# Budesonide Versus Fluticasone for Treatment of Eosinophilic Esophagitis

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# *BUDESONIDE VERSUS FLUTICASONE FOR TREATMENT OF EOSINOPHILIC ESOPHAGITIS*

**Principal Investigator:**
Evan S Dellon, MD, MPH
Associate Professor of Medicine
University of North Carolina School of Medicine
Chapel Hill, NC

**Co-Investigators:**
John A Baron, MD
Professor of Medicine
University of North Carolina School of Medicine
Chapel Hill, NC

Joseph A Galanko, PhD
Research Assistant Professor of Medicine
University of North Carolina School of Medicine
Chapel Hill, NC

Nicholas J Shaheen, MD, MPH
Professor of Medicine and Epidemiology
Director, Center of Esophageal Diseases and Swallowing
University of North Carolina School of Medicine
Chapel Hill, NC

John T Woosley, MD
Professor of Pathology and Lab Medicine
University of North Carolina School of Medicine
Chapel Hill, NC

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**Initial version:** June 21, 2016
**Version 2.0:** March 16, 2017
**Version 3.0:** March 14, 2019

## Changes from Previous Version

This version includes the following changes to the previous version of the protocol, Version 2.0 (16Mar2017).

| | |
|---|---|
| Sections 3.2, 3.3, 3.4, 7.1 | Modified study endpoints to reference outcomes, and clarified primary, secondary, and exploratory outcomes. |
| Entire document | Administrative changes to protocol version number and date |

# Table of Contents

## Protocol Signature Page

I have read and agree to the protocol entitled '*BUDESONIDE VERSUS FLUTICASONE FOR TREATMENT OF EOSINOPHILIC ESOPHAGITIS*' dated 14 March 2019. I am aware of my responsibilities as an Investigator under the guidelines of GCP, local regulations (as applicable) and the trial protocol. I agree to conduct the trial according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the trial.

**Principal Investigator:**

Evan Dellon
Print Name

Professor of Medicine
Title


Signature

3/20/19
Date

# List of Abbreviations

| Item | Definition |
|---|---|
| AUC | Area Under the Curve |
| CEDAS | Center for Esophageal Diseases and Swallowing |
| EC | Ethics Committee |
| eCRF | Electronic Case Report Form |
| EGD | Esophagogastroduodenoscopy |
| EoE | Eosinophilic Esophagitis |
| EoG | Eosinophilic Gastroenteritis |
| Eos | Eosinophils |
| EREFS Score | EoE Endoscopic Reference Score[104] |
| FFPE | Formalin-fixed paraffin embedded |
| H&E | Hematoxylin & Eosin |
| HPF | High Power Field |
| IDS | Investigational Drug Service (at UNC) |
| IRB | Institutional Review Board |
| MDI | Multi-dose Inhaler |
| NEB | Nebulized and then Swallowed |
| OVB | Oral Viscous Budesonide |
| PPI | Proton-Pump Inhibitor |

# Study Summary

| | |
|---|---|
| Title | Budesonide versus Fluticasone for Treatment of Eosinophilic Esophagitis |
| Short Title | TREET trial (**TR**eatment of **E**o**E** with **T**opical steroids) |
| Methodology | Prospective, randomized, double-blind, double-dummy, single center clinical trial comparing oral viscous budesonide (OVB) to fluticasone multi-dose inhaler (MDI) for treatment of eosinophilic esophagitis (EoE). |
| Study Duration | 3 years |
| Study Center(s) | University of North Carolina, Chapel Hill, NC |
| Objectives | *Primary objective:* To determine whether viscous budesonide is more effective than fluticasone MDI for improving esophageal eosinophil counts and symptoms of dysphagia in patients with EoE after an initial treatment course.<br><br>*Secondary objective*: To determine whether treatment with viscous budesonide results in less symptomatic and histologic recurrence than fluticasone MDI one year after the initial treatment course. |
| Number of Subjects | 200 |
| Diagnosis and Main Inclusion Criteria | *Inclusion Criteria*:<br>1) Age 16-80 years old<br>2) New diagnosis of EoE as per consensus guidelines. Cases must have symptoms of dysphagia, persistent esophageal eosinophilia (≥ 15 eosinophils in at least one high-power field) after 8 weeks of treatment with a twice daily proton-pump inhibitor, and other competing causes of esophageal eosinophilia excluded<br>*Exclusion Criteria*:<br>1) Medical instability that precludes safely performing upper endoscopy<br>2) Ongoing or recent symptoms of intestinal bleeding (throwing up blood, passing blood in the stool)<br>3) Concomitant eosinophilic gastroenteritis (EoG)<br>4) Esophageal narrowing or stricturing that will not allow a standard 9 mm upper endoscopy scope to pass<br>5) Cancer in the esophagus, stomach, or intestine<br>6) Prior surgery on the esophagus (e.g., removal of part of the esophagus)<br>7) Esophageal varices (dilated blood vessels in the esophagus)<br>8) Current use of blood thinners like Plavix or Coumadin that are not stopped prior to endoscopy procedures<br>9) Corticosteroid exposure within the four weeks prior to the baseline endoscopy. Exclusionary corticosteroid exposure is defined as any swallowed topical steroids for EoE or systemic steroids for any condition within the four weeks prior to the baseline endoscopy. Corticosteroids used for asthma or intranasal corticosteroids are not an exclusion and are allowable.<br>10) Pregnancy<br>11) Inability to read or speak English |
| Study Product, Dose, Route, Regimen | Oral Viscous Budesonide (OVB), 1 mg swallowed twice daily<br>Fluticasone Multi-Dose Inhaler (MDI), 4 puffs (880mcg) swallowed twice daily |
| Duration of administration | 8 weeks |

| | |
|---|---|
| Reference therapy | Reference therapies are a placebo viscous slurry and a placebo MDI. All patients will receive either active OVB/placebo MDI, or placebo slurry/active MDI, in double blind fashion. |
| Statistical Methodology | To test whether OVB is more effective than fluticasone MDI for improving eosinophil counts, the mean post-treatment maximum eosinophil count will be compared between the OVB and MDI groups using a two-sample t-test. To test whether OVB results in less symptomatic recurrence than fluticasone MDI, survival analysis will be performed with the interval between treatment end (week 8) and recurrent symptoms or study end (week 60) as the time of interest. Symptoms will be measured with the Dysphagia Symptom Questionnaire, a validated instrument in EoE. |

# 1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

## *1.1 Background*

***Eosinophilic esophagitis is a previously rare condition with a rapidly increasing incidence***

Eosinophilic esophagitis (EoE) is an immune-mediated clinicopathologic entity whereby abnormal infiltration of eosinophils into the esophageal mucosa leads to dysphagia, progressive esophageal stenosis, and food impaction.[1-3] First described in 1978[4] and initially felt to be rare,[5] estimates in multiple populations, including our center, show that incidence has increased more than four-fold in the last five to ten years (Figure 1).[6-9] Because EoE is chronic, the prevalence is also increasing.[6, 7, 10, 11] Overall, between 5% and 16% of patients undergoing endoscopy for dysphagia will have EoE,[12-15] and more than 50% of patients presenting to an emergency room with food impaction are now diagnosed with EoE.[3, 16] Because of this dramatic change in epidemiology and the increasing burden of disease attributable to EoE, the NIDDK-sponsored National Commission on Digestive Diseases has made research in EoE a priority.[17]

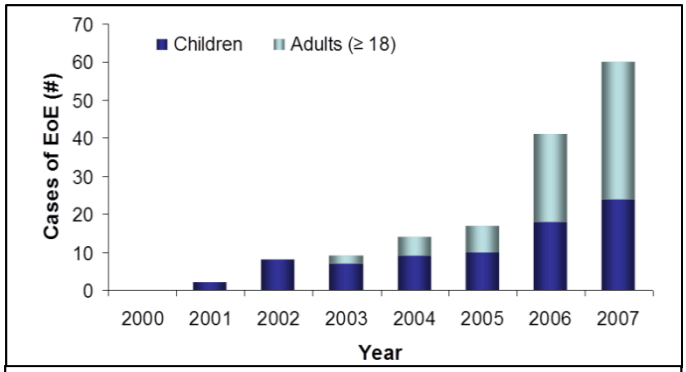



**Figure 1:** Rapid increase in new cases of EoE at UNC in both adults and children. This increase persists when normalized for endoscopy and biopsy volume, and mirrors national and international trends

***EoE is diagnosed by clinical and pathologic criteria***

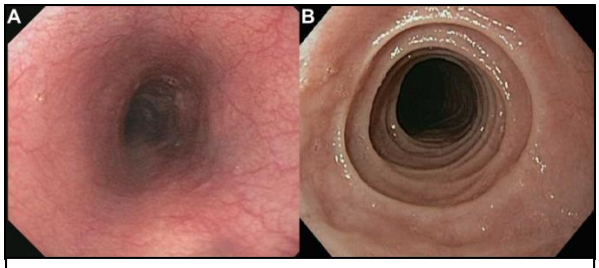



**Figure 2:** Endoscopic views of **(A)** the normal esophagus and **(B)** EoE with rings and narrowing.

While dysphagia is the clinical hallmark and most common symptom of EoE in adolescents and adults,[1, 18, 19] other symptoms can include heartburn, reflux, and chest pain.[2, 20, 21] When suggestive symptoms are present, upper endoscopy with biopsy is required to make the diagnosis.[2] Endoscopic signs of EoE can include esophageal rings, strictures, luminal narrowing, linear furrows, white plaques or exudates, and a loss of vascularity (Figure 2).[1, 18, 22, 23] On esophageal biopsy, demonstration of epithelial eosinophilia is required for diagnosis, and the current accepted threshold level is 15 eosinophils per high-power field (eos/hpf).[2] Because the symptoms and signs of EoE can be non-specific, diagnostic criteria have been published and recently updated (Table 1).[2] The guidelines require the presence of esophageal eosinophilia in the correct clinical setting. They also require exclusion of other conditions that may cause esophageal eosinophilia before a diagnosis of EoE can be formally confirmed.

**Table 1: Consensus diagnostic criteria for EoE[2]**

- Clinical symptoms of esophageal dysfunction
- ≥ 15 eosinophils in at least one high-power field
- Eosinophilia limited to the esophagus with other causes of esophageal eosinophilia excluded

***The treatment approach to EoE is rudimentary, but corticosteroids are the mainstay of therapy for EoE***

Despite the fact that EoE has become a major cause of upper gastrointestinal morbidity in both children and adults, the approach to treatment of EoE is rudimentary and evidence to guide practice is sorely needed (Table 2). Because data suggest that a Th2-mediated response to allergic pathogens governs esophageal eosinophil infiltration,[24] corticosteroids are currently the first-line pharmaceutical treatment option for patients with EoE.[2, 25] However, because no medications in this class have been specifically formulated for EoE, patients are prescribed asthma preparations such as fluticasone in a multi-dose inhaler (MDI) or aqueous budesonide. Patients are asked to swallow, rather than inhale, these medications to coat the esophagus and provide a topical anti-inflammatory effect. For fluticasone MDI, patients puff the medication into their mouth during a breath hold and then swallow it.[26, 27] For aqueous budesonide, patients mix the liquid into a slurry with a sugar substitute such as sucralose and then swallow it; this has been termed "oral viscous budesonide," or OVB.[28, 29] Another technique is to swallow the aerosolized droplets of aqueous budesonide after it has been nebulized.[30] These approaches were first described in observational studies,[28, 31-37] and several small randomized clinical trials have now shown that these treatment strategies can be effective for decreasing levels of esophageal eosinophilia and improving symptoms related to EoE.[26, 29, 30, 38, 39] However, the dose ranges, length of treatments, method of drug delivery, assessment of symptoms, and patient inclusion criteria vary widely between these studies,[25] so studies cannot be directly compared to draw conclusions about efficacy.

**Table 2: Major unanswered questions regarding EoE treatment**

1) What topical corticosteroid agent is the most effective?
   Importance: Doctors do not know what medication to use first.
2) After an initial treatment course, what is the durability of response?
   Importance: Patients cannot be properly informed about treatment outcomes and symptom recurrence.
3) Which patients are most likely to respond to topical corticosteroids?
   Importance: Patients who might not respond are subjected to unnecessary steroid exposure and provision of non-steroid therapies is delayed.

***The most effective topical steroid to use as a first line agent in EoE is unknown***

A major gap in the current knowledge regarding EoE treatment is that it is unknown which topical steroid is most effective. Because of this, practitioners do not know what medication to use first and cannot provide accurate information to patients about treatment outcomes. In a study of patterns of practice related to EoE performed by our group, the vast majority of gastroenterologists who prescribed a topical steroid chose fluticasone MDI as their initial steroid agent.[40] However, this choice is not supported by comparative effectiveness data, and it is unknown whether a different medication such as OVB might be more effective. This question has substantial practical implications. Topical steroids are not universally effective, with 13% to 50% of subjects failing to respond depending on the study and outcome measure.[26, 29, 30, 41] It is possible that poor treatment responses are due to difficulties administering the medication or to sub-optimal formulations. For example, fluticasone MDI is designed for pulmonary deposition and may be inefficiently delivered to the esophagus, whereas the entire dose of OVB is delivered to the esophagus.[42] Natural history data show that prolonged symptom duration in EoE is associated with increased risk of esophageal strictures,[43] so there is a strong rationale to treat with the most effective agent available at the time of diagnosis. However, providers need efficacy data to select the best medication to treat patients with EoE.

***The durability of the treatment response to topical steroid treatment for EoE is unknown***

Another major gap in knowledge is that the durability of treatment response in EoE is unknown. The few data available regarding durability of response are inconclusive. In a retrospective trial of adults treated with an initial two-week course of fluticasone MDI, 29 of 32 patients reported recurrent dysphagia at a

mean of 9 months.[44] In a prospective trial, subjects who previously responded to a two-week course of nebulized then swallowed budesonide were randomized to low dose budesonide or to placebo.[45] After 50 weeks of treatment, all 14 patients in the placebo arm had recurrent esophageal eosinophilia, and the median time to symptom relapse was 95 days. However, in this study the initial treatment course (2 weeks) was shorter than the current accepted standard of 8 weeks, so the recurrence rates might have been higher. Providers need high quality data from larger studies to accurately inform patients when recurrent symptoms and esophageal eosinophilia can be expected after an initial treatment course. A better understanding of durability of response will also inform decisions regarding which patients with EoE might need long-term therapy.[46]

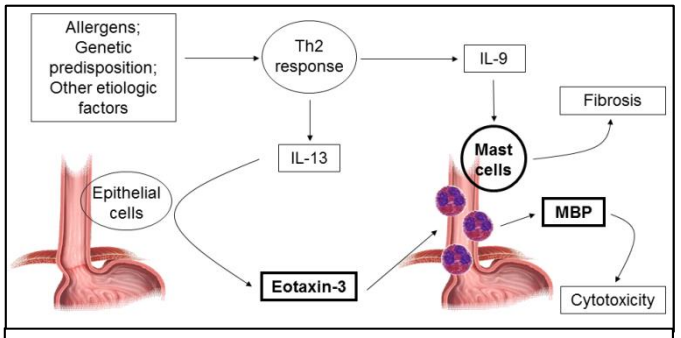



**Figure 3:** Proposed pathogenic pathway for EoE. Allergens or other yet to be determined etiologic factors, in the proper genetic milieu, stimulate a Th2 response leading to IL-13 production. IL-13 stimulates the esophageal epithelial cells to produce eotaxin-3, which in turn acts as a chemoattractant and activates eosinophils. Eosinophils release MBP, a cytotoxic granule that can injure the epithelium. The Th2 response also produces IL-9 which recruits mast cells. Mast cells are involved in promoting fibrosis, which leads to esophageal strictures.

***Predictors of response to steroid therapy are unknown, but candidate tissue biomarkers can be identified from the postulated pathogenesis of EoE***

A third key issue in the treatment of EoE is that predictors of response to topical steroid treatment have not been studied. Of the four published small randomized trials in EoE comparing topical steroids to placebo,[26, 27, 29, 30] only one attempted to assess predictors.[30] Of 10 clinical and histologic factors examined in that study, none were predictive, but there were only 36 patients in the trial so the null result could be due to type II error. Identification of predictors of treatment response is important not only to target therapy to those most likely to respond, but to minimize unnecessary steroid exposure and institute effective non-steroid alternatives such as dietary elimination in those least likely to respond.[2] Candidate biomarkers for prediction of treatment response can be selected based on the pathophysiology of EoE (Figure 3). The presence of eosinophils in the esophageal mucosa is abnormal,[47,48] and both murine and human data suggest a Th2-mediated response to allergen sensitization governs esophageal eosinophil infiltration and activation.[34, 49-56] In particular, IL-13 stimulates esophageal epithelial cells to produce eotaxin-3, a potent chemokine which is increased 50-fold in patients with EoE compared to controls.[56-60] Once activated, eosinophils degranulate and release factors such as major basic protein (MBP), which can disrupt epithelium and is increased in patients with EoE.[61-63] The Th2 response also results in mast cells infiltrating the esophagus, likely via IL-9.[64] In addition to increased numbers of mast cells in patients with EoE, mast cell-associated genes are upregulated in EoE, and mast cells are an important mediator of the esophageal remodelling and fibrosis that causes esophageal strictures in EoE.[54, 56, 57, 65-69] In addition, patients who successfully respond to topical steroid therapy have resolution of esophageal eosinophilia (and therefore a decrease in MBP release), normalization of eotaxin-3 gene expression, and resolution of esophageal mastocytosis.[26, 30, 60] While MBP, eotaxin 3, and mast cells are candidate markers of treatment response in EoE, they have yet to be studied for this application.

## *1.2 Investigational Agents*

Oral viscous budesonide (OVB) is a swallowed, or topical, steroid slurry. We will formulate this to be equivalent to what is used clinically: 1 mg/4 mL aqueous budesonide mixed with 10 g of sucralose.[28, 29, 42] Rather than asking the subjects to mix the slurry on their own and risk inconsistent formulations, a weakness of prior studies, the UNC investigational drug service (IDS) will provide pre-mixed OVB to all patients. For the purposes of compounding the medication to be dispensed as a one-month supply, the constituent elements of aqueous budesonide are combined in bulk with sucralose to yield the necessary concentration and consistency. The dose for OVB has been chosen because it is the most commonly studied dose, including our prior study, so we can accurately estimate response rates.[28, 29, 39, 42] Subjects randomized to this arm will also be instructed to use a placebo inhaler identical to the fluticasone MDI, with instructions to swallow 4 puffs twice daily.

Fluticasone MDI is also topical steroid. Subjects will swallow at a dose of 880 mcg twice daily (4 puffs of a 220 mcg inhaler twice daily). The dose for fluticasone MDI has been chosen because this is the most commonly used dose in adolescents and adults with EoE, so effect estimates are also available.[2, 25, 27, 101] Subjects randomized to this arm will also be instructed to take 4 mL twice daily of a placebo slurry of sucralose identical in consistency and taste to the OVB.

## *1.3 Preclinical Data*

**Preliminary data and study feasibility**

The number of EoE cases seen at UNC continues to increase, providing an expanding source population for the proposed study. Through the UNC Center for Esophageal Diseases and Swallowing (CEDAS), one of the largest esophageal referral centers in the country, and two UNC GI procedure units, the PI has a rich patient population to draw from for research. Over the past 7 years, there has been a trend towards an increasing number of EoE cases, including more than 65 new (incident) cases of EoE annually for the past 3 years (Table 3). In addition, the number of cases in patients 16 years and older, the study population for this trial represents the majority of these cases, and is similarly increasing. The PI tracks EoE cases in an EoE Patient Registry and an EoE Clinicopathologic Database. This latter resource now has complete clinical and histologic information on more than 400 patients with EoE.

**Table 3: Incident cases of EoE at UNC**

| Year | Total # of new EoE cases | New EoE cases in ages ≥16 years |
|---|---|---|
| 2006 | 41 | 26 |
| 2007 | 60 | 39 |
| 2008 | 56 | 33 |
| 2009 | 54 | 30 |
| 2010 | 68 | 36 |
| 2011 | 70 | 43 |
| 2012 | 88 | 52 |

In addition, the PI has recently completed a prospective study of the prevalence of esophageal eosinophilia and EoE in the UNC GI procedure units. Of 173 patients undergoing endoscopy for dysphagia, [65] (38%) had esophageal eosinophilia with ≥ 15 eos/hpf, and 40 (23%) were confirmed to have a new diagnosis of EoE after a PPI trial as per consensus diagnostic guidelines (Table 1).[98] This is the highest prevalence of EoE yet to be reported in patients undergoing endoscopy for dysphagia.[13-15] These statistics document how commonly EoE is seen at our center, given that in 2012 more than 1000 upper endoscopies were done for patients with dysphagia between our two procedure units. Taking these data together, it is clear that our unit can support a steady recruitment of newly diagnosed EoE patients into a clinical trial.

Enrollment rates in multiple prior prospective studies of EoE at UNC have been excellent, indicating that EoE patients seen by the PI and his care team are highly willing to participate in clinical studies. In the PI's prospective study of eosinophil inflammation and activation funded by an UNC institutional NIH KL2 award (KL2 RR025746), a total of 276 patients were approached for screening and 223 (81%) agreed to participate. In the PI's prospective study of risk factors and biomarkers for diagnosis of EoE funded by an NIH K23 award (K23 DK90073), a total of 183 subjects have been screened to date with only 8 (5%) refusals. For both of these studies, patients undergoing outpatient upper endoscopy were approached for the first time prior to the procedure, and were remarkably willing to participate. For patients newly diagnosed with EoE, rates are even better. In the randomized study of OVB vs nebulized/swallowed budesonide that we conducted (see below), a total of 34 incident EoE cases were screened and 9 did not meet eligibility requirements. Of the remaining 25 cases, none refused participation. Similarly, we are a lead site for an industry-sponsored randomized trial of budesonide syrup vs placebo for treatment of adolescents and young adults with EoE. To date we have approached 14 patients with EoE for participation; only one patient refused to be enrolled. These high recruitment rates demonstrate the

feasibility and high likelihood of enrolling EoE patients into the proposed trial at UNC. To further confirm this, we performed a feasibility assessment for the proposed study. A total of 20 patients who met this study's inclusion criteria were approached and 19 (95%) indicated that they would be willing to be randomized.

The PI has helped to standardize methodology for determining esophageal eosinophil counts, the primary histologic outcome used in clinical trials of EoE. Because esophageal eosinophilia is required for diagnosis of EoE and is central to the pathophysiology of the condition, the eosinophil count is the most commonly used outcome for EoE treatment trials. In early work, the PI identified a number of shortcomings in the way in which eosinophil counts had been determined, including inconsistent methodology to quantify cells and variable definitions of microscope high-power fields.[21] To address these issues, in collaboration with the study pathologist (Dr. Woosley), a protocol using digitized histology slides to quantify eosinophil counts was developed and validated. This method has been documented to have excellent intra- and inter-observer reliability for determining cell counts (see appendix and Aim 1 methods, below),[82] and will form the basis of the histologic analysis used for the proposed study.

The PI has participated in a multicenter collaboration to develop and validate a dysphagia symptom score in adults and adolescents with EoE which will be used as a primary symptom outcome in EoE studies. While a number of clinical trials of topical steroids in EoE have attempted to assess symptoms as an outcome, none have used a validated symptom score.[26, 29, 30, 38, 39, 42] Recently, the PI has participated in a multicenter study that developed and validated the Dysphagia Symptom Questionnaire in adolescents and adults with EoE, the first such measure that is available.[99] This is a daily symptom diary with three questions that assess the frequency and severity of dysphagia. The one and two week composite scores strongly correlate with the frequency of dysphagia, distinguish patients on topical steroids from those not treated (by showing that untreated patients had more dysphagia), and strongly correlate with a dysphagia measure that has been responsive in a previous trial of EoE.[30] This instrument will form the basis of the symptom analysis used for the proposed study.

## *1.4 Clinical Data to Date*

The PI has conducted the only randomized clinical trial comparing two formulations of topical steroids, demonstrating that OVB is superior to nebulized/swallowed budesonide for improving esophageal eosinophilia. In this study, a viscous slurry of budesonide (OVB) was compared with budesonide that was nebulized and then swallowed (NEB).[42] This was the first study to compare two topical steroid formulations, and also the first to use OVB in an adult population. These two formulations were chosen because OVB has been shown to be effective in children and NEB has been shown to be effective in adults.[29, 30] In addition, because we were assessing medication deposition in the esophagus, these formulations were amenable to being tagged with a radiotracer.

A total of 25 patients were randomized, 13 to NEB and 12 to OVB, and 11 were analyzed in each group. Even with this small sample size, the results were dramatic. First, the post-treatment esophageal eosinophil counts were significantly lower in the OVB group compared with NEB (Figure 4). Second, this response was largely explained by the difference in esophageal medication contact time (Figure 5). Specifically, the OVB group had a significantly higher esophageal medication contact time (as measured by the area under the esophageal emptying curve) compared with the NEB group. Responders in both groups had increased esophageal medication contact time compared to non-responders.

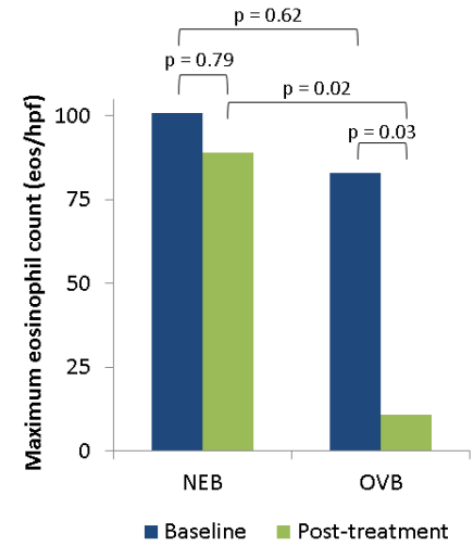



**Figure 4:** Histologic response in EoE with NEB vs OVB. Baseline eosinophil counts (blue bars) are similar between OVB and NEB. After treatment (green bars), there is near normalization of the eosinophil count in OVB, but no change in NEB.

These data strongly implicate the medication formulation as a key variable in the response of EoE to topical steroids, and provide support for our hypothesis that OVB will be more effective than fluticasone MDI, which is analogous to NEB in that it is a medication formulation that is optimized for pulmonary deposition. We have selected fluticasone MDI as a comparator for OVB rather that NEB because fluticasone MDI is the most commonly used topical corticosteroid in EoE[40] and because patients in our previous trial study found NEB to be a cumbersome delivery method. In fact, of the 11 patients randomized to NEB, all but one opted to switch to OVB when given the choice at study end. Overall, data from this study provide strong justification for moving forward with the proposed study and show that the assembled research team has already successfully completed a randomized trial of topical steroid use in EoE, and therefore will also be able to complete the proposed study.

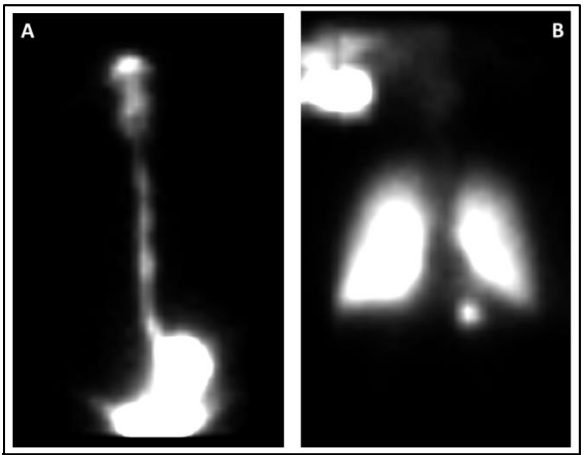



**Figure 5:** Representative nuclear scintigraphic studies showing cumulative esophageal deposition of OVB **(A)** and NEB **(B)** over 10 minutes after dosing. OVB coats the esophagus and enters the stomach. NEB has pulmonary uptake with poor esophageal deposition.

The PI has demonstrated that staining esophageal biopsies for MBP, eotaxin-3, and mast cell tryptase is technically feasible and has substantial diagnostic utility for EoE. We have conducted two case-control studies of the utility of using immunohistochemistry (IHC) to stain esophageal biopsies for biomarkers to diagnose EoE. In the first, tryptase was used to stain mast cells, and this stain had an excellent utility for distinguishing EoE and GERD patients (area under the receiver operator characteristic curve (AUC) = 0.84).[69] In the second, both MBP and eotaxin-3 were assessed, and the combination of these two stains had outstanding utility for diagnosing EoE (AUC = 0.96).[62] These published data show not only that we have technical expertise in staining for and quantifying these biomarkers (Figure 6) but that these stains have clinical utility for diagnosis of EoE.

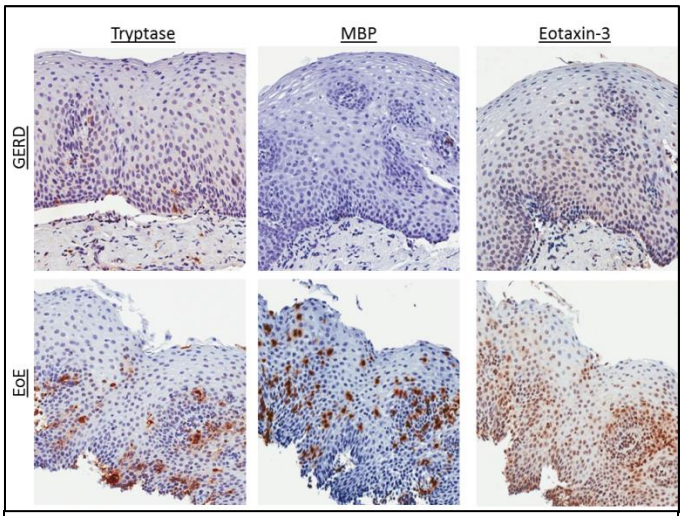



**Figure 6:** IHC staining for tryptase, MBP, and eotaxin-3 in a representative GERD and EoE patient. In all cases, increased staining is seen in EoE.

Preliminary data suggest that increased staining of eotaxin-3 and tryptase, but not MBP, in esophageal biopsies at baseline prior to treatment with topical steroids is associated with treatment response. While no published studies have examined MBP, eotaxin-3, and tryptase staining at baseline to predict outcomes of topical steroid therapy, we have generated preliminary unpublished data that suggests that these markers do, in fact, have promise for this. Using stored samples in the UNC EoE Registry and Biobank, we identified patients who did (n = 20) and did not (n = 20) have a complete histologic response (defined as < 5 eos/hpf) to topical steroid therapy. We stained the baseline (pre-steroid treatment) biopsies for MBP, eotaxin- 3, and mast cell tryptase. The results showed that EoE patients who responded to topical steroid therapy had

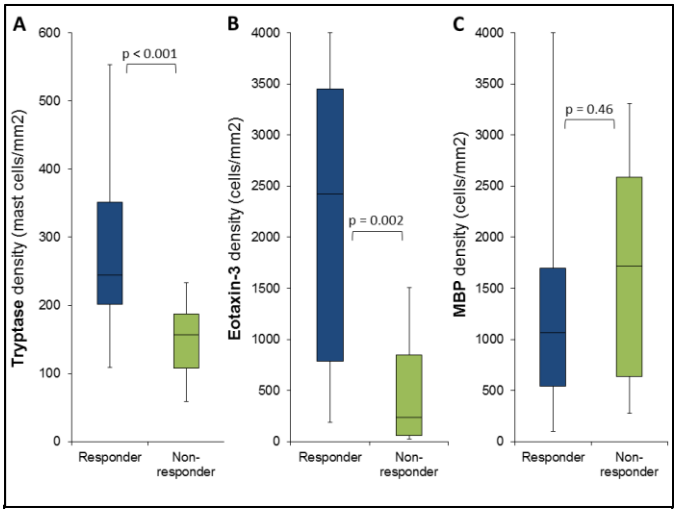



**Figure 7:** EoE patients who responded to topical steroids (blue bars) had higher levels of staining for tryptase **(A)** and eotaxin-3 **(B)** then non-responders (green bars), but this was not the case for MBP **(C)**

significantly higher levels of staining for eotaxin-3 and tryptase than did patients who did not respond (Figure 7). This was not the case with MBP. While the underlying mechanism for this finding is not known, we hypothesize that patients with a more inflammatory milieu in the esophagus as evidenced by increased eotaxin-3 levels and the presence of mast cells, are more steroid responsive than those who do not have this milieu. Because MBP levels correlate closely with eosinophil levels,[62] and because it is thought that the baseline severity of esophageal eosinophilia itself does not predict treatment response,[2] it is not surprising that MBP was not associated with treatment response. However, we plan to include MBP in our panel of biomarkers to confirm our finding and to provide a negative control stain. These preliminary data provide justification for including Aim 3 in the proposed study.

## *1.5 Dose Rationale and Risk/Benefits*

OVB dose of 1 mg twice daily is a slurry equivalent to what is used clinically: 1mg/4mL aqueous budesonide mixed with 10 g of sucralose. The dose for OVB has been chosen because it is the most commonly studied dose, including our prior study, so we can accurately estimate response rates. [28,29,39,42]

Fluticasone MDI dose of 880 mcg twice daily (4 puffs of a 220 mcg inhaler twice daily) has been chosen because this is the most commonly used dose in adolescents and adults with EoE, so effect estimates are also available.[2, 25, 27, 101]

For both arms, the slurry will be administered first, the MDI will be administered 15 minutes later, and patients will take nothing by mouth for an additional 30 minutes. This schedule is based on our previously published esophageal emptying data for OVB demonstrating that the half-life for OVB in the esophagus is <2 minutes.[42] Therefore, the swallowed slurry will be out of the esophagus prior to swallowing the MDI, so interaction between the two is not a concern. For both arms, the treatment period will be 8 weeks. There is no placebo arm in this trial because the goal is to compare two active agents and determine which is more effective. Of note, no dietary changes or changes in baseline PPI medication dose will be allowed during the study period.

There are risks associated with the study medications. Both budesonide and fluticasone are corticosteroids, and while these medications have been shown to be well-tolerated in several prior studies of EoE when compared to placebo, [26,29,38,39] adverse effects are still possible. Local effects such as mouth irritation or sore throat are expected in less than 5% of subjects. Oral candidiasis is also expected in less than 5% of subjects. Candidal esophagitis can occur in 10-20% of subjects treated with swallowed corticosteroids, with most cases detected on follow-up endoscopy. This is readily treated with an antifungal agent such as nystatin or fluconazole. Adrenal insufficiency is a theoretic concern with any corticosteroid medication. However, at the doses proposed for this study and with an 8 week treatment period, there have been no reports of adrenal axis suppression in EoE and we do not expect this adverse event during the proposed study.[29,38,39,42] Other steroid-related side effects such as bone mineral loss, cataracts, skin fragility, and diabetes have not been reported with initial short-term topical steroid use in EoE.[26,29,38,39,42] It is important to note that while there are some risks associated with taking these medications, for patients diagnosed with EoE it is likely that they would be treated with one of these agents even if they were not participating in this study, so the medication risks related to the study are may not be higher than those of routine clinical care.

The major direct potential benefit to the patient is that all subjects will receive active medication to treat EoE. Another benefit is that they will receive structured follow-up care and active monitoring for symptom recurrence. There is also potential benefit to society is based on scientific knowledge to be gained. Eosinophilic esophagitis is a newly recognized disease entity, and data supporting the best approach to pharmacologic treatment are lacking in the medical literature.

Given the discussion of the risks above, many of which are related to testing and treatments that could be ordered during the course of routine care were the patients not participating in this study, we feel the risks to subjects are acceptable in relation to the potential benefits.

# 2 Study Objectives

Specific Aim 1. To determine whether viscous budesonide is more effective than fluticasone MDI for improving esophageal eosinophil counts and symptoms of dysphagia in patients with EoE after an initial treatment course.

Specific Aim 2. To determine whether treatment with viscous budesonide results in less symptomatic and histologic recurrence than fluticasone MDI one year after the initial treatment course.

Specific Aim 3. To determine whether increased baseline staining of esophageal biopsies for major basic protein, eotaxin-3, and mast cell tryptase is associated with histologic response in EoE patients treated with topical corticosteroid therapy.

# 3 Study Design

## *3.1 General Design*

This is a prospective, randomized, double-blind, double-dummy, clinical trial comparing OVB to fluticasone MDI for treatment of EoE. This over study design will generate data for all three Aims, reporting will comply with the CONSORT statement[100] and will be registered with clinicaltrials.gov.

## *3.2 Aim 1 Study Outcomes*

The primary outcome for the study will be the post-treatment maximum eosinophil count (measured in eos/hpf). Eosinophil counts will be determined by the study pathologist both for the screening (baseline) and post-treatment exams using our previously validated protocol.[82] In brief, 4 esophageal biopsies will be obtained from both the distal (3 cm above the gastroesophageal junction) and proximal (15 cm above the junction) esophagus to maximize sensitivity of detecting eosinophils.[102] On each biopsy fragment, 5 high-power fields (hpf area = 0.24mm2) will be examined and the maximum eosinophil count determined at each level. The overall maximum count in the esophagus will be the primary outcome measure because there is no consensus in the literature about what eosinophil cut-point constitutes a “histologic non-responder” and different studies use different definitions.[25, 103]

The co-primary outcome for the study will be the dysphagia score, as measured by the DSQ.[99] This is a composite score generated by a symptom diary completed daily over the two weeks immediately prior to randomization (weeks -2 to 0) such that baseline symptoms take into account any dilation performed at the screening endoscopy. The diary will be repeated over the two weeks immediately prior to the follow endoscopy (weeks 6-8). Subjects will be automatically emailed a daily secure link to the three question survey to complete each night. The DSQ score (range: 0-6; higher is more severe) is calculated by dividing the sum of the daily scores by the number of days in which the diary was filled out. The two-week observation period will minimize the effect of symptom variation. Subjects will also be given the option of completing the diary and questionnaires on paper; capturing the date and time of each response.

Pre-specified secondary outcomes include: 1) Endoscopic findings of EoE, including esophageal rings, white plaques/exudates, linear furrows, edema/decreased vascularity, and strictures, will be measured using the recently validated EoE Endoscopic Reference Score (EREFS);[104] 2) Levels of histologic response (ie <15 eos/hpf; 3) Medication compliance as measured by percentage of medication appropriately used in each arm; and 4) symptoms of dysphagia as measured by the Eosinophilic Esophagitis symptom Activity Index (EEsAI).

## *3.3 Aim 2 Study Outcomes*

Secondary outcomes to be assessed under Aim 2 are:
- Symptomatic recurrence will be defined as at least a 1 point increase in the DSQ score over the post-treatment score. When the study coordinator receives a report of dysphagia, the DSQ will be re-administered over a 2 week period to determine if the subject meets the criteria for recurrent symptoms.

- Histologic recurrence will be defined as recurrent esophageal eosinophilia ≥15 eos/hpf. This will be detected on the follow-up endoscopy, using the identical biopsy protocol and pathology interpretation protocol as described in Aim 1.

- Other secondary outcomes will include: 1) Endoscopic findings of EoE measured by the EREFS; and 2) Levels of recurrent esophageal eosinophilia as measured by the maximum eosinophil count (eos/hpf).

## *3.4 Aim 3 Study Outcomes*

For the IHC sub-analysis to be performed after study completion, the exploratory outcome of histologic response will be defined as a maximum eosinophil count <15 eos/hpf on post-treatment biopsies (the same definition asin Aim 1).
For the methods, quantification of IHC staining will be performed with a protocol that mirrors the one used for the eosinophil counts and that has been successfully used in our previous studies.[62, 69, 82] The IHC glass slides will be scanned, converted to digital slides, and viewed with Aperio ImageScope (Aperio Technologies, Vista, CA).[82] The maximum density of cells that stained positive for each antibody of interest in the esophageal epithelial layer will be quantified (cells/mm2) in five microscopy fields using the Aperio Positive Pixel Count Algorithm (version 9.1, Aperio Technologies).[62, 69, 76]

# 4 Subject Selection and Withdrawal

## *4.1 Inclusion Criteria*

1) Age: 16-80 years
2) Subject is having a clinically indicated endoscopy (Baseline visit 1) for suspicious EoE and has been on BID PPI for at least 8 weeks. **OR** New diagnosis of EoE as per consensus guidelines.[2] Cases must have symptoms of dysphagia, persistent esophageal eosinophilia (≥ 15 eosinophils in at least one high-power field) after 8 weeks of treatment with a twice daily proton-pump inhibitor, and other competing causes of esophageal eosinophilia excluded.

## *4.2 Exclusion Criteria*

1) Medical instability that precludes safely performing upper endoscopy
2) Ongoing or recent symptoms of intestinal bleeding (throwing up blood, passing blood in the stool)
3) Concomitant eosinophilic gastroenteritis (EoG)
4) Esophageal narrowing or stricturing that will not allow a standard 9 mm upper endoscopy scope to pass
5) Cancer in the esophagus, stomach, or intestine
6) Prior surgery on the esophagus (e.g., removal of part of the esophagus)
7) Esophageal varices (dilated blood vessels in the esophagus)
8) Current use of blood thinners like Plavix or Coumadin that are not stopped prior to endoscopy procedures
9) Corticosteroid exposure within the four weeks prior to the baseline endoscopy. Exclusionary corticosteroid exposure is defined as any swallowed topical steroids for EoE or systemic steroids for any condition within the four weeks prior to the baseline endoscopy. Corticosteroids used for asthma or intranasal corticosteroids are not an exclusion and are allowable.
10) Pregnancy
11) Inability to read or speak English

## *4.3 Subject Recruitment and Screening*

Patients with a new diagnosis of EoE or who are undergoing routine care upper endoscopy for a clinical suspicion of EoE will be screened by research personnel. These patients will be identified by screening the endoscopy and CEDAS clinic schedules or via referrals from other physicians. Once a potential subject is identified, study personnel will contact the potential subject to describe the study and gauge interest in participating. Initial contact may be in person or on the phone using an IRB approved phone script. If the patient is interested in participating, then the study coordinator or other study staff will obtain written informed consent from the subject. In practice, the vast majority of patients who would qualify for this study are seen by the PI in clinic prior to endoscopy, and this greatly simplifies the logistics of identifying patients, enrolling them, and collecting baseline data. At the time of enrollment, the coordinator will collect information about subject demographics, symptom duration, and concomitant atopic conditions (asthma, atopic dermatitis, allergic rhinitis/sinusitis, and food allergies).

## *4.4 Early Withdrawal of Subjects*

### 4.4.1 When and How to Withdraw Subjects

Subjects have the right to withdraw from the study at any time, for any reason, and without repercussion. The sponsor-investigator, Dr. Evan Dellon, has the right to withdraw a patient from the study in the event of an intercurrent illness, adverse event (AE), treatment failure, protocol violation, and for administrative or other reasons. An excessive rate of withdrawals would reduce the amount of data available for analysis and limit the ability to interpret the study results; therefore, unnecessary withdrawal of patients should be avoided.

If a subject withdrawals prematurely, either due to voluntary withdrawal or discontinuation by the sponsor-investigator, Dr. Evan Dellon, then they will be asked to return for a final visit to return unused study drug as well as to complete safety assessments (adverse events).

### 4.4.2 Lost to Follow-Up

A subject will be considered lost to follow-up after documentation has been made of at least two documented attempts to contact (via phone or email). At that point a certified letter should be mailed to the subject's home address. If there is still no response after the certified letter is delivered, then the subject will be withdrawn from the study as lost to follow-up.

### 4.4.3 Data Collection and Follow-up for Withdrawn Subjects

Patients who withdraw prematurely from the study may be asked to complete an end of study visit per the study procedures. In addition, any ongoing adverse drug reactions or study-related adverse events will be followed until resolution or documentation of why there will be no resolution if the event will be ongoing.

# 5 Study Drugs

## *5.1 Description*

Oral viscous budesonide (OVB) topical steroid slurry 1 mg/4 mL aqueous budesonide mixed with 10g of sucralose. At total of 300mL of slurry will be provided to the patient in a 16oz plastic bottle every 4 weeks during the 8 week treatment period. For the purposes of compounding the medication to be dispensed as a one-month supply, the constituent elements of aqueous budesonide are combined in bulk with sucralose to yield the necessary concentration and consistency. The placebo oral suspension will also be 300mL of an indistinguishable solution also provided in a 16oz. plastic bottle every 4 weeks during the 8 week treatment period.

Fluticasone MDI will be provided in a 220 mcg metered dose inhaler that has 120 actuations. Subjects will receive 2 MDIs every 4 weeks during the 8 week treatment period. As similar number of placebo inhalers will be provided as well. For the inhalers, the UNC IDS pharmacy will prepare and blind these. All inhalers will be provided in a featureless white plastic shell with no labels. The containers with medication or placebo will also have no labels, and will be sealed inside the container with tamper-proof tape.

## *5.2 Treatment Regimen*

Arm 1: OVB 1 mg swallowed at a dose of 1mg twice daily and placebo inhaler 4 puffs twice daily for 8 weeks.

Arm 2: Fluticasone 220 mcg MDI is swallowed at a dose of 880 mcg twice daily (4 puffs of a 220 mcg inhaler twice daily) and placebo slurry 4mL twice daily for 8 weeks.

## *5.3 Method for Assigning Subjects to Treatment Groups*

Subjects will be randomized in 1:1 fashion to either OVB + placebo inhaler or fluticasone MDI + placebo slurry using a blocked randomization protocol with computer-generated variable block sizes. The randomization sequence will be provided to the UNC investigational drug service (IDS) and allocation will be concealed from all investigators, subjects, and data analysts. The IDS will ensure that all study medications are appropriately blinded. At the time of randomization (study visit 2; Figure 8), the study coordinator will be provided with the appropriate study medications for the patient, but will not know the allocation.

## *5.4 Preparation and Administration of Study Drug*

Triangle Compounding Pharmacy will provide OVB 1mg/4ml aqueous suspension and placebo suspension to the UNC IDS. The OVB will be the equivalent of a slurry of 1mg/4mL aqueous budesonide

mixed with 10g of sucralose. The placebo OVB slurry will be aqueous sucralose. Triangle Compounding Pharmacy will provide all of the necessary ingredients and devices to compound the medications, such that they are indistinguishable by sight, appearance, and taste. For the purposes of compounding the medication to be dispensed as a one-month supply, the constituent elements of aqueous budesonide are combined in bulk with sucralose to yield the necessary concentration and consistency. The medications will be prepared in a powder containment hood following USP 795 guidelines. The UNC IDS will purchase fluticasone MDI, and the PI has purchased the equivalent placebo inhalers and has provided them to the IDS. The IDS will then blind the inhalers, as noted above. All study drug and placebos will be stored and dispensed from the UNC IDS. UNC IDS will determine randomization and dispense blinded study drug and placebo to a study coordinator who will then dispense to the subject.

There may be instances in which the participation of an eligible subject is limited only by the ability to come to the site for study only visits (Treatment Start Visit 2,and/or Mid-Treatment Visit 3. In these cases, study drug may be shipped to the subject only after Study PI approval, using the IDS approved Shipping Standard Operating Procedure. Visit assessments would be completed by phone, email, and on paper as applicable.

UNC Investigational Drug Services: 919-966-8739

## *5.5 Subject Compliance Monitoring*

Budesonide medication compliance will be measured by residual OVB slurry remaining in the bottle. Study coordinator will measure the amount and calculate volume remaining to compare to expected volume remaining. The fluticasone medication compliance will be measured using an actuation counter as well as by weight of the inhaler before and after use. Medication compliance will be assessed at the mid-treatment visit, and subjects who are non-compliant will be trained on appropriate study medication dosage and counseled on the importance of being compliant with the study medication. Medication compliance will also be assessed at the end of treatment visit.

## *5.6 Prior and Concomitant Therapy*

All past and current EoE medical therapies (including medication, dilation, etc.) will be collected along with all current concomitant medications. Any swallowed topical corticosteroid exposure for EoE or systemic steroids for any condition within the 4 weeks prior to baseline EGD and during the treatment phase are not permitted for the study. No dietary changes or changes in baseline PPI medication dose will be allowed during the study period.

## *5.7 Packaging and Labeling*

UNC IDS will dispense active study medication and placebo. Specifically, they will provide the pre-mixed 16oz bottles containing 300mL of OVB slurry, the identical-tasting 16oz bottles containing 300mL OVB placebo slurry, the 220mcg fluticasone inhaler, and the placebo inhaler. All inhalers will be provided in a featureless white plastic shell with no labels. The containers with medication or placebo will also have no labels, and will be sealed inside the container with tamper-proof tape. Based on the blinded randomization schedule, IDS will assemble and label subject drug kits to include either:

1 16oz bottle of OVB slurry with 1 measuring device (syringe) and 2 placebo MDIs or

1 16oz bottle of placebo with 1 measuring device (syringe) and 2 fluticasone MDIs

All investigational products dispensed for this study will be in compliance with labeling requirements per 21CFR312 and include the following statement: “Caution: New Drug-Limited by Federal law to investigational use”

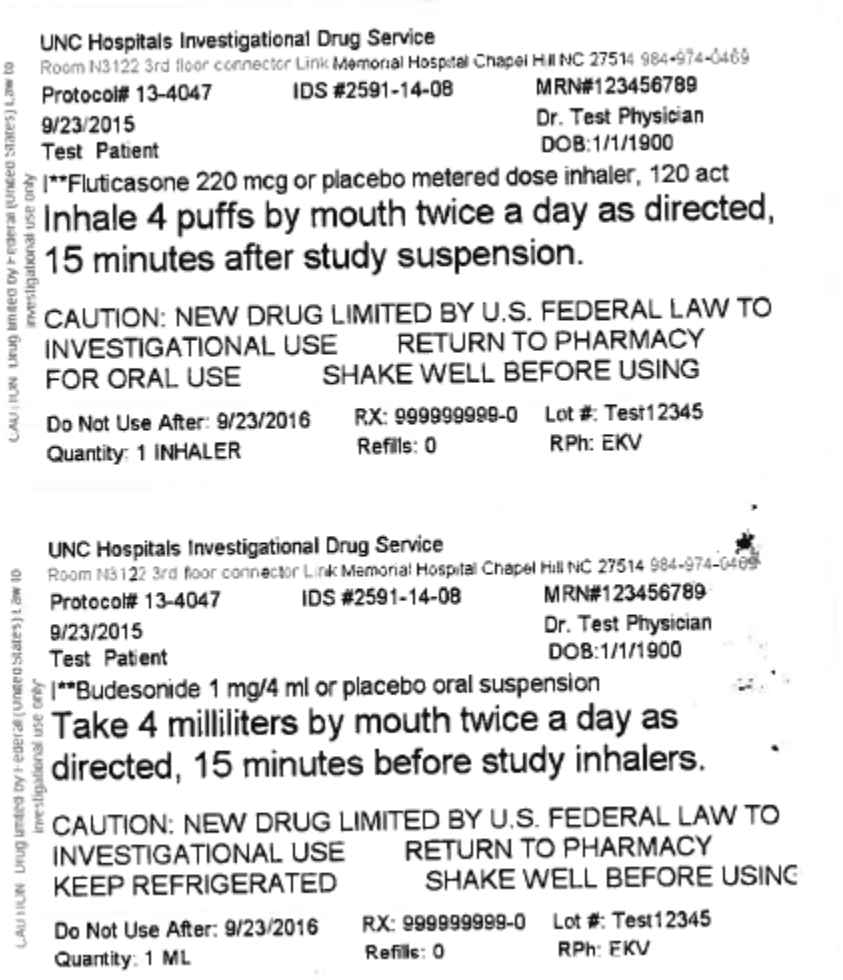
In addition, product labeling will include:

- IDS contact information
- Study IRB number
- Study IDS number
- Subject medical record number

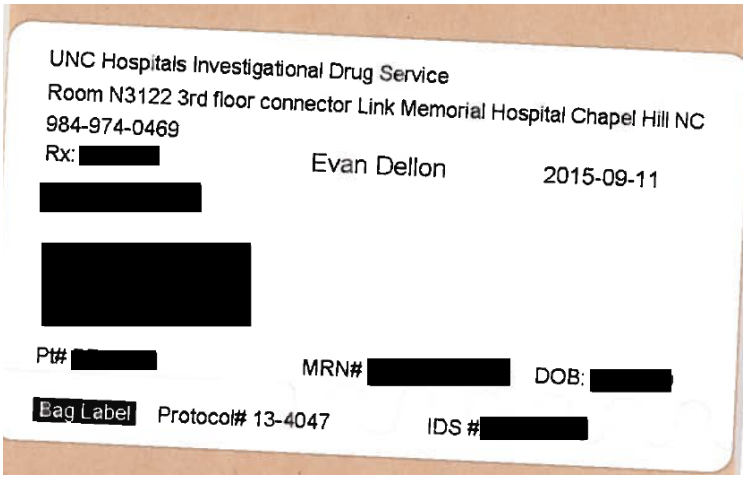
- Subject name
- Dosing instructions
- Drug expiration date
- Prescription number
- Lot number
- Quantity
- Number of refills
- Pharmacist information

Sample labels are provided below.

IP Label:



Bag Label:




## *5.8 Blinding of Study Drug*

UNC IDS will implement the blinding of the study drugs. All study medication will be labelled with unique study identification numbers, and the only link between the study ID and the randomization sequence will be kept on file by the IDS. In this manner, study subjects and all other study personnel (investigators, endoscopists, nurses, statisticians, and study staff) will remain masked as to allocation.

## *5.9 Receiving, Storage, Dispensing and Return*

### 5.9.1 Receipt of Drug Supplies

Triangle Compounding Pharmacy will deliver OVB and OVB placebo to IDS as patients are enrolled in the study. Upon receipt of the of the study treatment supplies, IDS will inventory the drug and a drug receipt log will be filled out and signed by the person accepting the shipment. IDS study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

### 5.9.2 Storage

All slurry and inhalers will be stored at room temperature in the IDS.

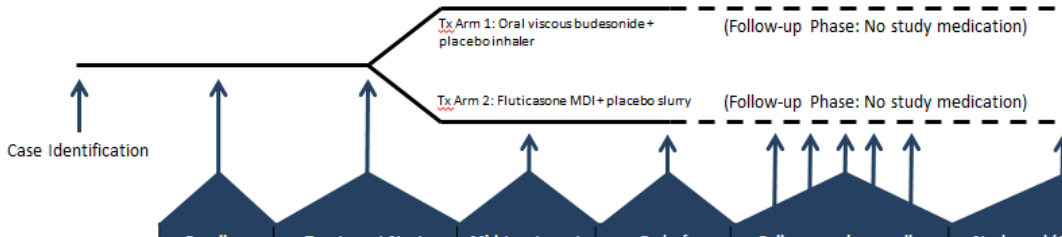
### 5.9.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form, and signed and dated by IDS. UNC IDS is responsible for maintaining all drug accountability including receipt, dispensation, and destruction.

### 5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files. UNC IDS is responsible for maintaining all drug accountability including receipt, dispensation, and destruction.

# 6 Study Procedures




| | Baseline Visit | Treatment Start | Mid-treatment | End of treatment | Follow-up phone calls | Study end (60 weeks or recurrent symptoms) |
|---|---|---|---|---|---|---|
| Time (weeks) | -6 to -2 | 0 | 4 | 8 | 12,20,30,40,50 | 60 (maximum) |
| Visit # | 1 | 2 | 3 | 4 | (Phone call or Email) | 5 |
| **Procedures** | | | | | | |
| Informed consent | X | | | | | |
| Inclusion/exclusion criteria review | X | X | | | | |
| EoE medical history/demographics | X | | | | | |
| Concomitant meds | X | | | | | X |
| EGD with EREFS score and path biopsies | X | | | X | | X |
| Research blood/biopsy | X | | | X | | X |
| Randomization | | X | | | | |
| Adverse events | X | | | X | | X |
| DSQ administration | X | | | X | X | X |
| TREET trial qstnr | | X | | X | | X |
| EEsAI questionnaire | | X | | X | | X |
| Symptom assessment | | | | | X, X, X, X, X | X |
| Study med Dispensed | | X | X | | | |
| Study med compliance | | | X | X | | |

**Figure 9.** Study overview of procedures at each visit. Baseline Visit: Clinically indicated routine care EGD to determine eligibility. Treatment Start Visit: If subject is eligible, subject is randomized and starts study medication. End of Treatment Visit: Clinically indicated routine care EGD to determine response to treatment. Follow-up Phase: No study medication taken; subject symptoms are assessed by phone calls or via email. Study End: Occurs 60 weeks post Treatment Start Visit or when dysphagia symptoms recur. Of note, all CFRs will be entered in a custom electronic data capture and management system designed expressly for this trial.

## *6.1 Case Identification*

During case identification, eligibility is assessed (based on inclusion/exclusion criteria) and those eligible and interested in participating will be consented during the Baseline Visit. Potential cases will be identified by screening the endoscopy and CEDAS clinic schedules. This will require approval of a limited HIPAA waiver to access the personal health information (PHI) of potential research subjects prior to their formal enrollment during Baseline Visit.

## *6.2 Baseline Visit (Visit 1)*

The baseline visit is a clinically indicated and clinically scheduled EGD appointment that is performed per routine care. The subject is responsible for all associated costs of the EGD.

The following procedures will be completed during the baseline visit:

- Informed consent
- Inclusion/exclusion criteria review to determine eligibility
- EoE medical history review and demographics collection
- Concomitant medications collected
- EGD with EREF score and biopsies for pathology, per routine care
- Sample collection, research related
- Adverse events noted
- Daily Symptoms Questionnaire (DSQ) administration – DSQ will be explained and subject will be instructed to begin completing daily entries in the evening for 2 weeks after receipt of pathology results and confirmation of eligibility by Dr. Dellon

The Baseline Visit Case Report Form (CRF) will be completed by the study coordinator. This form captures demographics including race, ethnicity, gender, and date of birth, adverse events, EoE medical history including documentation of endoscopic procedures to date as well as pathology findings and concomitant medications related to EoE and current concomitant medications. CRF will also include EGD data with EREFS score. The Pathology CRF will be completed by the study pathologist. This form captures eosinophil counts and associated histologic findings.

Patients may also be identified after routine care EGD by the study physician or a UNC GI colleague. Patients that are referred will be contacted by Dr. Dellon to discuss the option to participate in EoE research studies. If subjects have already had a confirmatory EGD for EoE, subjects may be consented into the study by phone and email; sending a signed consent form to study personnel before beginning DSQ. See section 6.2.1 for details. Pathology review will occur using previous clinical or research samples.

### 6.2.1 Consenting Procedure

If a subject is screened eligible and interested in the study, the subject will be consented on the study prior to any study procedure. Written informed consent will be obtained by qualified study personnel. Documentation of the consent process will be maintained in the subject's research record.

Subjects will be given ample time to review the consent document and ask any questions they may have. A copy of the written consent form will be provided to the subject and the original maintained in the subject's research record.

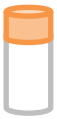
If subjects meet all inclusion and none of the exclusion criteria and consent to the study, they will be enrolled in the study. Subjects will be assigned a unique subject code.

If a previous EGD result is being used for eligibility purposes, consent will be obtained before all other Baseline study procedures. Subjects will be provided the consent form by mail or email and the consent interview will be conducted by telephone while the subject can read the consent form during discussion. The subject will then sign and date consent forms and email, mail, or return signed copy at next visit. The subject will be provided with a staff signed copy of the consent form.

### 6.2.2 Biopsy Collection and Processing, Research Related

Biobanking will be performed during this study to archive specimens for future research purposes. During the clinically indicated EGD, clinical biopsies will be obtained for pathology assessment. In addition, 4 research-specific biopsies will be obtained from each of the following locations: Distal esophagus (3cm from TGF), middle esophagus (8cm from TGF), and proximal esophagus (13cm from TGF). Additionally, 2 research-specific biopsies will be obtained from the duodenum, and 2 research-specific biopsies will be obtained from the stomach (1 from antrum, 1 from body). The 4 distal biopsies will be separated into 4

cryovials: 2 filled with formalin for histology, 1 filled with RNALater, and 1 empty to be frozen immediately in liquid nitrogen.

Cryovial depiction: 

**Research Related Biopsy Collection Protocol:**

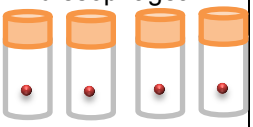
| Biopsy Location | Description | Processing/Labeling Instructions |
|---|---|---|
| 4 distal esophageal  | 4 single biopsies 3cm from GEJ | • 2 formalin: **Label:** PID[1]_eh[2,3]_d[4]_date[5] and PID_eh_d2_date<br>• 1 RNALater: Refrigerate, store long term in -80deg.F **Label:** PID_er_d_date<br>• 1 frozen: **Label:** PID_ef_d_date |
| 4 mid esophageal  | 4 single biopsies 8cm from GEJ | • 1 formalin: **Label:** PID_ eh_m_date<br>• 1 RNALater: **Label:** PID_er_m_date<br>• 2 frozen: **Label:** PID_ef_m_date and PID_ef_m2_date |
| 4 proximal esophageal  | 4 single biopsies 13cm from GEJ | • 2 formalin: **Label:** PID_eh_p_date and PID_eh_p2_date<br>• 1 RNALater: **Label:** PID_er_p_date<br>• 1 frozen: **Label:** PID_ef_p_date |

**Figure 10.** Research related biopsy tissue collection outline. Specimen labels include: participant identifier[1]_tissue type[2]_ fixitive/sample processing[3]_ esophageal location[4]_date collected[5]

[1]participant identifier (PID)
[2]tissue type: "e" is esophageal
[3]fixitive/sample processing: r, h, or f where "r" is RNALater, "h" is histology/formalin, and "f" is frozen/empty cryovial
[4]esophageal location: d, m, or p where "d" is distal, "m" is middle, and "p" is proximal
[5]date collected: MMDDYYYY

A reduced number of biopsies may be collected at the discretion of the endoscopist performing the procedure. Biopsy samples missed will not be considered protocol deviations or violations.

### 6.2.3 Blood Sample Collection and Processing

| Blood tube | Processing/Labeling Instructions |
|---|---|
| 2 red top serum separators | Centrifuge, aliquot serum into 9 cryovials, store in refrigerator and transfer to -80<br>**Label**: PID_s1, PID_s2, PID_s3, etc. |
| 1 yellow top plasma buffy separator | Centrifuge, aliquot plasma into 9 cryovials<br>**Label**: PID_p1, PID_p2, PID_p3, etc. |

| | |
|---|---|
| | buffy into 1 cryovial<br>**Label**: PID_b |
| 1 purple top whole blood | Aliquot whole blood into 5 cryovials<br>**Label**: PID_w |
| 1 PAXgene blood RNA | Store PAXgene tube upright at room temperature for a minimum of 2hrs and maximum of 72hrs before transferring to refrigerator then freezer<br>**Label**: PID |

Missed blood samples are allowable, and will not be considered protocol deviations or violations.

Urine or saliva samples may be collected for future testing at Enrollment, End of Treatment, and Study End Visits.

## *6.3 Treatment Start (Visit 2)*

At treatment start, the subject will come for a study visit, and the following procedures will be performed:

- Inclusion/exclusion criteria will be reviewed
- TREET Trial Questionnaire, completed by subject by email (or provided on paper)
- EEsAI Questionnaire, complete by subject on paper
- Study Medications dispensed to subject- the subject will be provided with specific instructions about how to use the medications and the timing of administration. Additionally, an information sheet will be provided with this information.
    - Prior to this visit, the coordinator will receive the set of study medications from the IDS that are labelled and coded for that patient, according to the randomization scheme. The coordinator, subject, and all study personnel remain blinded to treatment allocation. If determined applicable by the Study PI, the medication can be mailed to the subject with confirmation of receipt provided to the study staff.

Mid treatment (visit 3) and end of treatment (visit 4) appointments will be scheduled. The Treatment Start CRF will be completed by the Study Coordinator.

## *6.4 Mid-treatment (Visit 3)*

At the mid treatment visit, the subject will come for a study visit, and the following procedures will be performed:

- Adverse Events noted
- Subject will return used medications
- The coordinator will assess compliance based on subject report, and identify any barriers to compliance with the subject, including retraining on medication administration if necessary
- Coordinator will measure the remaining medications returned for compliance assessment, as noted above.
- Study medications dispensed to subject. Prior to this visit, the coordinator will receive the set of study medications from the IDS. If determined applicable by the Study PI, the medication can be mailed to the subject with confirmation of receipt provided to the study staff.

The mid-treatment CRF will be completed by the Study Coordinator.

## *6.5 End of treatment (Visit 4)*

This visit is similar to the treatment start visit. It is a clinically indicated and clinically scheduled EGD appointment that is performed per routine care for follow-up of treatment response. The subject is responsible for all associated costs of the EGD.

The following procedures will be completed during the baseline visit:

- EGD with EREF score and biopsies for pathology, per routine care
- Sample collection, research related
- Adverse events noted
- Daily Symptoms Questionnaire (DSQ) administration confirmation – DSQ will have been sent 2 weeks prior to the endoscopy.
- TREET Trial follow-up questionnaire and the EEsAI, will be completed by subject
- Subject will return used medications
- The coordinator will assess compliance based on subject report
- Coordinator will measure the remaining medications returned for compliance assessment, as noted above.

The Follow-up Visit Case Report Form (CRF) will be completed by the study coordinator. This form captures adverse events, and EGD data with EREFS score. The Pathology CRF will be completed by the study pathologist. This form captures eosinophil counts and associated histologic findings.

After this visit, the PI will review biopsy results. Subjects who do not have a histologic response (defined by <15 eos/hpf) will be exited from the study and can resume routine clinical care. Subjects with a histologic response will continue on in the follow-up phase of the study, and will be observed without any study medication.

## *6.6 Follow-up phone calls and emails*

During the follow-up phase, subjects will receive automated email follow-up questions from the electronic data management system at weeks 12, 20, 30, 40, and 50. They will also be instructed to contact study staff with any recurrent symptoms or issues. Participation in this phase will last up to one year (study week 60) or until symptoms recur, whichever is first. During the follow-up phase, the patients will be contacted by email and/or phone five times by the study staff to screen for adverse events and symptom recurrence. If there is symptom recurrence, the patients will fill out a repeat DSQ, and then will proceed to endoscopy. If there is no symptom recurrence, the patients will proceed to surveillance endoscopy in 1 year (see section 6.7).

## *6.7 Study end (Visit 5)*

This visit occurs if symptoms have recurred during the 1 year follow-up phase, or at study week 60, whichever comes first. This visit is similar to the treatment start visit. It is a clinically indicated and clinically scheduled EGD appointment that is performed per routine care for follow-up of treatment response. The subject is responsible for all associated costs of the EGD.

The following procedures will be completed during the baseline visit:

- Concomitant medications collected
- EGD with EREFS score and biopsies for pathology, per routine care
- Sample collection, research related
- Adverse events noted
- Daily Symptoms Questionnaire (DSQ) administration confirmation – DSQ will have been sent 2 weeks prior to the endoscopy.
- TREET Trial follow-up questionnaire
- EEsAI questionnaire
- Symptom assessment

The End-of study Case Report Form (CRF) will be completed by the study coordinator. This form captures adverse events, and EGD data with EREFS score. The Pathology CRF will be completed by the study pathologist. This form captures eosinophil counts and associated histologic findings.

After this procedure, the subject has completed the study and will return to routine care with their referring provider.

# 7 Statistical Plan

## *7.1 Sample Size Determination*

The overall study is powered for the co-primary histologic and symptom outcomes. Based on estimates of histologic improvements from our study of topical budesonide formulations as well as the other published studies of topical steroids in adults and children (Table 4),[26, 27, 29, 39, 41, 42, 101, 105] we estimate that average baseline maximum eosinophil counts will be 80 eos/hpf and that average post-treatment maximum eosinophil counts will be 10 ± 10 eos/hpf in the OVB arm and 20 ± 20 eos/hpf in the fluticasone MDI arm. To detect this different with a power of 0.9, 53 subjects per arm are needed (Table 5). Assuming a drop-out rate of 15%, which is what we observed in our budesonide trial,[42] we will randomize to treatment 61 subjects in each arm, for a total of 122. This sample size will also allow us to detect with a power of 0.9 a DSQ difference of as little as 1 point, which was the average difference between those patients on topical steroids and those not on topical steroids in the validation study of the DSQ.[99] This is a clinically significant difference equivalent to having one day less of dysphagia per week. We are planning a 36 month enrollment phase, which averages to 40 subjects per year. This is substantially less than the number of incident cases in the study age range diagnosed at UNC in the last two years (Table 3) and is a realistic goal.

For Aim 2, based on estimates in the literature, we would expect at least 80% of subjects in the OVB arm,[29, 30, 39, 42] and at least 50% of subjects in the fluticasone MDI arm,[26, 27, 41, 101, 105] to have a symptomatic and histologic response (<15 eos/hpf) after the initial treatment period. Therefore, approximately 42 subjects in the OVB arm and 27 subjects in the MDI arm will enter the follow-up period. There are no prospective comparative data on symptomatic or histologic recurrence rates for these two medications. However, based on these sample sizes and estimating a recurrence rate of 80% in the MDI group, we would be able to detect a hazard ratio for symptomatic recurrence of 0.43 or lower with a power of 0.8 for OVB compared with MDI. Similarly, for histologic recurrence, we would be able to detect a difference as low as 36% with a power of 0.8 for OVB compared with MDI.

For Aim 3, based on estimates in the literature and our own preliminary data, we expect 60% of subjects in the OVB arm,[29, 39, 42] and 40% of subjects in the MDI arm,[26, 27, 41, 101, 105] to have a complete histologic response of <5 eos/hpf. Therefore, we expect a total of 53 histologic responders and 53 non-responders. With this sample size, we will be able to detect a difference between the responders and non-responders as low as 100 cells/mm$^2$ for tryptase staining and 1250 cells/mm$^2$ for eotaxin-3 staining with a power of 0.9. Both of these values are less than or equivalent to the differences we observed in our preliminary data. While we do not expect to see a difference in MBP staining, with this sample size we would be powered to find a difference of 750 cells/mm$^2$ or greater if such a difference exists

## *7.2 Statistical Methods*

For Aim 1, to test whether OVB is more effective than fluticasone MDI for improving eosinophil counts, the mean post-treatment maximum eosinophil count will be compared between the OVB and MDI groups using a two-sample t-test. The pre- and post-treatment counts will also be compared within study groups using a paired t-test. To test whether OVB is more effective than MDI for improving symptoms of dysphagia, the mean DSQ scores will be compared between the OVB and MDI groups using a two-sample t-test, and within groups using a paired t-test. For the secondary outcomes, means will be compared for the post-treatment endoscopy score with t-tests, and proportions will be compared with chi-square for the levels of histologic response. For all analyses, if data distributions are not normal, non-parametric testing will be used. All analyses will be by intention-to-treat and performed using SAS (version 9.2). All

**Table 5:** Sample size calculations for primary histologic outcome

| Post-treatment eosinophil counts (mean eos/hpf)* | | | | |
|---|---|---|---|---|
| OVB | MDI | α | Power | N per group |
| 15 | 20 | 0.05 | 0.8 | 157 |
| 10 | 20 | 0.05 | 0.8 | 40 |
| 5 | 20 | 0.05 | 0.8 | 18 |
| 15 | 20 | 0.05 | 0.9 | 211 |
| ***10*** | ***20*** | ***0.05*** | ***0.9*** | ***53*** |
| 5 | 20 | 0.05 | 0.9 | 24 |

tests will be two-sided with a significance level set at $p < 0.05$. To account for the possibility that baseline subject characteristics are unevenly distributed between study groups, we will perform an additional analysis with multiple linear regression to control for possible confounders including co-existing atopic disease (asthma, atopic dermatitis, allergic rhinitis/sinusitis, food allergies), symptom duration prior to diagnosis, esophageal stricture on baseline endoscopy, and esophageal dilation on baseline endoscopy.

For Aim 2, to test whether OVB results in less symptomatic recurrence than fluticasone MDI, survival analysis will be performed with the interval between treatment end (week 8) and recurrent symptoms or study end (week 60) as the time of interest. A Kaplan-Meier curve will be constructed comparing the time until symptom recurrence in both study groups using the log-rank test. Hazard ratios will be calculated using Cox proportional hazards models, and we will adjust for potential confounders including age, gender, atopic status, symptom duration prior to EoE diagnosis, and presence of esophageal strictures on endoscopy. To test whether OVB results in less histologic recurrence than fluticasone MDI, the proportion of subjects with ≥15 eos/hpf at follow-up endoscopy in each group will be compared using chi-square. This analysis cannot be time-dependent because the eosinophil count is not known for all subjects until the follow-up exam is complete.

For Aim 3, to test the hypothesis that elevated levels of eotaxin-3 and tryptase, but not MBP, will predict histologic response after treatment with topical steroids, we will perform three identical analyses. In the first, the mean baseline cellular staining for eotaxin-3 will be compared between the responder and non-responder groups using a two-sample t-test. This same analysis will be repeated for tryptase and MBP. We also plan a multivariable analysis to determine if these biomarkers independently predict response. For this, logistic regression modeling will be performed controlling for factors such as age, gender, atopic status, symptom duration prior to EoE diagnosis, and presence of esophageal strictures on endoscopy. For the secondary analyses, we will assess staining using other levels of histologic response (partial response with 5-14 eos/hpf and non-response with ≥15 eos/hpf) and by formulation of topical steroid (OVB vs fluticasone MDI). We will also determine if baseline staining was associated with decreased post-treatment symptoms of dysphagia measured by the DSQ, and whether the biomarkers correlate with each other. For all analyses, if data distributions are not normal, non-parametric testing will be used. All tests will be two-sided with a significance level set at $p < 0.05$.

### *7.3 Subject Population(s) for Analysis*

The subject population for analysis will be by intention-to-treat, and will include all patients who are randomized and who complete the protocol to have follow-up data available.

# 8 Safety and Adverse Events

### *8.1 Definitions*

**Unanticipated Problems Involving Risk to Subjects or Others (UPIRSOs)**
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

**Adverse Event**
An ***adverse event*** (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, experience, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Adverse events encompass both physical and

psychological harms. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

**Serious Adverse Event**
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

**Serious vs. Severe**
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

**Adverse Drug Reaction (ADR)**
Any adverse event caused by a drug. An ADR can be considered a "suspected" adverse drug reaction if there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

**Unexpected Adverse Drug Reaction**
An adverse drug reaction, the nature or severity of which is not consistent with the applicable product information (e.g. package insert or investigator's brochure).

**Adverse Event Reporting Period**
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

**Preexisting Condition**
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

**General Physical Examination Findings**
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

**Post-study Adverse Event**
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

**Abnormal Laboratory Values**
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

**Hospitalization, Prolonged Hospitalization or Surgery**
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

## *8.2 Recording of Adverse Events*

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

## *8.3 Reporting of Serious Adverse Events and Unanticipated Problems*

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

### 8.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of study-related serious adverse event, must be reported to the study sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to the PI, and the study coordinator, who in turn will communicate to the UNC IRB, and if necessary, NIH.

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

### *8.3.2* Notifying the UNC IRB

Dr. Evan Dellon is responsible for reporting adverse events to the UNC IRB per the UNC IRB SOPs for reporting adverse events. Federal regulations require investigators to report unanticipated problems involving risks to subjects or others to the IRB. Historically, there has been confusion about what needs to be reported. OHRP and FDA have issued guidance that clarifies that investigators need only report “unanticipated problems involving risks to subjects or others” (or UPIRSOs). The UNC-Chapel Hill policy is based on this guidance. “Adverse events” that are not UPIRSOs are not required to be reported to the IRB.

#### 8.3.2.1 Differentiating between an UPIRSO and an Adverse Event

By definition, an UPIRSO is unexpected, whereas an “adverse event” may be anticipated or unanticipated. Additionally, an UPIRSO may involve the increased risk of harm—whether or not any actual harm occurred. In order to decide which events or circumstances constitute an UPIRSO, it is important to bear in mind the following:

- Not all Adverse Events are UPIRSOs. Only a small subset of “adverse events” occurring in FDA-regulated clinical trials and other types of studies constitute UPIRSOs. Many events that are required to be reported to the sponsor or federal agency are not UPIRSOs.
- An UPIRSO may not be an adverse event. It is possible for an event that does not involve actual physical, psychological, social, or economic harm to a research subject or another person nevertheless to constitute an UPIRSO that must be reported to the IRB. This is the case if the event places subjects or others at increased or different risk of harm, regardless of whether actual harm has occurred.

There are other types of incidents, experiences and outcomes that occur during the conduct of human subjects research that represent UPIRSOs but are not considered adverse events. Some UPIRSOs involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, UPIRSOs place subjects or others at risk of harm, but no harm occurs. For example, an investigator conducting behavioral research collects individually identifiable sensitive information about illicit drug use and other illegal behaviors by surveying college students. The data are stored on a laptop computer without encryption, and the laptop computer is stolen from the investigator's car. This is an UPIRSO and must be reported because the incident was (a) unexpected (i.e., the investigators did not anticipate the theft); (b) related to participation in the research; and (c) placed the subjects at a greater risk of psychological and social harm from the breach in confidentiality of the study data than was previously known or recognized.
Other examples of UPIRSOs that should be reported to the IRB, even though they are not adverse events, include:

- Publication in the literature, safety monitoring report (e.g., DSMB report), interim result, or other finding that indicates an unexpected change to the risk/benefit ratio of the research;
- Breach in confidentiality resulting from a disclosure of confidential information or from lost or stolen confidential information;
- Unresolved complaint of a participant, family member or other individual;
- Laboratory or medication errors that may involve potential risk to that individual or others;
- Change in FDA labeling because of adverse consequences or withdrawal from marketing of a drug, device, or biologic used in a research protocol;
- Disqualification or suspension of investigators;
- Accidental or unintentional change to the IRB-approved protocol that involves risks or has the potential to recur;
- Deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant
- Any deviation from the IRB-approved protocol that increases the risk or affects the participant's rights, safety, or welfare.

#### 8.3.2.2 UNC IRB Reporting Timelines

Reporting is required of all UPIRSOs, including those which may occur after the participant has completed or is withdrawn from the study, or following study closure. Reporting is completed via IRBIS, UNC's online IRB information system.

Events that meet the criteria for an UPIRSO and are also serious adverse events should be reported to the IRB within **one (1) week** of the investigator becoming aware of the event.

Any other events that meet the criteria for a UPIRSO should be reported to the IRB within **two (2) weeks** of the investigator becoming aware of the problem.

If the report cannot be completed in its entirety within the required time period, a preliminary report should be submitted. The report should be amended once the event is resolved and/or more information becomes available.

### 8.3.3 Notifying the FDA

As the sponsor, Dr. Evan Dellon is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event per 21CFR312.32:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug

- unexpected,
- fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening
    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

**Additional reporting requirements**
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

**Reporting Process**
Adverse events requiring reporting to FDA per the above may be submitted on FDA Form 3500A or in a narrative format. If supplied as a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3.

## *8.4 Unblinding Procedures*

If an AE or other clinical event necessitates unblinding the patient, this decision will be made by the PI in consultation with the DSMB. Actions will be reported to the UNC IRB and the DSMB.

## *8.5 Stopping Rules*

This study does not have stopping rules, and there are no plans for an interim analysis.

## *8.6 Medical Monitoring*

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10). Medical monitoring will include a regular assessment of the number and type of adverse events.

### 8.6.1 Independent Data and Safety Monitoring Board

The DSMB will regularly review interim data to assess compliance, monitor toxicity, and recommend whether the trial should continue. Members will be independent experts not otherwise affiliated with the trial or UNC as an institution, and will be chosen on the basis of their expertise and scientific rigor. The areas of expertise for the DSMB members span the disciplines relevant to the conduct of GI clinical trials, including epidemiology, trial design and conduct, and clinical care of EoE patients. The members will be:

| | | |
|---|---|---|
| Ikuo Hirano (Chair) | David A. Katzka | Gary W. Falk |
| Professor of Medicine | Professor of Medicine | Professor of Medicine |
| Northwestern University | Mayo Clinic | University of Pennsylvania |
| Chicago, IL | Rochester, MN | Philadelphia, PA |

The DSMB will meet via conference call at the beginning of the study, and then at 6-month intervals until the study is complete. The PI (Dr. Dellon) as well as the study biostatistician (Dr. Galanko) will participate in the open portion of the meetings. If needed, the DSMB's voting members may then choose to discuss

issues in a closed session kept confidential from the investigators. The DSMB has the responsibility to review the research protocol and to evaluate the progress of the trial overall. It will also evaluate participant risk and benefit as the trial progresses, considering evolving scientific discoveries or treatment options that may affect the desirability of continued treatment. At the conclusion of each meeting, the DSMB will recommend whether the trial be continued. The specific information it will review include: current status of study enrollment; level of medication compliance; medication-related adverse events; endoscopy-related adverse event; and subject-reported events. Unexpected serious adverse events will be reported to the DSMB between meetings as needed, as well as to the UNC IRB as noted above.

# 9 Data Handling and Record Keeping

## *9.1 Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

## *9.2 Source Documents*

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

## *9.3 Case Report Forms*

The study will utilize electronic case report forms (eCRFs). All data requested on the CRF must be recorded. All missing data must be explained in the comments section of the eCRF. The electronic data capture system (EDC) will maintain an audit trail.

## *9.4 Records Retention*

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

# 10 Study Monitoring, Auditing, and Inspecting

## *10.1 Study Monitoring Plan*

This study will be monitored regularly by the coordinator and investigator to ensure accurate data entry and reporting. If other agencies choose to monitor the site such as the sponsor (NIH), FDA, or institution (UNC), then the Investigator will ensure the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.
The monitor is responsible for ensuring:

(a) the rights and well-being of trial participants are protected,
(b) reported trial data are accurate, complete, and verifiable from source documents,
(c) the conduct of the trial is in compliance with the currently approved protocol/amendments, with GCP, and with the applicable regulatory requirements, and
(d) data reported are verifiable to support meeting the study objectives.

Procedures and sources of information:
Protection of the rights and well-being of study subjects by verifying:

1. Investigator(s) have adequate qualifications, education and training, and resources necessary to conduct the study.
2. Verify the investigator and his staff follow the approved protocol and all approved amendments, if any
3. The investigator personally conducts or adequately supervises his study staff
4. Study staff are adequately trained and study functions are delegated to authorized individuals
5. Written informed consent was obtained before each subject's participation in the study
6. Enrollment of only eligible subjects
7. Subjects are instructed in the proper use, storage and return of the investigational product
8. Adequate and accurate case histories that record all observations are maintained
9. Unanticipated adverse events are reported in accordance with the protocol

Trial data are accurate, complete and verifiable from source documents as verified from review of subject file, case report forms, and medical procedures reports

1. Verify that the investigative staff are performing the specific trial functions in accordance with the protocol
2. Checking the accuracy and completeness of case report form entries, source documents and other trial related records against each other
3. Adequate and accurate case histories that record all observations are maintained
4. All withdrawals and dropouts are properly reported and explained
5. Investigator is informed of protocol deviations, data entry errors and omissions and illegibility

Study is conducted in compliance with the currently approved protocol/amendments, with GCP, and applicable regulatory requirements

1. Verify the investigator follows the approved protocol and all approved amendments, if any
2. Ensuring that an IRB approval is obtained prior to study initiation and that the IRB is kept informed of changes in research activity and unanticipated problems involving risk to subjects
3. Determine if investigator is maintaining essential documents in a regulatory binder
4. Investigational product is properly procured, stored and destroyed accordingly
5. Documenting deviations from the protocol, SOPs, GCP and applicable requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.
6. Monitor will provide a written report after each study visit or study visit communication

### *10.2 Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

## 11 Ethical Considerations

This trial will be conducted in compliance with institutional review board (IRB) and ICH GCP Guidelines including Title 21 Part 56 of the United States of America (USA) Code of Federal Regulations (CFR) relating to IRBs and GCP as described in the United States Food and Drug Administration (FDA) CFR (21 CFR § 50, 56, 312) in accordance with applicable ICH regulations regarding clinical safety data management (E2A, E2B(R3)), with ICH regulations regarding scientific integrity (E4, E8, E9 and E10) and with FDA regulations regarding financial disclosure (21 CFR § 54). In addition this trial will adhere to all local regulatory requirements, and requirements for data protection.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided an IRB-approved consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

## 12 Study Finances

### *12.1 Funding Source*

This study is funded by a grant from NIH (R01 DK101856).

### *12.2 Conflict of Interest*

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of North Carolina investigators will follow the University conflict of interest policy.

### *12.3 Subject Stipends or Payments*

Subject incentives are graded, with increasing payments for each phase of the study to maximize subject retention in the trial ($25 at the baseline visit, $25 at randomization, $50 at the mid-treatment visit, $50 at treatment end, and $50 at the study end/recurrent symptoms visit). Subjects are paid only for visits completed. This total of $200 per subject for 122 subjects randomized to treatment will be distributed over the 36 month enrollment period.

# 13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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