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Oral Viscous Budesonide Is Effective in Children with Eosinophilic Esophagitis in a Randomized, Placebo-Controlled Trial

Ranjan Dohil, M.D., Robert Newbury, M.D., Lyman Fox, MS.,
John Bastian, M.D., Seema Aceves M.D., PhD.

From the Departments of Pediatrics and Pathology, University of California, San Diego, La Jolla, California and Rady Children's Hospital San Diego

Address reprints and correspondence to: Ranjan Dohil, MD.
UCSD Medical Center,
Hillcrest,
200 West Arbor Dr.,
San Diego, CA 92103-8450
Tel: 619 543 7544
Fax: 619 543 7537
E-Mail: [**rdohil@ucsd.edu**](mailto:rdohil@ucsd.edu)

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ABSTRACT

Background & Aims: Eosinophilic esophagitis (EoE) is caused by immunologic reactions to ingested/inhaled allergens. The diagnosis is considered if ≥ 15 eosinophils/hpf are detected in mucosal biopsies. Placebo-controlled studies have not been conducted to evaluate the safety and efficacy of oral viscous budesonide (OVB).

Method: Children with EoE were randomly assigned to groups that were given oral viscous budesonide (OVB; $n=15$) or placebo ($n=9$). Patients <5 ft and ≥ 5 ft tall received 1mg and 2mg OVB daily, respectively. All patients received lansoprazole. The duration of treatment was 3 months, followed by repeat endoscopy and biopsies. Patients were classified as responders if their peak eosinophil counts were ≤ 6 eos/hpf, partial-responders with 7–19 eos/hpf, or non-responders with >20 eos/hpf. Baseline and post-treatment symptoms and endoscopic and histologic features were scored.

Results: Thirteen (86.7%) children given OVB ($P<0.0001$) and none that received placebo ($P=0.3$) were classified as responders. Mean pre/post-treatment peak eosinophil counts were 66.7 and 4.8 eos/hpf, respectively, in the group given OVB ($P<0.0001$); they were 83.9 and 65.6 eos/hpf, respectively, in the group given placebo ($P=0.3$). In the group given OVB, there were significant reductions from baseline values in proximal ($P=0.002$), mid- ($P=0.0003$) and distal- ($P=0.001$) esophageal eosinophilia. Following OVB therapy, compared with baseline, the mean symptom ($P=0.0007$), endoscopy ($P=0.0005$), and histology scores improved ($P=0.0035$) significantly.

Conclusions: OVB is effective treatment for pan-esophageal disease in children with EoE. OVB improves symptoms, endoscopic and histologic features. PPI single therapy did not significantly improve esophageal eosinophilia or symptoms of EoE.

Key Words. Topical corticosteroids; lansoprazole; lamina propria fibrosis; scoring tools.

Eosinophilic esophagitis (EoE) is a clinicopathologic disease characterized by esophageal eosinophilia and gastrointestinal symptoms. It most likely represents an immunologic reaction to ingested and inhaled allergens¹⁻⁵. The prevalence of EoE is increasing with rates now ranging from 6 to 30 cases per 100,000.⁶⁻⁹ Although this may also represent increased physician-recognition, it is likely that the rise in prevalence is real and mirrors the recent increase in prevalence of atopic disorders such as asthma^{8, 10}. The diagnosis of EoE is dependent upon finding ≥ 15 eosinophils per high power field (eos/hpf) in esophageal mucosal biopsies^{11, 12}. Symptoms of EoE often mimic gastroesophageal reflux disease (GERD) but are often refractory to acid-suppression therapy. Symptoms include regurgitation, vomiting, pain, anorexia and dysphagia^{10, 11}. Although the optimal therapy for EoE is unclear, a major concern for untreated EoE is esophageal remodeling and the development of strictures which are reported in 16-40% of adult patients¹³⁻¹⁵. A likely prerequisite of esophageal remodeling is the presence of lamina propria (LP) fibrosis as seen under light microscopy and measured by levels of Transforming Growth Factor- β_1 (TGF β_1 and phosphorylated Smad2/3)¹⁶⁻¹⁸. We recently reported that LP remodeling associated with EoE diminishes with topical corticosteroid therapy^{16, 17}, and also that patients with specific polymorphisms in the TGF β_1 -promotor gene may respond better to corticosteroid therapy¹⁷.

Treatment options for EoE are dietary restriction including those identified by skin testing⁵, six-food elimination diet¹⁹ and elemental formula^{1, 20}. Systemic corticosteroids²¹ are effective but are seldom warranted. Topical corticosteroids such as fluticasone propionate, administered through a metered-dose inhaler (MDI) and oral viscous budesonide suspension (OVB), can safely induce and maintain low esophageal eosinophil levels²²⁻²⁸. We previously reported our retrospective experience of 20 children with EoE treated with OVB for 3-4 months²⁷. Eighty percent of these patients achieved the histologic study endpoint and also had significant improvement of their symptoms and endoscopic findings²⁷.

In this report we describe the results of the first placebo-controlled study using OVB for the treatment of EoE in children. Histologic, symptomatic and endoscopic responses to OVB therapy are reported. In addition, esophageal biopsies containing LP were evaluated for vascular activation and TGF β_1 .

METHODS

This randomized, double-blinded, placebo-controlled study to determine the efficacy and safety of OVB was approved by Rady Children's Hospital, San Diego (RCHSD) and University of California at San Diego (UCSD) Human Research Protection Program and informed consent was obtained for each participant. A separate informed consent was obtained for genotyping. This study was conducted under Investigator Investigational New Drug Application (IND) #77,022.

Screening. Patients were referred to the Eosinophilic Esophagitis Clinic at RCHSD by pediatric gastroenterologists and allergists. Patients had an established diagnosis of EoE including, when necessary, a previous challenge with proton pump inhibitor (PPI) therapy with repeated endoscopy and biopsies to exclude GERD. At initial evaluation patients were offered alternative non-study therapeutic options. Individuals with esophageal peak eosinophil counts ≥ 20 eos/hpf were asked to participate by the study coordinator. Biopsies were obtained less than two months before study entry and no changes in diet, asthma or allergy therapy were allowed in the interim or during the study. Specific dietary restrictions are reported in Table 1. Baseline and post-treatment morning cortisol levels (normal range 7-25ug/dL) were measured to estimate adrenal suppression using an Automated Chemiluminescent Immunoenzymatic Assay System (BeckmanCoulter, Brea, CA).

Randomization and Treatment. OVB is a viscous liquid consisting of budesonide nebulizer suspension (Pulmicort respulesTM, AstraZeneca) mixed with sucralose (SplendaTM). Using a validated computer-based random number generator (Proc Plan in SAS version 9.1) subjects were randomized 2:1 into OVB and placebo groups, respectively. Both groups received lansoprazole solutabs (PrevacidTM, TAP Pharmaceuticals). Subjects $< 5'$ tall received OVB 1mg and those $\geq 5'$ tall received OVB 2mg. Subjects < 10 years old received lansoprazole 15mg twice daily and those ≥ 10 years old received 30mg twice daily. Treatment lasted 3 months. Physicians, pathologists, study coordinators, statisticians and patients were blinded to treatment during the study. Using appropriate precautions, budesonide suspension (0.5mg/ 2ml) or the same volume of placebo (sterile water) were placed by the investigational pharmacist into sealed light-protective vials. Formal stability analysis on budesonide suspension had confirmed the drug to be stable for at least 3 months in the vials. Subjects received a study number which was affixed to each vial of drug/placebo which were dispensed monthly. Only the

pharmacist had access to the randomization code. Immediately before ingestion, preferably at bedtime, the contents of each vial was mixed with Splenda™ (10 one gram packets to 1mg budesonide to create approximately 8ml volume). Patients did not eat/drink for 30 minutes after drug ingestion.

Patients were withdrawn from the study if they were non-compliant with therapy (<50% drug) or if they required systemic corticosteroid therapy for reasons including acute asthma. Compliance was determined every month by unopened vial count. During the study collected data was stored in a locked cabinet. One month after study completion the database was “locked” and the randomization code revealed.

Outcome Measures. The primary outcome measure was improvement of esophageal eosinophilia. The response to therapy was determined by comparing baseline and final treatment peak counts/hpf under light microscopy (x400). Patients were categorized into *responders* (0-6 eos/hpf), *partial-responders* (7-19 eos/hpf) and *non-responders* (≥ 20 eos/hpf) based on final treatment biopsy results. Secondary outcome measurements included response of symptoms and endoscopic and histologic features to treatment. These were ascertained using previously reported, but as yet non-validated tools²⁹⁻³¹. (See Table 2).

Upper Gastrointestinal Endoscopy and Endoscopy Scoring Tool (EST). Repeat endoscopy was undertaken using the Olympus P160 endoscope by (RD) at 3 months of treatment. Mucosal biopsies were taken from the distal (2-3cm above the gastroesophageal junction), proximal (2-3cm below the cricopharyngeus muscle) and mid (midway between distal and proximal) esophageal levels. Gastric and duodenal biopsies were also obtained. An EST, previously used to differentiate the endoscopic features of EoE from GERD²⁹, was modified for this study. The endoscopy score was determined by RD who performed over 80% of baseline EGDs and all follow-up procedures. The other baseline endoscopy scores were determined using endoscopy reports and photographs. The maximum endoscopy score was 15.

Patient Monitoring and Symptom Scoring Tool (SST). The SST, devised originally for children with acid-peptic disorders, is used regularly in our clinic^{27, 30, 31}. The SST has been used to differentiate between EoE, GERD and patients with other atopic and non-atopic disorders.²⁹ Physical examination and symptom questionnaire completion occurred at monthly clinic visits. In addition, the subjects/guardians were contacted every week and the SST was recorded (max. 14 points). Baseline symptom scores were estimated

from the questionnaires and from physician-dictated clinical records taken within one month of study enrollment. There was no baseline criterion for minimum SST severity. Final symptom scores were calculated from the mean of the symptom scores recorded during the last month of treatment.

Biopsies and Histology Scoring Tool (HST). Proximal, mid and distal esophageal biopsies, taken before and after treatment, were processed routinely and evaluated using light microscopy (x400) by a pediatric pathologist (RN). Before entering the study all baseline biopsies were reviewed again by a blinded observer (RN). Two biopsies per level yielded between 15-20 evaluable hpfs. An HST was used to score features within the epithelium and lamina propria separately²⁹ because the latter was not present in each biopsy. When LP was present, features of fibrosis including the presence of fibroblasts, thickness of collagen bundles and collagen accumulation were evaluated¹⁶. (Table 2).

Transforming Growth Factor- β_1 and TGF β_1 Promotor Genotype. Lamina propria remodeling is associated with increased TGF β_1 -positive and pSmad2/3-positive eosinophils and also increased expression of TGF β_1 signaling transcription factor in the sub-epithelial space¹⁶⁻¹⁸. Following staining of biopsies containing LP with TGF β_1 and pSmad2/3 primary antibodies (Santa Cruz Biochemicals, Santa Cruz, CA) positive-stained cells were enumerated in 3-5 hpf at x400 magnification¹⁶. The mean value was calculated and expressed as TGF β_1 -positive and pSmad2/3-positive cells/hpf.

A polymorphism (TT genotype) of the C-509T promoter region of TGF β_1 has been associated with increased TGF β_1 levels and also more severe airways obstruction in asthmatics³². Patient's DNA was isolated from peripheral blood and the C-509T SNP of the TGF β_1 promoter was analyzed. The TGF β_1 promoter genotype was assessed using forward (5'-GGGGACAGTAAAATGTATGGG-3') and reverse (5'-GTCACCAGAGAAAGAGGAC-3') TGF β_1 promoter primers followed by digestion with DdeI to yield 242, 189, and 53 base-pair products analyzed by gel electrophoresis¹⁷. The frequency of the CC, CT and TT alleles in the study group was evaluated and correlated, when possible, with the severity of LP fibrosis and the response to OVB therapy.

Statistical Analysis. The *a priori* power of the primary endpoint analysis was estimated using Fisher's exact test with a 0.05, two-tailed significance level. Assuming a histologic response rate of 80% in the OVB group and 20% in the placebo group, there was 85% power to detect a significant between-group difference with sample sizes of 20 and 10, respectively.

All statistical analyses were conducted using SAS® Version 9.1. Inferential statistical testing was performed with two-tailed tests at the 0.05 significance level. Efficacy analyses for histology, endoscopy, and symptom score variables were conducted on the modified intent-to-treat analysis set, which was defined as all subjects who received at least one dose of study drug and completed both pre- and post-treatment biopsy assessments.

Hypothesis tests for the primary efficacy endpoint (i.e., the percentage of histologic responders) were analyzed using Fisher's exact test. Discrete secondary efficacy variables including the percentage of subjects with $\geq 50\%$ reduction in the clinical symptom score from baseline at each week, and the percentage of subjects with complete histologic resolution and complete resolution of symptoms were also analyzed using Fisher's exact test. Change from baseline in maximum peak eosinophil count, total endoscopy score, and absolute and percent change from baseline in clinical symptom score were analyzed using an analysis of covariance (ANCOVA) model with a main effect for treatment and the baseline score as a covariate. For those instances in which deviations may have occurred from the statistical assumptions underlying the ANCOVA model (e.g., non-normality or heterogeneity of variance), a sensitivity analysis was also performed and consisted of ranking the scores/counts across the two treatment groups, and then performing an ANCOVA on the ranked data³³. The resulting p-values from this nonparametric analysis are identified and provided alongside the ANCOVA p-values, where applicable.

RESULTS

Between February 2008 and July 2009 thirty-one patients with EoE were randomized, 24 completed the study and were included in the final analysis. Of these 24 subjects, 15 received OVB and PPI and 9 received placebo and PPI. One patient, with a prior diagnosis of EoE, had symptoms, pan-esophageal furrowing and exudates on endoscopy but a peak eosinophil count of 15 eos/hpf (#30) was also enrolled. There were 20 males and 4 females (mean age was 7.8 years, range 1-17 yrs, median 7yrs). Eighteen children had asthma, eczema and/or allergic rhinitis, 11 had food/aeroallergen allergy with positive skin and/or RAST testing. There was no significant difference between the OVB and the placebo groups for race/ ethnicity, age, presence of atopy or food/aeroallergen

sensitization, previous use of PPIs, height and weight (before or after treatment). (See Table 1).

Final analysis was not possible in seven patients who withdrew from the study without repeat endoscopy. Reasons for withdrawal included not wanting to take SplendaTM, acute asthma requiring systemic corticosteroids, non-compliance with therapy, adverse event (transient rash attributed to lansoprazole, #10). No serious adverse events were reported in 24 study-completers. One patient (#20) developed oral candida which responded to nystatin. Esophageal candida was not found at follow-up EGD in any of the subjects.

There was no significant difference between pre-/post-treatment mean morning cortisol levels which were 12.7 ug/dL (range 4.1-21) and 9.1 ug/dL (range 4.4-16), respectively, for the OVB group and 10.1 ug/dL (range 5.8-14) and 10.4 ug/dL (range 5.3-16), respectively, for the placebo group ($p=0.31$).

Primary Outcome

Peak Esophageal Eosinophil Count.

Oral viscous budesonide and PPI therapy significantly reduced the mean peak eosinophil count from baseline when compared with placebo and PPI ($p=0.0001$; nonparametric test $p=0.0003$). This reduction in peak eosinophil count in the OVB group, compared with placebo, was significant at all esophageal levels including proximal (0.0024), mid ($p<0.0001$) and distal ($p=0.0001$). Peak eosinophil count responses to study drug are shown in Figure 1. All gastric and duodenal biopsies were normal. Mean compliance with study treatment for both groups group was 80%. (See Table 3 and 4).

Oral Viscous Budesonide Group. Thirteen of 15 patients (86.7%) who received OVB were *responders* ($p<0.0001$), one was a *partial-responder* (#26) and one a *non-responder* (#32). The mean pre- and post-treatment peak eosinophil counts for the OVB group were 66.7 (range 22-125) and 4.8 eos/hpf (range 0-25), respectively. When biopsies, taken pre-/post-treatment from the same levels, were compared there was a significant reduction in peak eosinophil counts in the proximal ($p=0.002$), mid ($p=0.0003$) and distal ($p=0.001$) esophagus, respectively. The peak eosinophil count was 0-1 eos/hpf in biopsies from the distal, mid and proximal esophagus in 8, 10 and 11 subjects, respectively. The peak count was ≤ 6 eos/hpf from distal in 13, mid in 14 and proximal in 14. OVB was therefore effective in reducing pan-esophageal eosinophilia. All patients <5 ft tall ($n=11$, mean age 6.3yrs, range 2-10yrs) responded to therapy. Of the 4 patients ≥ 5 ft tall (mean 15.1yrs,

range 13-17yrs), two responded, one was a *partial-responder* (#26), one was a *non-responder* (#32). Patients #26 and #32 returned 30% of the OVB vials unopened.

Placebo Group. None of the 9 patients (0%) who received placebo were *responders*. The mean pre-/post-treatment peak eosinophil counts for the group was 83.9 (range 15-140) and 65.6 eos/hpf (range 7-130)($p=0.3$), respectively. When biopsies, taken pre-/post-treatment from the same levels, were compared there was no significant reduction in peak eosinophil counts in the proximal ($p=0.96$), mid ($p=0.56$) and distal ($p=0.34$) esophagus. Therefore, placebo and PPI therapy did not significantly reduce the eosinophilia at any esophageal level. One patient (#6) was a *partial-responder* (post-treatment peak eosinophil count was 7 eos/hpf with basal zone hyperplasia (BZH) in esophageal biopsies). After study completion, subject #6 commenced open-label OVB therapy and eosinophils and BZH were absent in repeat biopsies.

Secondary Outcomes

1. Upper Gastrointestinal Endoscopy Score.

The EST was used to measure the endoscopy score. The mean total endoscopy score fell significantly following OVB compared with the placebo group ($p=0.041$; nonparametric test $p=0.0418$). (See Table 3 and 4).

Oral Viscous Budesonide Group. There was a significant improvement in the mean endoscopy score from 4.6 (range 0-9) before and 1.5 (range 0-7) after treatment ($p=0.0005$). In 9 of the 15 patients the endoscopy score normalized after treatment. None of the group had an esophageal stricture.

Placebo Group. Although, the mean endoscopy score improved from 7.8 (range 4-9) before to 5.4 (range 1-9) ($p=0.041$) after treatment, none of the endoscopy scores completely normalized. One patient (#19) had an esophageal stricture.

2. Symptom Score.

The most commonly reported symptoms were dysphagia, vomiting, regurgitation and abdominal pain. The mean total symptom score decreased significantly from baseline following OVB compared with the placebo group ($p=0.031$; nonparametric test $p=0.0458$). When the mean symptom scores, taken from all patients within the group and averaged for months 1, 2 and 3 were compared with baseline, the mean scores improved significantly in the OVB group but not in the placebo group. (see Figure 2).

Oral Viscous Budesonide Group. The mean symptom score improved from 3.5 (range 0-10) at baseline to 1.2 (range 0-7) after treatment ($p=0.0007$). By study week 3, there was a significant change in mean symptom score compared with baseline ($p < 0.05$). Symptom categories that significantly improved compared with baseline were heartburn/regurgitation ($p=0.008$), nausea/ vomiting ($p=0.004$) and dysphagia ($p=0.017$). Seven patients had a post-treatment symptom score of zero.

Placebo Group. The mean symptom score was 2.7 (range 1-6) before and 1.8 (range 0-7) after treatment ($p=0.22$). None of the symptom categories showed significant improvement from baseline. Placebo and PPI did not significantly improve symptoms in most patients. Only two patients had a post-treatment symptom score of zero.

3. Epithelial and Lamina Propria Histology Score (EHS and LPHS).

The EHS was measured in all patients and the LPHS was measured in 13 and 6 subjects before and after treatment, respectively. There was an improvement in EHS ($p=0.0035$) and LPHS over baseline in the OVB group when compared with the placebo group.

Oral Viscous Budesonide Group. The decrease in mean EHS from baseline (6.3 to 1.0) following treatment was significant ($p<0.0001$). Paired (from the same subject) pre- and post-treatment biopsy samples containing LP were available for 2 patients in the OVB group. There was a decrease in mean LPHS from baseline of 3.8 to 0.4. These results suggest that both LP eosinophilia and fibrosis improves with oral viscous budesonide. (See Table 3/Figure 3)

Placebo Group. The change in mean EHS (8.1 to 6.8, $p=0.11$) from baseline was not significant. Paired pre- and post-treatment biopsy samples containing LP were available for 3 patients in the placebo group. There was a decrease in mean LPHS from baseline of 4.0 to 3.5.

TGF β ₁ and TGF β ₁ Promotor Genotype. TGF β ₁ promotor C-509T SNP was evaluated in 11 patients in the OVB group. TGF β ₁ and pSmad2/3 staining were evaluated in biopsies with LP (11 biopsies from 8 patients; 7 pre, 4 post) including 3 with paired pre/post (1 OVB, 2 placebo group) biopsies.

Genotype. Of the 9 OVB-responders who were evaluable for genotyping, 7 had the genotype CC/CT (2 CC, 5 CT), the *partial-responder* was CC, the *non-responder* was TT. One patient in the placebo group was TT genotype and had an esophageal stricture;

one placebo patient was CC and subsequently responded to open label OVB. The remaining genotyped placebo patients (6) were CT.

TGF β ₁. Quantitative immunohistochemistry of biopsies demonstrated that OVB reduced the numbers of TGF β ₁ and pSmad2/3 positive cells in the lamina propria (Figure 3c). Prior to therapy, OVB and placebo patients had similar numbers of TGF β ₁ (106 versus 78) and pSmad2/3 (73 versus 105) positive cells. In contrast to OVB treated patients, patients on placebo continued to have elevated numbers of TGF β ₁ (OVB: 29, placebo: 131) and pSmad2/3 positive cells (OVB: 58, placebo:108). In this limited number of patients, OVB decreased the fibrosis score from 2.5 to 0 following treatment, placebo treatment did not (mean=2.3 before and 2.5 following therapy). Both TGF β ₁ ($r=0.89$, $p=0.002$) and pSmad2/3 ($r=0.65$, $p=0.03$) positive cells correlated with the severity of fibrosis.

DISCUSSION.

Oral viscous budesonide is a thick liquid which is intended to coat the full length of the esophagus and therefore treat pan-esophageal eosinophilia²⁷. In a previous retrospective study of OVB in children the primary histologic endpoint (≤ 7 eos/hpf) was achieved in 80% of patients²⁷. In our present study, which is the first randomized, double-blind, placebo controlled study using OVB, the histologic end-point of ≤ 6 eos/hpf was achieved in 87% of children with EoE. In addition, patients receiving OVB, had a significant improvement in symptom score, endoscopy score, epithelial and LP histology scores. A recent placebo-controlled study using fluticasone, administered through an MDI, showed that 50% of patients responded to therapy compared with 9% in the placebo group ($p=0.047$); however, when the same histologic endpoint as used in our study (≤ 6 eos/hpf) was applied, a statistically significant difference between fluticasone and placebo groups was not achieved²⁸. The patients that did respond to fluticasone were younger (i.e., mean age 5.9yrs for responders versus 11.3yrs for non-responders), shorter (i.e., mean height of 118.3 for responders and 146.2cm for non-responders) and non-allergic patients²⁸. Response to fluticasone was particularly poor in the distal esophagus compared with OVB (mean distal eosinophil counts were 82.2eos/hpf before and 45.7 eos/hpf after fluticasone, compared with 48.9 eos/hpf before and 4.1 eos/hpf after OVB therapy). In our study we show that OVB is an effective therapy for pan-esophageal disease with 87% of OVB post-treatment patients having ≤ 6 eos/hpf in the distal esophageal biopsies and

100% having ≤ 6 eos/hpf in proximal esophageal biopsies. All patients < 5 ft tall (mean age 6.3 yrs, mean height 119.4cm, range 81.7-175.2cm) receiving OVB responded to therapy. Of the 4 patients ≥ 5 ft tall (mean age 15.1 yrs) two responded (mean height 169.1cm), one patient failed and another had a partial response to OVB (mean height of partial- and non-responder was 173.2cm). The non-responder failed to respond only in the distal esophagus (before OVB treatment his eosinophil counts in proximal, mid and distal biopsies were 31, 35 and 48 eos/hpf, respectively, and after treatment were 0, 1 and 17 eos/hpf, respectively) suggesting that in this tall child adequate amounts of drug may not have reached the distal esophagus in this patient. Clearly some of the taller patients do respond to therapy and it is unlikely that height alone determines response to therapy with OVB as other factors such as drug compliance will play a role. Nine (60%) of the 15 patients in the OVB group had negative food/aeroallergen skin prick/ RAST allergy testing and all (100%) of these patients responded to therapy. Of the six who tested positive and received OVB, 4 responded, one was a *partial-responder* and the other a *non-responder*. The *non-responder* and *partial-responder* were older, taller, male allergic children (15 and 17yrs) who returned 30% of there study drug vial unopened.

The threshold esophageal eosinophil count required for the diagnosis of EoE is not clearly established although peak levels from ≥ 15 to ≥ 24 eos/hpf are used^{10-12, 16, 27-29, 34-37}. Some would stipulate that a trial of PPI therapy is mandatory before the diagnosis of EoE is made as GERD may also cause esophageal eosinophilia. This statement was initially predicated on findings in only three patients³⁶. However, in most cases of GERD esophageal eosinophilia is uncommon and relatively mild. In a recent retrospective study of patients with esophageal eosinophilia, patients with ≥ 20 eos/hpf, including those with abnormal 24h pH studies, were shown to respond poorly to PPI therapy³⁷. In our own study none of the study patients receiving placebo plus PPI actually reached the end point of the study. Although one patient did, however, come close with peak counts falling from 75 to 7 eos/hpf, it was only after post-study treatment with OVB that the esophageal eosinophilia and BZH disappeared completely. Compared with baseline, the placebo group in our study did not show a significant reduction in peak eosinophil count at any esophageal level, or significant improvement in EHS and LPHS (see Table 3). This would suggest that treating all patients routinely with PPI therapy before establishing a diagnosis of EoE may not be indicated and will in most patients only prolong the eosinophilic inflammation and associated symptoms.

Although, symptoms and endoscopic features do not always correlate with esophageal eosinophilia^{10, 27} we have, in this study, utilized previously published tools²⁹ to monitor the symptomatic, endoscopic and histologic response to therapy. Patients in the OVB group had a significant improvement in mean symptom score compared both to baseline ($p=0.0007$) and also to the change in symptom score seen in the placebo group ($p=0.031$). Symptoms of nausea/vomiting, heartburn/regurgitation and dysphagia significantly improved with OVB. The mean total endoscopy score significantly improved in the OVB treated patients when compared with baseline ($p=0.0005$) and also to placebo ($p=0.041$). The EHS and the LPHS improved notably with OVB therapy, but not with placebo. This finding in the LP tissue was particularly important as it supports our recently reported observations that responders to OVB have a reduction in LP remodeling as measured by decreased profibrotic factors $TGF\beta_1$ and $pSmad2/3$ ¹⁷. Immunologic staining confirmed that $TGF\beta_1$ -and $pSmad2/3$ -positive cells decrease significantly in LP following OVB but not with placebo; however, the small numbers of patients with paired pre/post LP was not amenable to statistical analysis. $TGF\beta_1$ -and its signaling pathway $pSmad2/3$ are important mediators of fibrosis^{16, 18}. The number of $TGF\beta_1$ and $pSmad2/3$ positive cells correlated strongly with the severity of LP fibrosis. Our findings would suggest that reduction in esophageal eosinophilia induced by topical corticosteroids may be associated with reduced LP fibrosis and possibly remodeling.

Genetic polymorphisms (CC, CT, TT) at 509 in the $TGF\beta_1$ promoter region may be associated with increased $TGF\beta_1$ levels and also more severe airway obstruction in asthmatics³². In a recent report, EoE patients with the CC genotype all responded to OVB and also had fewer LP $TGF\beta_1$ -positive cells^{16, 17}. It was postulated that the CC genotype is a less fibrotic, more steroid-responsive form of EoE than the CT or TT genotype. In our present study 11 of 15 patients who received OVB were genotyped. Three were CC with 2 *responders* and 1 *partial-responder*. One patient in the placebo group was CC and later responded to open label OVB therapy. Although the numbers in this study are very small and larger studies are indicated, the findings are consistent with our previous observation that most CC patients are responsive to topical budesonide therapy while CT and TT patients have a variable response to topical corticosteroid therapy.

Oral viscous budesonide is an effective treatment for pan-esophageal eosinophilia in patients with EoE and reduction in eosinophil count (to ≤ 6 eos/hpf) correlates well with symptomatic and endoscopic improvement. Although patients with negative

food/aeroallergen skin prick/ RAST allergy testing appear to respond better, patients testing positive do also respond and should also be considered for OVB therapy. In our small group of patients, subepithelial fibrosis was seen to improve with OVB therapy. Whether or not this has implications for preventing future stricture formation is not known but merits further evaluation. Although 3 months of therapy with OVB is short and does not allow for an evaluation of long-term outcomes and the influence on the natural history of EoE, data from this study does support the need for a proprietary OVB medication specifically for the treatment of EoE.

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Legend.

Table 1. Patient data. Atopic patients had asthma (As), eczema (Ec) and allergic rhinitis (AR). Patients were deemed food or aeroallergen sensitized/allergic if RAST or skin-prick testing was positive. Patients <5ft tall received 1mg OVB and those $\geq$ 5ft received 2mg. Patient #18 was randomized and collected drug, but, was treated with steroids for asthma and did not take study drug. Patients #25, 28 and 32 had received intermittent topical steroid therapy for asthma before, but not during the study. Dietary restrictions before baseline biopsies were maintained during the study and are shown above (M=milk, E=eggs, S=soy, W=wheat, N=nuts, F=fish).

Table 2. Scoring tools for endoscopy, histology and symptoms were taken from previous reports²⁹⁻³¹. For the symptom score, subjects requiring urgent hospital attention for esophageal food impaction or gastrointestinal bleeding within the previous 2 months received 2 points.

Table 3. Individual patient data is shown for subjects in the OVB and placebo groups both before and after treatment.

Table 4. Mean values for peak eosinophil counts, endoscopy, symptom and histology scores. Mean morning cortisol levels are shown in umol/dl. Baseline and post-treatment comparisons are made both within the groups and between the OVB and placebo groups. Statistical significance is noted by *italicized P value.

Figure 1. Individual pre- and post-treatment peak eosinophil counts following 3 months treatment with OVB and PPI (n=15) and placebo plus PPI (n=9).

Figure 2. Mean symptom scores during months 1, 2 and 3 of the study for patients in the OVB and placebo groups. Comparing changes from baseline, the mean symptom score improved significantly in the OVB group during months 1, 2 and 3., but not in the placebo group.

Figure 3a. Pre-treatment distal esophageal mucosal biopsy. Above, left, showing several intraepithelial eosinophils, basal zone hyperplasia and intercellular edema (H&E x 200). Above right, lamina propria with fibrosis and eosinophilic inflammation (H&E x 400).

Figure 3b. Post-treatment with OVB, distal esophageal biopsy from the same patient. Above left, unremarkable epithelium (H&E x 100). Above right shows lamina propria with no evidence of fibrosis or eosinophilic inflammation (H&E x 400).

Figure 3c. Esophageal mucosal biopsies (x400 light microscopy) stained for pro-fibrotic mediators showing LP in a *responder* patient. Upper images show TGF β ₁ positive (brown) cells and lower images show phosphorylated Smad2/3 positive (red) cells pre- and post-treatment with OVB.

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correlating symptoms with inflammation. *Ann Allergy Asthma Immunol* 2009;103:401-6.

PATIENT	AGE YRS	SEX	RACE/ ETHNICITY	HEIGHT	ATOPY	ALLERGY +RAST/SPT	DIETARY RESTRICTIONS	PREVIOUS PPI	LONG TERM THERAPY	ADVERSE EVENTS
Oral Viscous Budesonide + PPI Group										
#3	3	M	Non Hisp White	<5ft	As, Ec	No	-	Y	No	-
#4	6	M	Hispanic White	<5ft	AR	Yes	-	N	No	-
#5	15	F	Non Hisp White	≥5ft	Ec	No	M	Y	No	Emesis (wk 1)
#9	8	M	Non Hisp White	<5ft	As, AR, Ec	Yes	-	Y	Albuterol	-
#12	7	M	Non Hisp Afr Am	<5ft	No	Yes	-	N	Abilify	-
#13	13	M	Non Hisp White	≥5ft	As, AR, Ec	No	-	N	Zyrtec	-
#16	5	M	Non Hisp White	<5ft	No	No	M	N	No	-
#20	1	M	Hispanic White	<5ft	No	No	N	Y	No	Oral Candida
#21	5	F	Non Hisp White	<5ft	No	No	-	N	No	-
#22	10	M	Hispanic White	<5ft	AR	Yes	N/S/F	Y	Zyrtec	Transient headache
#25	4	F	Hispanic White	<5ft	As	No	M/E/Beef	Y	Singular, Flovent	-
#26	13	M	Non Hisp White	≥5ft	AR	Yes	-	N	No	-
#28	3	M	Hispanic White	<5ft	As, AR	No	-	Y	Claritin, Pulm Neb	-
#29	7	M	Non Hisp White	<5ft	AR	No	-	N	No	-
#32	17	M	Hispanic White	≥5ft	AR	Yes	-	Y	Flovent	-
Placebo + PPI Group										
#2	6	M	Non Hisp White	≥5ft	AR	No	-	Y	No	-
#6	10	F	Non Hisp Asian	<5ft	AR	No	Apple	Y	No	-
#14	2	M	Non Hisp Indian	<5ft	AR, Ec	Yes	W/E/S/N/Peas	Y	No	Eczema worse
#17	8	M	Non Hisp White	<5ft	No	Yes	Gluten	Y	Sulfasalazine	Chest infection
#19	16	M	Non Hisp White	≥5ft	As	Yes	-	N	No	Mild Abd Pain
#24	3	M	Hispanic White	<5ft	As	Yes	-	N	Zantac	Transient headache
#27	10	M	Non Hisp White	<5ft	As, AR	Yes	-	N	No	-
#30	12	M	Non Hisp White	<5ft	No	No	-	Y	No	-
#31	2	M	Hispanic White	<5ft	As	No	-	Y	Albuterol	Transient diarrhea
Withdrawal from Study										Reason
#1	15	M	Non Hisp White	≥5ft	As, AR,	Yes	-	N	Flovent	Sucralose Issue (week 1)
#7	15	M	Non Hisp White	≥5ft	Ec	Yes	W	N	Flovent	Steroids for asthma
#8	15	M	Non Hisp White	≥5ft	AR, Ec	Yes	-	N	No	Poor compliance

#10	10	M	Non Hisp White	<5ft	AR	No	-	Y	Albuterol	Rash (week 1)
#11	6	M	Hispanic White	<5ft	As,AR,Ec	Yes	-	N	Singular, Albuterol	Steroids for asthma
#15	5	M	Non Hisp White	<5ft	As	Yes	M/W/E/N	N	No	Sucralose Issue (week 1)
#23	5	F	Non Hisp White	<5ft	No	Yes	W/N	Y	Zyrtec, Nasonex	Lost to follow-up

Table 1. Patient data. Atopic patients had asthma (As), eczema (Ec) and allergic rhinitis (AR). Patients were deemed food or aeroallergen sensitized/allergic if RAST or skin-prick testing was positive. Patients <5ft tall received 1mg OVB and those $\geq$ 5ft received 2mg. Patient #18 was randomized (OVB group) and collected drug, but, was treated with steroids for asthma and did not take study drug. Patients #25, 28 and 32 had received intermittent topical steroid therapy for asthma before, but not during the study. Dietary restrictions before baseline biopsies were maintained during the study and are shown above (M=milk, E=eggs, S=soy, W=wheat, N=nuts, F=fish).

Eosinophilic Esophagitis Tool and Categories	Score in each Esophageal Level Proximal Mid Distal	Maximum score per Level/ Symptom	Maximum Total score
Endoscopy Scoring Tool (EST)			
- Mucosal pallor / reduced vasculature	0 = Absent 1 pt = present	3 pts	15 pts
- Linear furrows/ mucosal thickening			
- White plaques			
- Concentric rings / stricture			
- Friability / “tissue paper” mucosa			
Histology Scoring Tools			
Epithelial Histology Score			
- Peak Eosinophil Count	0 = 0 Eos/hpf 1 pt = 1-10 Eos/hpf 2 pts = 1-20 Eos/hpf 3 pts = 21-40 Eos/hpf 4 pts = 41-60 Eos/hpf 5 pts = >61 Eos/hpf	12 pts	Calculated as a mean of all three levels. Therefore maximum is 12 pts
- Basal zone hyperplasia	0 = < 20% of epithelial thickness 1 pt = 21-50% 2 pts = 51-75% 3 pts = >75%		
- Dilated intercellular spaces	0 = Absent 1 = Present		
- Epithelial desquamation	0 = Absent 1 = Present		
- Eosinophil Clusters/ Abscesses	0 = Absent 1 = Present		
- Degranulated eosinophils.	0 = Absent 1 = Present		
Lamina Propria Histology Score			
- Peak Eosinophil Count	0 = 0 Eos/hpf 1 pt = 1-5 Eos/hpf 2 pts = 6-20 Eos/hpf 3 pts = >20 Eos/hpf	6 pts	Calculated as a mean of all three levels. Therefore maximum is 6 pts
- Lamina Propria fibrosis	0 = Absent 1 pt = Mild 2 pts = Moderate 3 pts = Severe		
Including fibroblast and collagen bundles			
Symptom Scoring Tool			
- Heartburn/ regurgitation	0 = Absent 1 pt = Mild symptoms that are intermittent and do not interrupt daily activities. 2 pts = Severe symptoms that occurred every day and/or interrupted daily activities.	2 pts	14 pts
- Abdominal pain			
- Nausea/ Vomiting			
- Anorexia / Early satiety			
- Dysphagia			
- Symptom induced nocturnal wakening			
- Gastrointestinal bleeding			

Table 2. Scoring tools for endoscopy, histology and symptoms were taken from previous reports²⁹⁻³¹. For the symptom score, subjects requiring urgent hospital attention for

esophageal food impaction or gastrointestinal bleeding within the previous 2 months received 2 points.

ORAL VISCOUS BUDESONIDE + PPI

Patient	Response	Comp- liance %	Peak Eosinophil Count / hpf		Symptom Score (max. 14 pts)		Endoscopy Score (max. 15 pts)		Epithelial Histology Score (12pts)		Lamina Propria Histol Score (6)		Morning Cortisol (umol/dL)	
			Pre	Post	Pre	Post*	Pre	Post	Pre	Post	Pre	Post	Pre	Post
#3	Responder	100	27	0	4	6	4	0	4.3	0	4		-	4.4
#4	Responder	98	45	0	4	2	7	0	9	0	4		-	7
#5	Responder	83	125	4	4	2	4	2	4.3	2.7			15	8
#9	Responder	90	124	6	3	0	6	6	10.5	1.3	4		7.6	9.8
#12	Responder	64	25	2	9	0	6	0	5	0.7	6	1	7.1	4.7
#13	Responder	67	32	4	2	0	9	5	7	0.7			-	5.4
#16	Responder	93	74	4	1	3	0	0	9	1.3	3.3	0	16	10
#20	Responder	75	66	4	6	0	0	0	9.3	0.7			12	8.4
#21	Responder	68	85	0	2	1	6	0	7.7	0			14	9.2
#22	Responder	75	110	3	3	1	3	0	6	0.3			17	8.6
#25	Responder	83	110	3	4	0	5	0	7.3	1			13	16
#26	Partial	71	48	17	3	1	5	1	7	1.3	4		10	8.6
#28	Responder	78	20	0	6	3	0	0	1.5	0		0.3	4.1	9.9
#29	Responder	90	60	0	0	0	7	0	4	0	4		21	14
#32	Non-responder	67	47	25	2	0	7	7	2.7	5	1		14	12
Mean	86.7%	80.1	66.6	4.8	3.5	1.2	4.6	1.5	6.3	1.0	3.8	0.4	12.7	9.1

PLACEBO + PPI

#2	Non-responder	100	105	100	5	6	9	9	10.3	9.3	3		7.1	5.3
#6	Partial	82	75	7	4	3	5	2	4.5	1.5			10	12
#14	Non-responder	90	67	118	1	2	9	9	8.7	9.1	4	5	8.9	6.8
#17	Non-responder	82	75	35	2	0	9	4	8.7	5.3			11	14
#19	Non-responder	72	98	130	6	2	7	7	11.3	11			12	8.4
#24	Non-responder	62	90	82	3	0	9	1	8	8.7	4		14	16
#27	Non-responder	81	90	28	2	1	6	6	9	4.3			5.8	6
#30	Non-responder	90	15	42	0	2	7	6	3.7	5.7	3	3	13	15
#31	Non-responder	65	140	48	1	1	9	5	8.7	6	6	2.5	9.3	-

Mean	0%	80.4	83.9	65.6	2.7	1.8	7.7	5.4	8.1	6.8	4.0	3.5	10.1	10.4
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Table 3. Individual patient data is shown for subjects in the OVB and placebo groups both before and after treatment.

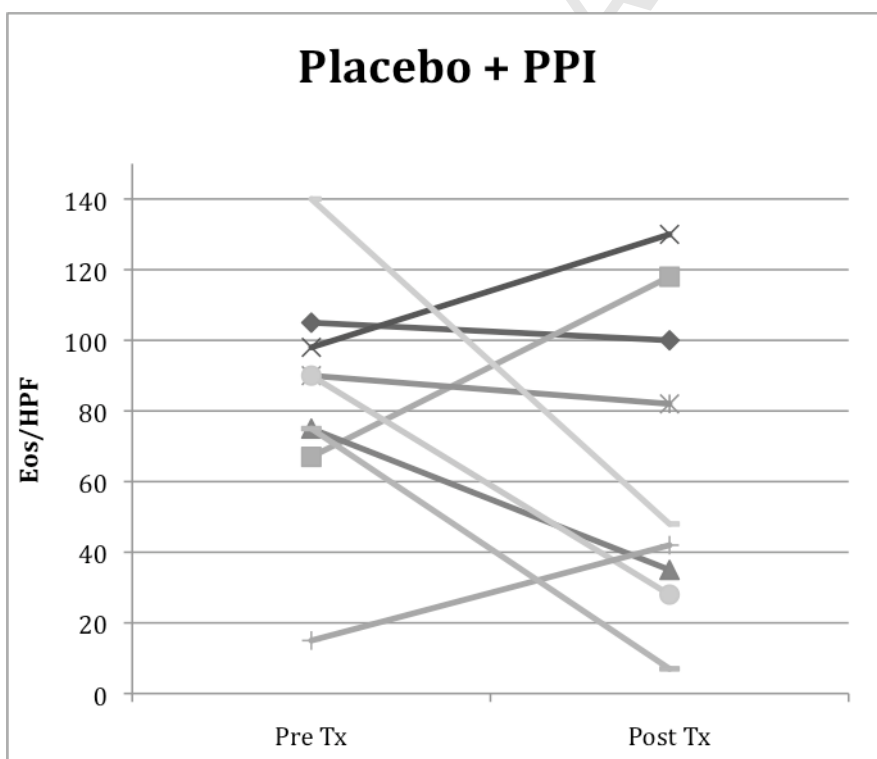
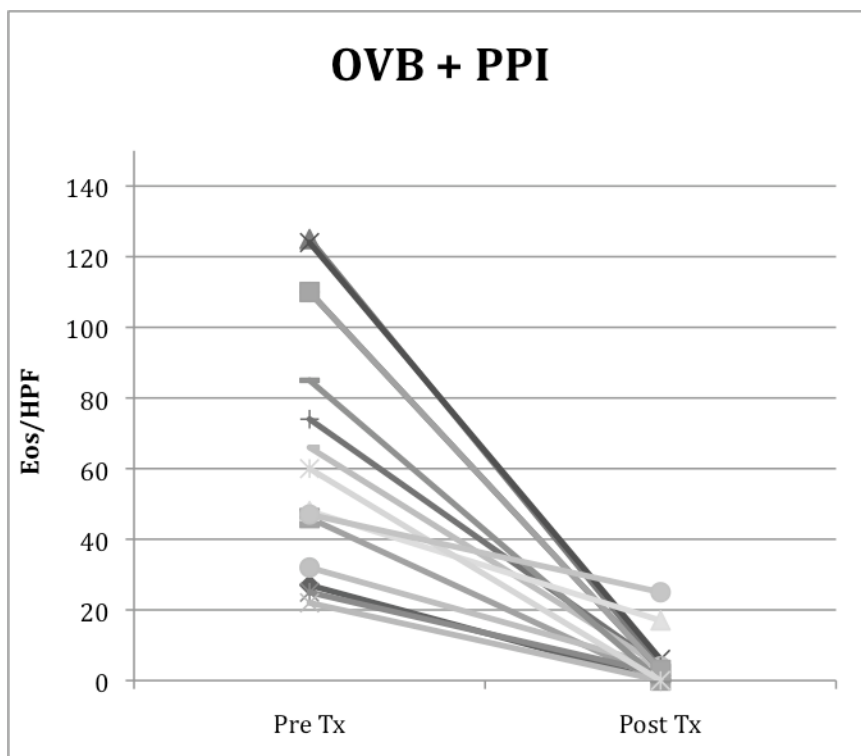
Represents a mean of the symptom scores measured during the last month of treatment (i.e., at weeks 9-12).

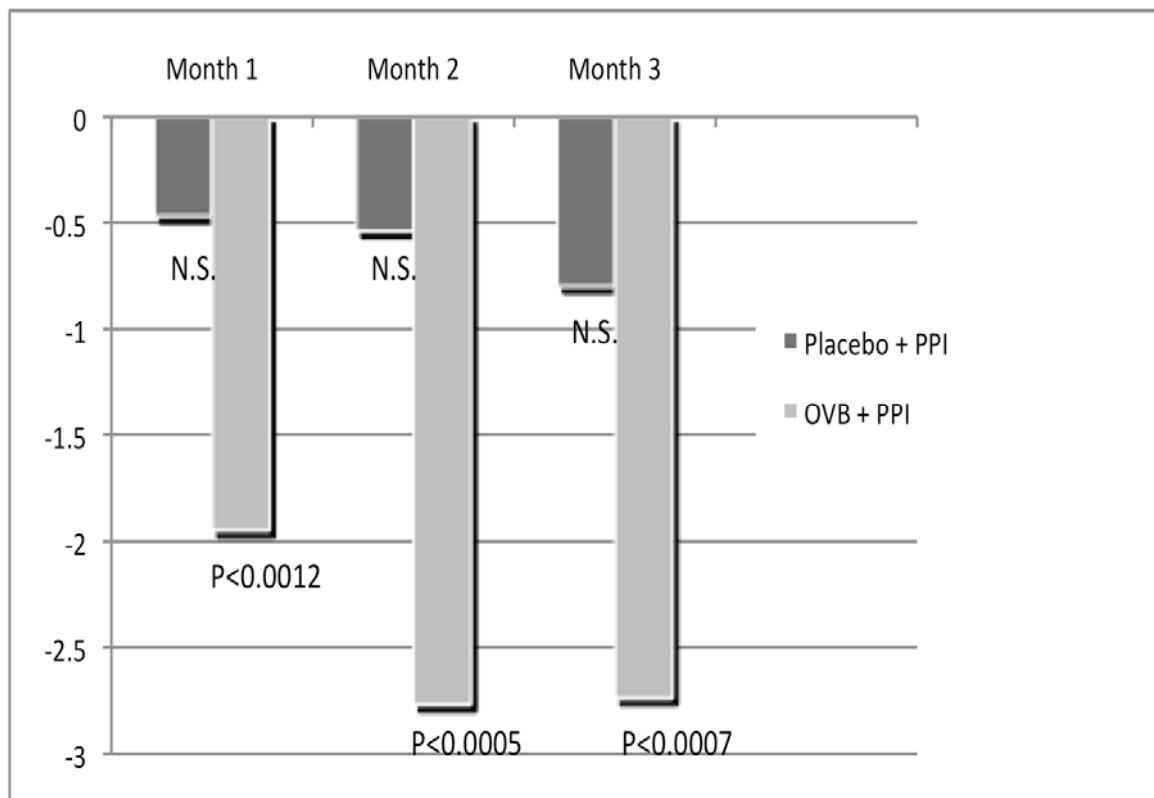
Category According to Feature or Esophageal level	OVb +PPI Mean Values (SD/Median) Change from Baseline within Group		Placebo +PPI Mean Values (SD/Median) Change from Baseline within Group		Change From Baseline OVb Group Superior to Placebo Group
	Pre	Post	Pre	Post	
Eosinophil Count					
- Total (all levels)	66.7 (36.4/ 60)	4.8 (7.0/ 3)	83.9 (33.7/ 90)	65.6 (43.3/ 48)	
	*P <0.0001		P = 0.30		*P = 0.0001
- Proximal Esophagus	28.1 (27 / 20.5)	1.1 (1.5 / 0)	44 (32.7 /30)	43.3 (40.9 /35)	
	*P = 0.0024		P = 0.96		*P = 0.0024
- Mid Esophagus	46.4 (34.5/ 36)	2.9 (6.1/ 1)	79.6 (36.9/ 87)	62 (46.2/ 48)	
	*P = 0.0003		P = 0.56		*P = <0.0001
- Distal Esophagus	48.9 (37.6/ 43.5)	4.1 (7.3/ 1)	60.3 (28/ 58)	46.9 (31.9/ 34)	
	*P = 0.001		P = 0.34		*P = 0.0001
Endoscopy Score					
- Total (maximum 15)	4.6 (2.8/ 5)	1.5 (2.5/ 0)	7.8 (1.6/ 9)	5.4 (2.8/ 6)	
	*P = 0.0005		*P = 0.04		*P = 0.041
- Pallor/ reduced vascular	2 (1.3/ 3)	0.67 (1.1/ 0)	2.9 (0.3/ 3)	2.2 (1.2/ 3)	
	*P = 0.002		P = 0.11		*P = 0.025
- Furrows/ Thickening	1.67 (1.2/ 2)	0.6 (1.1 / 0)	2.9 (0.3/ 3)	2 (1.2/ 3)	
	*P = 0.007		P = 0.052		P = 0.076
- Plaques	0.9 (1.0/ 1)	0.07 (0.3/ 0)	1.67 (1.4/ 2)	1 (1.5/ 0)	
	*P = 0.004		P = 0.26		P = 0.07
- Conc. Rings/Stricture	0 (0/ 0)	0.07 (0.3/ 0)	0.2 (0.4/ 0)	0 (0/ 0)	
	P = 0.33		P = 0.33		P = 0.49
- Friability/Tissue paper	0 (0/ 0)	0.07 (0.3/ 0)	0.1 (0.3/ 0)	0.1 (0.3/ 0)	
	P = 0.33		P = 0.35		P = 0.48
Symptom Score					
- Total (maximum 14)	3.5 (2.3/ 3)	1.2 (1.87/ 0.7)	2.67 (2/ 2)	1.85 (1.8/ 1.8)	
	*P = 0.0007		P = 0.22		*P = 0.031
- Heartburn/Regurgitation	0.79 (0.8/ 1)	0.23 (0.45/ 0)	0.33 (0.5/ 0)	0.32 (0.4/ 0.3)	
	*P = 0.008		P = 0.89		*P = 0.033
- Abdominal pain	0.8 (0.8/ 1)	0.39 (0.6/ 0.3)	0.56 (0.7/ 0)	0.3 (0.4/ 0)	
	P = 0.10		P = 0.28		P = 0.87
- Nausea/Vomiting	0.73 (0.8/ 1)	0.07 (0.2/ 0)	0.67 (0.7/ 1)	0.2 (0.3/ 0)	
	*P = 0.004		P = 0.065		P = 0.11
- Anoexia/ Early satiety	0.33 (0.7/ 0)	0.28 (0.56/ 0)	0.33 (0.7/ 0)	0.32 (0.7/ 0)	
	P = 0.66		P = 0.85		P = 0.76
- Dysphagia	0.73 (0.8/ 1)	0.18 (0.5/ 0)	0.67 (0.9/ 0)	0.3 (0.5/ 0)	
	*P = 0.017		P = 0.20		P = 0.46

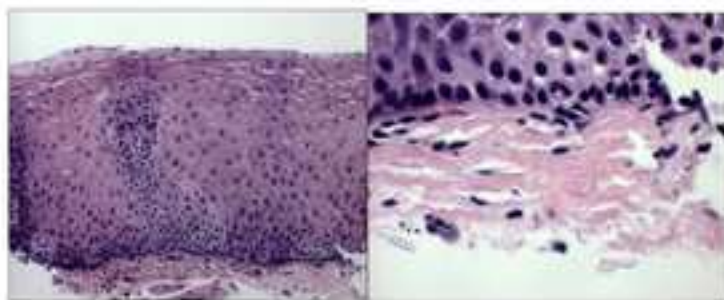
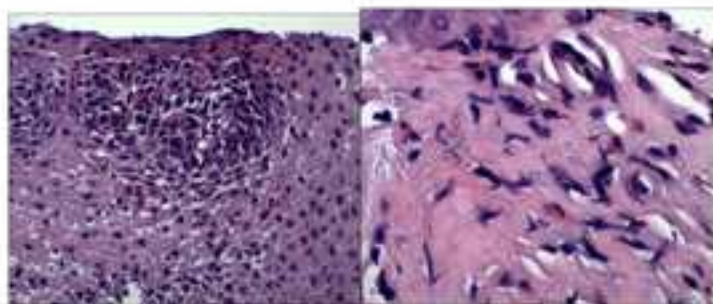
- Nocturnal wakening	0.2 (0.6/ 0)	0.05 (0.1/ 0)	0.1 (0.3/ 0)	0.37 (0.3/ 0.5)	
	P = 0.34		<i>*P = 0.047</i>		<i>*P = 0.001</i>
- GI bleeding	0 (0/ 0)	0 (0/ 0)	0 (0/ 0)	0 (0/ 0)	
	P = 0.33		P = 0.33		P = 0.48
Epithelial Histology Score	6.3 (2.6/ 7)	1 (1.3/ 0.7)	8.1 (2.5/ 8.7)	6.8 (3.0/ 6)	<i>*P = 0.0035</i>
	<i>*P = <0.0001</i>		P=0.11		
L.P. Histology Score	3.8 (1.4/ 4.0)	0.4 (0.5/ 0.3)	4.0 (1.2/ 4.0)	3.5(0.1.3/ 3)	**
	**		**		
Cortisol Levels	12.7 (5.0/ 13.0)	9.1 (3.2/ 8.6)	10.1 (2.9/ 9.3)	10.4 (4.3/10.2)	P = 0.31
	P=0.09		P=0.80		

Table 4. Mean values for peak eosinophil counts, endoscopy, symptom and histology scores. Mean morning cortisol levels are shown in umol/dl. Baseline and post-treatment comparisons are made both within the groups and between the OVB and placebo groups. Statistical significance is noted by *italicized P value.

** Inadequate number of LP samples for formal statistical analysis.







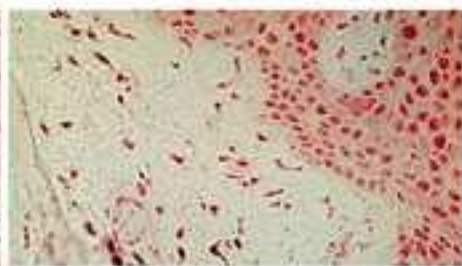
Pre



Post



Pre



Post

Flow diagram showing study patients.

