withdraws from the study before completing 12 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 will complete the study at this time while those with continued active inflammation will move on to the next phase, which consists of either 4FED (for Phase I 1FED non-responders) or swallowed fluticasone therapy at 880 mcg twice a day while back on an unrestricted diet (for Phase I 4FED non-responders).

7.6 Phase II Visit 1 (Week 13)

Those participants who have completed the Phase I diet protocol but have continued active inflammation will return to clinic once their biopsies have been read, within 1 week 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 those moving on to SGC
- Provide standardized instructions for 4FED to participants who will be on 4FED
- Provide diet or medication diary/log to participants

- Administer dietary questionnaire to all participants to measure *intake* of the food allergens. Review food diary (from Visit #5) for those who will be on 4FED
- Dispense the fluticasone to the participant for those moving on to SGC
- Measure morning cortisol (serum, ~1 tsp or 5 mL) for those moving on to SGC

7.7 Phone Visits, Phase II

A study staff member will conduct two phone visits (at weeks 14 and 24) during Phase II 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 all participants during week 14 and 24. The questionnaires will be used for therapy compliance assessment for those on 4FED.
    - A food diary will be completed by participants who are on 4FED during weeks 14 and 24 of the trial to measure adherence

7.8 End Of Treatment Phase II Or Early Withdrawal (Week 25)

For those participants who have completed the Phase II 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 and parent-reported outcome questionnaires
- Assess AEs
- Obtain up to 4 biopsies from the distal and proximal esophagus for research purposes (taken during SOC EGD)
- Assess participant compliance with concomitant medications/therapies
- Assess participant compliance with therapy (SGC)
- Pregnancy Test (urine)
- Measure morning cortisol (serum, ~1 tsp, or 5 mL) for those treated with SGC
- Research lab samples

- Esophageal biopsy for EDP
- Blood or saliva for future genetic testing may be collected at this visit

If a participant withdraws from the study before completing this 12 weeks of SGC or 4FED therapy, 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 4FED until results of biopsies are communicated to them.
Following the receipt of their biopsy results, the patients will return to standard clinical care.

7.9 Pregnancy
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.

7.10 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.

8 SELECTION AND WITHDRAWAL OF PARTICIPANTS

8.1 Participant Inclusion Criteria
At study enrollment, subjects will be eligible if they:

- Have diagnosis of EoE (based on consensus criteria)[3]
- Are aged 6 to 17 years
- Have histologically confirmed active disease $\geq$15 eosinophils/hpf in either distal or proximal esophagus within 12 weeks of enrollment visit
- PPI confirmation

- Symptomatic (have experienced symptoms within the last month prior to enrollment)
- Has 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). Subsequently, 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.

8.2 Participant Exclusion Criteria

Subjects will be excluded if they:

- Have been treated with topical swallowed steroids within the last 2 months or systemic steroids within the past 3 months
- Have eosinophilia in segments of the GI tract other than the esophagus
- Have been diagnosed with a GI malabsorption disorder (i.e., Inflammatory bowel disease, Crohn's disease) or Celiac disease
- Are currently on dietary therapy avoiding milk (i.e. on a 1FED), or milk, egg, soy, and wheat (i.e. on a 4FED)
- Have concurrent H pylori gastritis or parasitic infection
- Are unable to obtain EGD with esophageal biopsies at CCHMC or other participating institution within 4 weeks of study completion
- Have previously failed (in a clinical trial setting) dietary therapy with one of these regimens or topical steroid treatment with fluticasone at a total dose of 1760 mcg per day.
- Have definitely responded (in a clinical trial setting) to either dietary therapy avoiding these antigens or to swallowed fluticasone at a total dose of 1760 mcg per day
- Are concurrently receiving any of the prohibited medications listed in Table 2
- On immunotherapy for pollen (if not on maintenance therapy) or IgE-mediated food allergy.
- Not fluent in English

9 PARTICIPANT WITHDRAWAL CRITERIA

An intent to treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct

a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, large pleural effusion).
- Participants will be discontinued from the study if they are unable to comply with the dietary restrictions or develop severe complications from their EoE (e.g., esophageal strictures).

9.1 Replacement Of Participants

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 12 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.

9.2 Study Site Termination Criteria

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 or DSMB decision to terminate or suspend approval for the investigation or the investigator.

The DSMB will promptly inform the Principal Investigator and Sponsor conducting the study if the study is suspended or terminated for safety reasons, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. The investigator must inform the IRB promptly and provide the reason for the suspension or termination.

9.3 Study Discontinuation

The sponsor and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- *Early stopping rules have been met*
- *Accrual has been met*
- *The study objectives have been met*
- *The Study Chair / Study Investigators believe it is not safe for the study to continue*
- *The sponsor suspends or closes the trial*
- *The FDA suspends or closes the trial*

10 TREATMENT OF PARTICIPANTS AND DESCRIPTION OF STUDY INTERVENTION

We intend to conduct a 2-phase randomized prospective, non-blinded trial (each phase is 3 months in duration) to assess changes in patient reported outcomes in pediatric patients with EoE that utilizes one of two minimally restrictive dietary therapy regimens followed by topical swallowed steroid therapy (for 4FED non-responders), which is intended to provide data that will inform clinical management practices.

Empiric dietary therapy interventions for Phase I will consist of either a 1-food elimination (milk elimination alone) or a 4-food elimination (milk, egg, soy, and wheat) diet. Phase I 1FED non-responders will receive 4FED in Phase II, whereas Phase I 4FED therapy non-responders will receive topical swallowed steroid therapy (swallowed fluticasone at a dose of 880 mcg twice a day) for 3 months in Phase II.

10.1 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.

10.2 TABLE 2. Prohibited Medications And Washout Time

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

10.3Treatment Compliance

A participant will be considered compliant if he/she has remained adherent to the study dietary recommendations. Adherence to the fluticasone phase will be based on receiving at least 80% of prescribed doses. All non-compliance issues will be documented.

10.4Study Intervention Administration-Diet And Medication Instruction

All participants will receive standardized written dietary instructions with regards to the study dietary interventions. In addition, study dietitians will be available to answer questions from participants and their families as they arise. Similarly, all patients entering Phase II who will take SGC will receive standardized instruction on fluticasone administration.

10.5Efficacy Evaluation

We will calculate and compare efficacy for each dietary therapy (1FED or 4FED) as well as for fluticasone therapy for dietary non-responders.

10.6Primary Efficacy Endpoint:

- Pediatric EoE Symptom Score (PEESS, version 2). The PEESS total score change from pre-treatment to post-treatment is the primary efficacy endpoint.

10.7Secondary Efficacy Endpoints:

- Patient Related Outcome (PRO) metrics pre- and post-treatment
  - PedsQL EoE Module
  - PROMIS General Health Questionnaire and PedsQL
  - Individual domain PEESS scores
- Histology and endoscopy measures
  - Percent of participants who achieve histologic remission (<15 peak eosinophils/HPF) post therapy assessed after the end of the 3 months
  - Division of patients into complete (peak eosinophils≤1/HPF) or partial (≤6 or ≤10) remission
  - Histology Scoring system
  - Endoscopic scoring system (EREFS)
- Biomarkers pre- and post-treatment
  - Assessment of Eosinophil Diagnostic Panel EoE Score
- Clinical parameters and biomarkers to predict responsiveness to diet and SGC therapy
  - Correlation of clinical characteristics to response to therapy
  - Assessment of predictive value of skin prick and patch allergy skin testing
  - Correlation between presence of positive milk component allergy testing to response to 1FED
  - Predictive biomarker-analysis of EoE transcriptome from EDP analysis on baseline biopsies in correlation to response to treatment.

11 ASSESSMENT OF SAFETY

11.1 Safety Parameters

The Study Chair has primary oversight responsibility of this clinical trial and the Data Safety Monitoring Plan (DSMP) for this clinical trial. The Study Chair will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months.

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 II. Each site's Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate reports - detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed twice a year by the research team. A separate report detailing protocol compliance will also be available from the DMCC for site review on a

monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

11.1.1 Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) in conjunction with the Sponsor will develop and adopt a Charter which will delineate:

- Monitoring the safety of study participants by reviewing the incidence of clinically significant AEs;
- Monitoring compliance with the study protocol, especially as it relates to participant safety and the integrity of the study;
- Study stopping criteria as they relate to study drug-related AEs;
- Reporting the committee's deliberations to the Sponsor following scheduled and *ad hoc* meetings;

The DSMB members will review and approve the DSMB charter which defines the membership of the board, frequency of meetings, and roles and responsibilities.
The DSMB will meet:

- Twice during the conduction of the study (in person or via conference call), at a minimum; and
- On an *ad hoc* basis for safety concerns, per the DSMB Chair

The DSMB will be provided access to the safety data including AEs, study withdrawals, and SAEs. The DSMB may also review data on study progress including participant enrollment and disposition, especially as related to discontinuation at study site.

11.2Adverse Events

An adverse event is defined as any adverse experience (an unfavorable and unintended sign, symptom or disease) —including side effect, injury, toxicity, sensitivity reaction, intercurrent illness, or sudden death— whether or not it is considered related to the use of the study agent, that occurs any time after the participant signs informed consent/assent (Screening or Enrollment study visit) and will end after the cessation of the study agent (Week 29 [30 days after the last dose of the study agent]). This includes the onset of new illness and the exacerbation of pre-existing conditions other than the indication under study (i.e., EoE). A pre-existing condition is one that is present at the start of the study and is reported as part of the participant's medical history. A pre-existing condition should be reported as an AE only if its frequency, intensity or character worsens during study treatment. Participants (parents or legal guardians) should be instructed to report all AEs

to the investigator. All AEs must be appropriately documented in the participant's original source documents and on the CRF. Investigators should report syndromes rather than list symptoms. All AEs will be assessed for severity and relationship to the study agent. Expected EoE symptoms, including vomiting, regurgitation, abdominal pain, chest pain/heartburn, or dysphagia are not to be considered as AEs, except if they show a clear temporal relationship to the study agent administration or result in hospitalization. Hospitalization is to be reported as an SAE.

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol.

Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

11.3TABLE 3. Grading Of Adverse Events
The severity of each AE will be categorized using the following criteria:

Mild (Grade 1)	Awareness of sign, symptom or event, but easily tolerated; requires no special treatment and does not interfere with the participant's daily activities.
Moderate (Grade 2)	Discomfort enough to cause interference with usual activity and may warrant intervention.
Severe (Grade 3)	Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.
Life-threatening (Grade 4)	Places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, (i.e. it does not include a reaction that, had it occurred in a more severe form, might have caused death).

Death (Grade 5)	Death related to AE.

11.4TABLE 4. Relationship To Study Treatment

The investigator must assess the relationship of any AE to the use of the study agent, based on the available information, using the following guidelines:

Definitely not related	The event is definitely not associated with the study agent administration, and is judged clearly due to causes other than the study agent.
Probably not related	An event that follows a temporal sequence from administration of the study agent such that a relationship is not likely, and could be reasonably explained by the participant's clinical state or other modes of therapy administered to the participant .
Possibly related	An event that follows a reasonable temporal sequence from administration of the study agent, but that may be due to another cause and could also be reasonably explained by the participant's clinical state or other modes of therapy administered to the participant.
Probably related	An event that follows a reasonable temporal sequence from administration of the study agent, that is not easily explained by another cause such as known characteristics of the participant's clinical state or other treatment, and is confirmed by improvement on stopping or slowing administration of the study agent (de-challenge), if applicable.
Definitely related	

In the event of death, the cause of death should be recorded as the AE. "Death" is an outcome and is NOT the AE. The only exception is "sudden death" when the cause is unknown.

11.5Serious Adverse Event

The investigator is required to determine if each AE is a SAE. An SAE is any AE occurring between signing informed consent/assent and within 30 days after administration of the last dose of the study agent, that results in any of the following outcomes
(International Conference on Harmonization [ICH] Guide (E6) for GCP, 1996):

- Death;
- A life-threatening adverse drug experience;
- Inpatient hospitalization or prolongation of existing hospitalization, or
- A persistent or significant disability/incapacity;
- Congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be

considered SAEs when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. Reports of all SAEs will be communicated to the appropriate IRB and reported in accordance with local laws and regulations.

11.6Recording Adverse Events

All AEs will be recorded in the participant's source documents and CRFs. The onset and end dates, severity, and relationship to the study agent will be recorded for each AE. Any action or outcome (e.g., hospitalization, discontinuation of the study agent, etc.) will be recorded for each AE.

11.7Reporting Adverse Events

Participants (or parents or legal guardians) will be questioned and/or examined by the investigator or designee for evidence of AEs. The questioning of participants (or parents or legal guardians) with regard to the possible occurrence of AEs should be generalized such as: "How have you (or how has your child) been feeling since your last visit?"

Additional sources for AEs include, but are not limited to: direct observation, voluntary, spontaneous comments from the participant (parent or legal guardian), and clinically significant laboratory, physical examination, vital sign findings, or other test results. The investigator will exercise his/her medical and scientific judgment in deciding whether an abnormal clinical laboratory finding or abnormal assessment is clinically significant.
The presence or absence of specific AEs will not be solicited. Participants who experience AEs will be monitored with relevant clinical assessments and clinical laboratory tests as determined by the investigator. All AEs will be followed to satisfactory resolution or stabilization. Any actions taken and follow-up results will be recorded on the appropriate CRFs, as well as in the participant's source documentation. Follow-up laboratory results will be filed with the participant's source documentation. Non-serious AEs that occur from the time of consent/assent until 30 days after administration of the last dose of study agent will be reported. Any non-serious AEs will be considered as "continuing" if not resolved at this time. SAEs that occur from the time of informed consent/assent until 30 days after administration of the last dose of study agent will be reported. All SAEs should be followed until resolution or stabilization. For the purpose of reporting AEs, reporting will begin after informed consent/assent and will end after the cessation of the study agent [30 days after the last dose of

study agent]). Occasionally, AEs that occur during a study are unresolved at the time the participant's study participation ends. AEs are to be followed to resolution; until they resolve, disappear, or become stable up to 30 days. Note that any events which occur prior to the start of study agent are considered as medical history and should be recorded as such on the medical history screen of the CRF.

SAEs will be reported to regulatory authorities per 21 CFR 312.32 and institutional policy. Adverse events will be reported, in summary form, at the time of continuing annual review to the IRB, FDA, and DSMB.

11.8 Reporting Serious Adverse Events To Sponsor

Within 24 hours (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:

- Is considered life-threatening/disabling or results in death of subject
  -OR-
- Is Unexpected/Unanticipated

Investigators must report all other reportable SAEs within 5 working days (of learning of the event). All other (suspected) reportable AEs must be reported to the RDCRN within 20 working days of the notification of the event or of the site becoming aware of the event. Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

The research personnel should document the SAE on the source documents and CRFs.
The SAE information will include the following (as available):

- Participant identification number, site, investigator name; date site was notified of event
- SAE information: event term, onset date, event SOC, severity, local of event treatment, and casual relationship to study agent;
- Basic demographic information (e.g., age, sex, weight);
- The outcome(s) attributable to the event (death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant

disability/incapacity, congenital anomaly/birth defect, or other important medical event);

- A summary of relevant test results, pertinent clinical laboratory data, and any other relevant medical history;
- The first and last dates of the study agent administration;
- A statement whether the study agent was discontinued or the study agent administration was modified;
- A statement whether or not the SAE recurred after reintroduction of the study agent if administration had been discontinued or withheld;
- Supplemental information may include the following hospital records: clinical laboratory results, radiology reports, progress notes, admission and emergency room notes, holding/observation notes, discharge summaries, autopsy reports, and death certificate.

The SAE should be reported to the Sponsor within the expected reporting period with as much of the above information as available at that time. All initial and follow-up SAEs should be reported to the Sponsor via the DMCC AE reporting system. For the purpose of reporting SAEs, reporting will begin after informed consent/assent is signed and will end after the cessation of the study agent (Week 29 [30 days after the last dose of study agent]). Occasionally, SAEs that occur during a study are unresolved at the time the participant's study participation ends. SAEs are to be followed to resolution; until they resolve, disappear, or become stable. It is important for the investigator to realize that SAEs may occur in a study participant after the study follow-up period is complete, but which still merit reporting to the sponsor. The investigator together with the sponsor will try to determine if the SAE is related to the study agent.

11.9 DMCC Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event, 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 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 on a 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 STATISTICAL METHODS

12.1 Sample Size Calculation

Sample size was determined based on the primary endpoint of the trial: The overall PEESS score change from pre-treatment. Based on a preliminary report[18] we estimate that 40-50% of patients may respond to 1FED as our study is prospective compared with 65% response in this preliminary retrospective report and based on food reintroduction trial results[30]. Likewise, we would expect 75% of patients to respond to 6FED based on the previously reported studies, and from this we extrapolated a projected response of 65% to 4FED, given that the 4 eliminated foods are consistently identified as the most common triggering antigens[30]. As such, we estimate that 45% of patients will respond to 1FED and wish to detect an additional 20% increase for the 4FED (i.e. 65% response rate). A non-parametric Wilcoxon rank sum test will be applied for the primary efficacy endpoint comparison. We are assuming a difference in response rate of 45% vs 65% between the two treatment groups, and we further assume that those who respond to 1FED will naturally respond to 4FED. We also assume that those who responded will have a higher PEESS score change than those who do not respond. Then the estimated probability of 4FED scores higher than 1FED is about 0.60 (= 0.5 x prob. of response to both or neither + 1.0 x incremental response rate from 1FED to 4FED). This will require 131 patients per group to reach 80% power at 0.05 test level. By accounting for a 10% early dropout rate, we propose a sample size of 146 per group, or 292 total patients to be enrolled.

12.2Statistical Analyses: Aim 1

Demographic and patient characteristics 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, including the primary and secondary analyses.

There will be two sets of analysis populations in terms of efficacy assessment, the intent-to-treat and per protocol. All patients randomized to Phase I of the study who have at least one clinical observation post randomization will be considered in intent-to-treat set. Patients who are at least 80% compliant to dietary assignment during Phase I, and who do not have any major protocol violations will be considered in per protocol set. If there is more than 10% difference in patient numbers between these two populations, the primary efficacy analysis will be performed on both intent-to-treat analysis and per protocol populations. Otherwise, all analyses will be based on intent-to-treat population. Patients who do drop out of the trial before the end of Phase I will have their PEESS done at the time of dropout.

The primary endpoint is overall PEESS score change from pre-treatment to the end of Phase I. If normality assumption is approximately valid, a linear mixed effect model will be applied to the primary efficacy variable, account for site random effect, and other potentially important covariates, e.g. gender, race, and PPI use etc. Pertaining to the specific aim, the comparison of response (PEESS change from baseline) will be made between 1FED and 4FED groups via the above model. Because of non-normal nature of the scores observed in previous research, a Wilcoxon rank sum test may be applied to compare between two treatment groups when normality is violated.

As a secondary endpoint analysis, remission rates (as defined by the percentage of participants achieving histologic remission (eosinophils less than 15/HPF) at the end of Phase I will be compared between the two treatment groups using the generalized linear mixed effects model with the logit link (for binary outcome) that accounts for the clustering within sites. Important covariates will be analyzed in this model including gender, race, PPI use, atopy, age, and anthropomorphic features. Treatment group comparisons for secondary endpoints will be performed using the generalized linear mixed effects model using the link that is appropriate for the endpoint (i.e. logit for binary, log for Poisson count, identity for continuous). These models will account for the

clustering within sites and will include the same covariates as for the primary analysis. The remission rate for non-responders who proceed to Phase II will be summarized separately depending on the treatment patients received during Phase I and Phase II. The 95% confidence interval for the rates will be provided.

12.3 Statistical Analyses: Aim 2

To evaluate the ability of biomarkers and phenotypic characteristics to predict response to therapeutic intervention, multivariable logistic regression analysis will be conducted with histological evaluation of 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 I. It will be repeated at the end of Phase II by including those patients who also responded to SGC.

A sample size of 292 total patients will provide at least 80% power to detect an AUROC of 0.60. This calculation is based on the assumption that 161 patients (66 1FED, 95 4FED) will be in remission at the end of Phase I (and therefore not move on to phase II) and 131 patients will not be in remission (with an estimated 10% dropping out and not continuing on to Phase II).

Additionally, a correlation analysis between PEESS scores and biomarkers will be performed. Spearman's correlation coefficient and its confidence intervals will be computed to further assess predictive utility for the biomarkers of interest.

12.4 Direct Access To Source Date/Documents

Data generated by this study must be available for inspection by any regulatory authorities, by the Sponsor, DSMB, and the IRB as appropriate.

13 INVESTIGATOR REQUIREMENTS

13.1 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.

13.2 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.

13.3Source 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 (parent or legal guardian, as appropriate).

The patient and parent-reported outcome questionnaires will be recorded directly on the paper forms and will be considered as source data.

13.4Study 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).

13.5Audits 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.

13.6Institutional 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.
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.

14 ETHICS

14.1 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.

14.2 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.

14.3 Written Informed Consent And Assent

This study will be conducted in compliance with ICH E6 GCP: Consolidated Guidelines pertaining to informed consent and assent. At the first visit, prior to initiation of any study-related procedures, participants (parents or legal guardians, as appropriate) 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, in accordance with institutional requirements.

15 DATA HANDLING AND RECORDKEEPING

15.1 Inspection Of Records

Data generated by this study must be available for inspection by any regulatory authorities, and by the IRB as appropriate. At a participant's/parent's or legal guardian's request, medical information may be given to his or her (child's) personal physician or other appropriate medical personnel responsible for his or her (child's) 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.

15.2 Retention Of Records

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

15.3 Data Management

All study data will be collected via systems created in collaboration with the Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

15.4 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.

15.5 Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

15.6Data 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 DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The DMCC will monitor data delinquency on an ongoing basis.

15.7 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. On-line 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.

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