

ORIGINAL ARTICLE

GASTROINTESTINAL DISEASES

Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis

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Keywords

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Abstract

Background: Eosinophilic esophagitis (EoE) is a chronic, Th2-type inflammatory disease. Chemoattractant receptor-homologous molecule on Th2 cells (CRTH2) is a prostaglandin D₂ (PGD₂) receptor, expressed by Th2 cells and other inflammatory cells, including eosinophils and basophils, that mediates chemotaxis and activation. OC000459 is a selective CRTH2 antagonist and would be expected to suppress eosinophilic tissue inflammation. The purpose of this study was to evaluate the efficacy and safety of an OC000459 monotherapy in adult patients with active, corticosteroid-dependent or corticosteroid-refractory EoE.

Methods: In this randomized, double-blind, placebo-controlled trial, 26 adult patients (m/f = 22/4; mean age 41 years, range 22–69 years) with active EoE, dependent or resistant to corticosteroids, were treated either with 100 mg OC000459 (*n* = 14) or placebo (*n* = 12) twice daily. Pre- and post-treatment disease activity was assessed clinically, endoscopically, histologically, and via biomarkers. The primary end point was the reduction in esophageal eosinophil infiltration.

Results: After an 8-week OC000459 treatment, the esophageal eosinophil load decreased significantly, from 114.83 to 73.26 eosinophils per high-power field [(eos/hpf), *P* = 0.0256], whereas no reduction was observed with placebo (102.80–99.47 eos/hpf, *P* = 0.870). With OC000459, the physician's global assessment of disease activity improved from 7.13 to 5.18 (*P* = 0.035). OC000459 likewise reduced extracellular deposits of eosinophil peroxidase and tenascin C, the effects not seen with placebo. No serious adverse events were observed.

Conclusions: An 8-week treatment with the CRTH2-antagonist, OC000459, exerts modest, but significant, anti-eosinophil and beneficial clinical effects in adult patients with active, corticosteroid-dependent or corticosteroid-refractory EoE and is well tolerated.

Eosinophilic esophagitis (EoE) is a chronic (1), dominant Th2-type inflammatory (2, 3) disease of the esophagus

Abbreviations

CRTH2, chemoattractant receptor expressed on Th2 cells; EoE, eosinophilic esophagitis; eos, eosinophils; EPX, eosinophil peroxidase; hpf, high-power field; MC, mast cell; PGA, physician's global assessment of disease activity; PRO patient-reported outcome; TGF-β1, transforming growth factor-β1; TSLP, thymic stromal lymphopoietin; VDO, visual dysphagia questionnaire.

characterized clinically by symptoms related to esophageal dysfunction and, histologically, by an eosinophil-predominant inflammation (4–6). Eosinophilic esophagitis is rapidly increasing in both incidence and prevalence (7–9). Today, EoE is the second leading cause of chronic esophagitis, behind gastro-esophageal reflux disease (10), and likely the most frequent cause of dysphagia (4–6, 11, 12).

At least three reasons strongly advocate treating this chronic inflammatory esophageal disorder: Firstly, EoE patients have a substantially impaired quality of life due to

their dysphagia and the permanent risk of unforeseeable food impaction (1, 5, 6, 12); secondly, prolonged food impactions requiring emergency endoscopy risk severe esophageal injury (13) and should be avoided whenever possible; and thirdly, it has become clear that the persistence of unbridled eosinophilic inflammation evokes esophageal remodeling with ensuing functional impairment (1, 4–6, 12, 14–16). Standard recommendations for medical treatment of this chronic inflammation include systemic (17, 18) and topical corticosteroids (18–26). Despite the fact that the majority of patients can be treated sufficiently by one of these options, a substantial fraction of patients are nevertheless nonresponders, or require such high drug doses that side-effects become a concern (5), or they experience relapse despite consistent adherence to maintenance treatment (27). Unfortunately, several alternative therapeutic approaches, such as leukotriene antagonists (28), or monoclonal antibodies directed against IL-5 (29–31), or TNF- α (32), have not demonstrated clinical efficacy. The need for therapeutic alternatives thus remains.

Chemoattractant receptor expressed on Th2 cells (CRTH2) is a G-protein-coupled receptor expressed on Th2 lymphocytes, eosinophils, and basophils and mediates chemotaxis of these cells in response to prostaglandin D₂ (PGD₂), a key prostanoid in allergic responses largely produced by mast cells (MC) (33). In addition, CRTH2 receptors have also been described as being expressed by keratinocytes (34). OC000459 is a potent, selective and orally bioavailable CRTH2 antagonist, which blocks PGD₂'s ability to mediate chemotaxis and activation of CRTH2-expressing cells (35). Indeed, the clinical efficacy of OC000459 has been demonstrated in patients suffering from eosinophilic asthma (36). Therefore, the possibility that OC000459 could also suppress eosinophilic tissue inflammation associated with EoE presented a valid avenue for investigation.

The purpose of this study was to evaluate the efficacy, tolerability, and safety of an 8-week monotherapy with the selective CRTH2 antagonist, OC000459, in adult patients with severe and active EoE.

Methods

Study design and study participants

This randomized, placebo-controlled, parallel-group, single-center study tested whether an 8-week monotherapy with OC000459 was superior to placebo as an induction treatment in adult patients with active, corticosteroid-dependent or corticosteroid-refractory EoE, by determining the effect on the esophageal eosinophil load. We also investigated the reversibility of EoE symptoms and signs, as well as the tolerability and safety of the compound.

Adult patients, aged 18–75 years, with previously clinically, endoscopically, and histologically confirmed EoE, according to established criteria (4, 5), having an inadequate response to topical and/or systemic corticosteroid therapy (e.g., corticosteroid refractory, corticosteroid dependency or necessity of high doses to control the inflammation) were considered

eligible. Inclusion criteria were active EoE defined as (i) dysphagia with almost every intake of solids; (ii) relevant eosinophilic infiltration of the epithelial layer of the esophagus (mean cell density of ≥ 20 eosinophils (eos)/hpf in eight biopsies); and (iii) exclusion of other causes of esophageal or systemic eosinophilia. Gastro-esophageal reflux disease was excluded by proton pump inhibitor (PPI) pretreatment plus negative endoscopy for signs of reflux disease and pH monitoring (optional). After a screening examination, patients filled out a symptom questionnaire and discontinued all specific treatments for EoE (e.g., corticosteroids, leukotriene antagonists, histamine blockers, MC stabilizers). Previously established PPI therapies for secondary reflux were continued throughout the study period in a constant dose. After a wash-out period of at least 2 weeks, patients underwent a baseline examination. Patients who met the inclusion/exclusion criteria were randomized equally to either active treatment or placebo. During the subsequent 8-week treatment and 10-week follow-up-periods, patients attended the clinic at 2-week intervals. The following examinations and procedures were performed at baseline and repeatedly during the 8-week treatment period: structured assessment of symptoms, physical examination, laboratory analyses, including OC000459 plasma level determination and electrocardiogram. Upper endoscopy with structured tissue sampling was performed at baseline and at the end of the treatment period. In patients who achieved remission, an additional endoscopy was performed during the follow-up period as soon as symptoms reappeared, or 12 weeks after the end of the treatment period. All patients provided written informed consent. The study was conducted in accordance with International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) guidelines and approved by the local ethics committee and regulatory authorities, SwissMedic, Bern, Switzerland, and was registered at www.ClinicalTrials.gov (NCT01056783).

Study treatment

Patients ($n = 14$) randomized to the verum group received 100 mg OC000459 tablets, twice daily after meals for 8 weeks, whereas patients randomized to the placebo group ($n = 12$) received identical-appearing placebo tablets. Treatment compliance was calculated by counting the number of delivered and returned tablets.

Assessments and definitions

End points

The *primary end point* was defined as the reduction in the esophageal eosinophil load, defined as the mean eosinophil number measured in a total of 40 hpf from 2×4 biopsies taken from the proximal and distal esophagus. *Secondary end points* were the effects of OC000459 on patient-reported outcomes (PROs), on endoscopic alterations, and on EoE-relevant biomarkers in the esophagus and peripheral blood, as well as an assessment of the safety and tolerability of OC000459 in adult EoE patients.

Clinical manifestations of EoE

Patient-Reported Outcomes: To assess the clinical manifestations, the following, currently nonvalidated score was used weekly throughout the entire 18-week study period: The course of the leading EoE symptom 'dysphagia' was assessed using a visual dysphagia questionnaire (VDQ), an illustrated catalogue of nine well-known food categories, each having a defined and constant consistency, covering the whole spectrum from 'liquid' to 'solid'. Patients had to rate their current swallowing disturbances for each food category, ranking from 0 to 4 points. The second leading EoE symptom 'chest pain' was recorded using a previously published score (25). The VDQ (0–36 points) and the pain questionnaire (0–9 points) resulted in a total score ranging from a minimum of 0 to a maximum of 45 PRO points.

Physician's Global Assessment (PGA): In addition to the patient-oriented approach, a physician-based method was used to assess the disease activity. At the end of each visit, one EoE-experienced physician (AS) judged the disease activity, based on all clinical and endoscopic aspects of the disease, by using a 10-cm visual analogue scale with anchors at the extremes.

Endoscopy, tissue sampling, and histologic analysis

Endoscopies were performed in all patients by the same board-certified gastroenterologist who performed the PGA (AS). The global appearance of endoscopic abnormalities was assessed using a 10-cm visual analogue scale ranging from severe appearance to remission. Respecting the patchy nature of the disease (37, 38), at least four biopsy specimens were taken from the upper half and from the lower half of the esophagus. In all eight esophageal biopsies, eosinophils in the most densely infiltrated area were counted in five consecutive hpf (Zeiss Axiophot, Plan-Neofluar 40, ocular magnification 10 $\times$, area of microscopic field 0.3072 mm²).

Biomarkers

Eosinophil peroxidase (EPX), CD3, IL-13, CRTH2, tryptase, tenascin C, transforming growth factor (TGF)- β 1, and thymic stromal lymphopoietin (TSLP) biomarker analyses were performed as described previously (25, 27, 29). Briefly, to assess the effect of OC000459 treatment on the inflammatory cell infiltration, epithelial cytokine expression, molecular remodeling and expression of the drug target, we stained paraformaldehyde-fixed and paraffin-embedded proximal and distal esophageal sections by indirect immunofluorescence. Anti-eosinophil peroxidase (Lee Laboratory, Mayo Clinic, AZ, USA), anti-CD3 (DakoCytomation, Glostrup, Denmark and Lab Vision Corporation/NeoMarkers, Thermo Fisher Scientific, Fremont, CA, USA), anti-IL-13 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), anti-CRTH2 (Santa Cruz Biotechnology), anti-MC tryptase (DakoCytomation), antitenascin C (Monosan, Uden, The Netherlands), anti-TGF- β 1 (antitransforming growth factor β 1; R&D Systems Europe Ltd, Abingdon, UK), and anti-TSLP (antithymic stromal lymphopoietin;

Santa Cruz Biotechnology) were used as primary antibodies. Evaluation was performed using a confocal laser scanning microscope (LSM 510; Carl Zeiss, Jena GmbH, Jena, Germany). Eosinophils, MC, and T cells were evaluated in the five fields of highest inflammatory activity per biopsy (corresponding to a total of 40 fields (0.34 mm²) evaluated per patient and time point). The expressions of the drug target (CRTH2) and IL-13 by infiltrating T cells were assessed by double immunofluorescence in combination with CD3. Epithelial cytokine/receptor expressions (TGF- β 1, TSLP, CRTH2) were obtained by scoring the intensity of the staining in the 40 fields of highest inflammatory activity at 1000 $\times$ magnification. Extracellular tenascin C and EPX deposits were assessed using the same score of 0 (no activity) to 4 (very high activity). The scale used for the semi-quantitative assessment of the EPX deposits as well as the epithelial TGF- β 1 and TSLP expression is illustrated in Fig. 1A–C, respectively. Due to the expression pattern seen with tenascin C staining, we had to enlarge the area evaluated by using a 630 $\times$ magnification (corresponding to a total area of 0.86 mm² per time point and patient).

Safety

Safety was assessed with the use of adverse events reports, physical examination, laboratory tests (differential blood count, platelet count, hematocrit, hemoglobin, erythrocyte count, total protein, albumin, liver enzymes, and amylase), and electrocardiogram.

Statistical methods, sample size calculation, and data analysis

Patients were randomized in equal proportions to the verum and the placebo groups, using a computer-generated randomization schedule with a block size of 4. Patients, study center personnel, laboratory personnel, and the sponsor were blinded to study treatment allocation. All randomized patients were included in the assessment of the outcome (intention-to-treat analysis).

The sample size calculation was based on the analysis of the primary end point, the reduction in the mean esophageal eosinophil load. A primary analysis was based on comparing the percentage reductions in the mean number of eos/hpf between the OC000459 and placebo groups. Using two-sided testing with a 0.05 level of significance and 80% power of detecting a significant difference between placebo (mean 10% reduction, SD 50%) and OC000459 (mean 70% reduction, SD 25%) resulted in sample sizes of 9 and 9.

Demographic and host-based parameters were analyzed between treatment groups using the nonparametric Wilcoxon two-sample test. To compare the values between before and after treatment within groups, we used the paired *t*-test or Wilcoxon's signed-rank test. Correlations between the histologic status of the disease after treatment and the host-based parameters were analyzed. Spearman's rank correlation coefficient of 0.5–1 was considered as a strong, 0.3–0.5 as a weak,

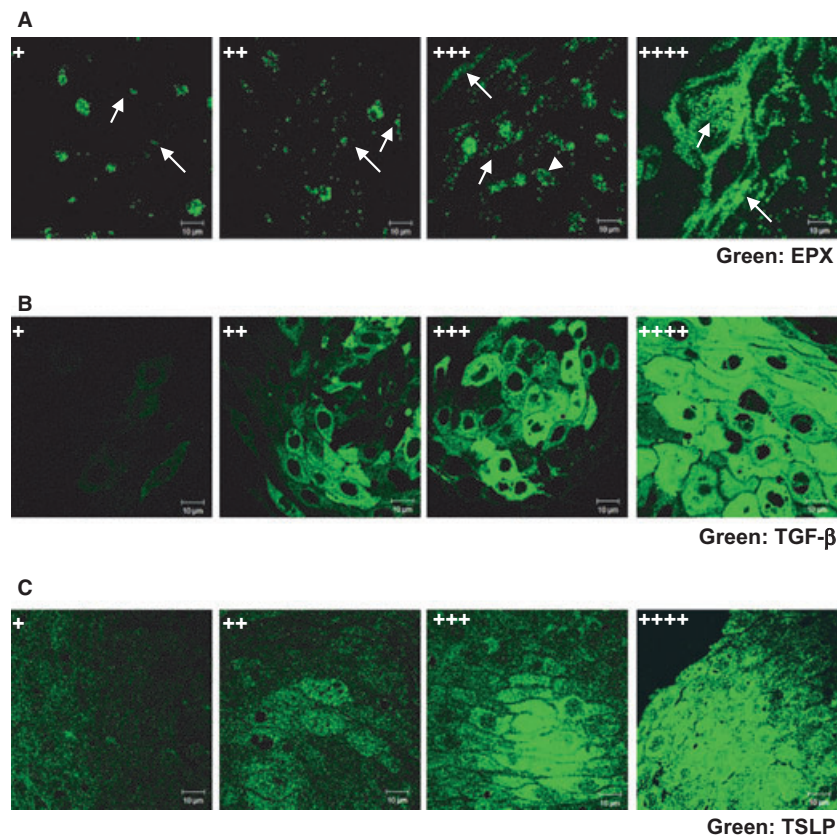


Figure 1 Scale used for the semi-quantitative assessment of the eosinophil peroxidase (EPX) deposits as well as the epithelial transforming growth factor- β 1 (TGF- β 1) and thymic stromal lymphopoietin (TSLP) expression. (A) EPX deposits (arrows) were distinguished from intact eosinophils (arrowheads) by morphological criteria. The deposits were divided into four categories depending on their size and number. (+) corresponds to at least two single deposits; (++) to at least two round clusters; (+++) to longitudinal

assemblies of clusters; and (++++ to large deposits. (B) The expression of TGF- β 1 by epithelial cells was analyzed by staining the tissue sections with anti-TGF- β 1 antibody. Staining intensity of the epithelium was assessed as weak (+); moderate (++); strong (+++); or very strong (++++). (C) The expression of TSLP by epithelial cells was analyzed using the same scale as described in panel B. Bars in all panels, 10 μ m.

and <0.3 as no correlation. A 0.05 level of significance was used throughout.

Results

Patient flow and characteristics

Between August 10, 2010, and April 4, 2011, 31 patients were screened for study participation; of whom, 26 fulfilled all of the inclusion and none of the exclusion criteria. Five patients represented screening failures due to low mean esophageal eosinophil counts (<20/hpf). At baseline, except for the gender distribution, no significant differences in demographic or disease-specific characteristics were noted between the two groups (Table 1). However, the overall male/female ratio is 3 : 1 for this disease. All 26 patients included completed the study according to the study protocol and were included in the analysis (Fig. 2). The treatment compliance rate was $96.94 \pm 3.76\%$ in the OC000459 group and $97.59 \pm 4.43\%$ in the placebo group, respectively.

Anti-inflammatory efficacy

After 8 weeks of treatment with OC000459, the baseline esophageal eosinophil load (114.83 eos/hpf) was significantly reduced by 36.20% to 73.26 eos/hpf ($P = 0.0256$), whereas under placebo, the density of the eosinophil infiltration [baseline 102.80 eos/hpf, post-treatment 99.47, ($P = 0.87$)] remained almost unchanged. The anti-eosinophil effect of OC000459 was slightly more pronounced in the proximal (−38.60%) ($P = 0.0335$) than in the distal esophagus (−34.08%) ($P = 0.0390$). This is even more prominent when the anti-EPX immunofluorescence data are considered (−43.01%, $P = 0.0025$ for proximal EPX staining) (Table 2 and Fig. 3). Moreover, the amount of extracellular EPX deposits was reduced after treatment with OC000459, but not after placebo. In contrast, neither the number of MC, CD3+ T cells nor the expression of TSLP by epithelial cells, a cytokine critically involved in the maturation of Th2 cells, changed significantly under the influence of the CRTH2 blockade (Table 2 and Fig. 3).

Table 1 Baseline demographics, clinical and disease-specific characteristics of 26 adult patients with active eosinophilic esophagitis

Characteristics	OC000459 (<i>n</i> = 14)	Placebo (<i>n</i> = 12)	<i>P</i> Value
Gender (M/F)	14/0	8/4	0.03*
Age (year)	43.71 ± 13.59	38.83 ± 14.58	0.36†
Duration of confirmed diagnosis (year)	5.43 ± 5.17	4.30 ± 3.16	0.94†
Weight (kg)	79.1 ± 13.2	74.3 ± 7.5	0.59†
Height (cm)	176.6 ± 4.6	175.1 ± 7.1	0.88†
BMI (kg/m ²)	25.28 ± 3.41	24.23 ± 2.13	0.35†
Heart rate (min ⁻¹)	64.3 ± 6.7	69.2 ± 6.6	0.07†
Blood eosinophils (G/l)	0.58 ± 0.26	0.41 ± 0.23	0.06†
Total IgE > 114 kU/l	6/13‡ (46.2%)	6/12 (50.0%)	1*
Total IgE (kU/l)	158.77 ± 160.53	442.67 ± 614.12	0.94†
History of airway allergies (AR, AC, AA) <i>n</i> (%)	6/14 (42.9%)	5/12 (41.7%)	1*
Esophageal peak eosinophils (per hpf)	189.93 ± 90.79	201.76 ± 108.45	0.82†

AR, allergic rhinitis; AC, allergic conjunctivitis; AA, allergic asthma.

Plus-minus values are means ± SD.

*Fisher's exact test.

†Wilcoxon two-sample test.

‡Data missing for one patient.

CRTH2 blockade with proton pump inhibition had a greater overall effect on the eosinophil load. To elucidate this, we compared the anti-eosinophil effect of OC000456 of the three patients who had a PPI co-medication (esomeprazole 40 mg/day) with the 11 patients who had OC000459 monotherapy. Although the results are not significant, there was a trend: The monotherapy led merely to a reduction in the eosinophil load by 24.17% (−23.38 eos/hpf), whereas the combination of OC000459 with PPI reduced the eosinophil load by 59.78% (−108.26 eos/hpf, *P* = 0.0844).

Clinical efficacy

At baseline, all patients suffered from swallowing disturbances with no significant group difference. In the OC000459 group, the PGA decreased significantly from 7.13 to 5.18 (*P* = 0.0350), whereas under placebo, only a nonsignificant drop from 6.74 to 5.80 (*P* = 0.3500) was observed. In contrast, the PRO revealed a significant reduction in both groups. In the OC000459 group, the score decreased from 16.50 to 10.79 (*P* = 0.0154) and in the placebo group, from 16.55 to 9.73 (*P* = 0.0144) (Table 3).

Endoscopic findings

Although not significant, the global assessment of the endoscopic abnormalities after 8 weeks of OC000459 treatment was slightly lower than at baseline (from 6.42 to 6.06) (*P* = 0.27), but remained almost unchanged in the placebo group (from 5.63 to 5.57) (*P* = 0.85) (Table 3).

CRTH2 receptor expression

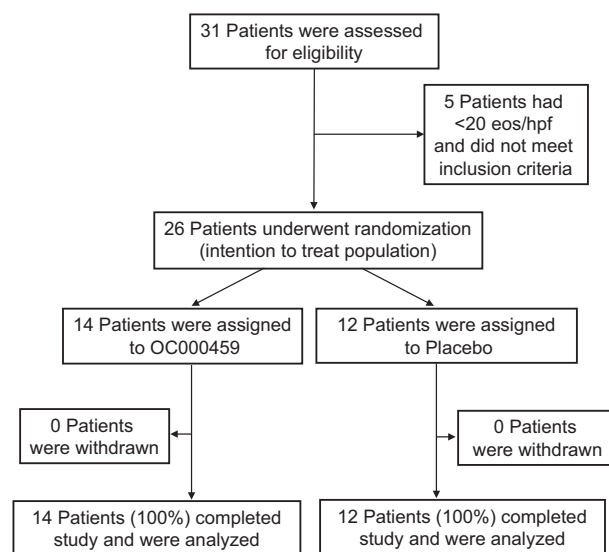
The absolute numbers of epithelial cells and CD3+ T cells expressing the CRTH2 receptor were not significantly reduced after 8 weeks of treatment with OC000459 (Table 2 and Fig. 4). Likewise, the proportion of CRTH2+ to CRTH2-CD3+ T cells did not significantly decline in the esophageal tissue under the treatment (Table 2).

Markers of tissue remodeling

Transforming growth factor-β1 and tenascin C are markers associated with tissue remodeling. We examined TGF-β1 expression by epithelial cells and tenascin extracellular deposits at baseline under inflammatory conditions and after OC000459 treatment. A significant increase in the epithelial expression of TGF-β1 and a marked reduction in tenascin C deposits were seen postverum therapy, while placebo again exerted no effect (Table 2).

Plasma concentrations of OC000459

At week 2, the mean plasma concentrations of the compound in the OC000459 group were 392 ± 259 ng/ml; at week 4, 421 ± 264 ng/ml; at week 6, 510 ± 356 ng/ml; and at week 8, 324 ± 233 ng/ml. The levels of drug in plasma were well in excess of those in the whole-blood *K_B* (10 ng/ml) and

**Figure 2** Flow chart of the patients included in the study.

Effect of PPI co-medication on OC000459 efficacy

Because the anti-eosinophil effect of OC000459 was somewhat more pronounced in the proximal than in the distal esophagus, we were interested to know whether the combination of

Table 2 Effect of treatment on eosinophil counts and biomarkers

End points	OC000459 (n = 14)	P Value*	Placebo (n = 12)
Primary			
Eosinophil load – entire esophagus (HE staining)			
Baseline (eosinophils/hpf)	114.83 ± 73.04	0.70	102.80 ± 55.34
P Value**	0.0256		0.87
Post-treatment (eosinophils/hpf)	73.26 ± 58.29	0.38	99.47 ± 69.95
Eosinophil load – proximal esophagus (HE staining)			
Baseline (eosinophils/hpf)	107.53 ± 72.03	0.60	89.89 ± 62.34
P Value**	0.0335		0.82
Post-treatment (eosinophils/hpf)	66.02 ± 66.11	0.17	94.75 ± 68.39
Eosinophil load – distal esophagus (HE staining)			
Baseline (eosinophils/hpf)	122.12 ± 80.07	0.98	116.19 ± 61.06
P Value**	0.0390		0.63
Post-treatment (eosinophils/hpf)	80.50 ± 58.70	0.56	104.19 ± 80.22
Eosinophil load (EPX staining)			
Baseline (eosinophils/mm ²)	1152.58 ± 692.49	0.98	1030.52 ± 429.58
P Value**	0.0044		0.80
Post-treatment (eosinophils/mm ²)	712.44 ± 389.67	0.26	987.09 ± 566.9
Eosinophil load – proximal esophagus (EPX staining)			
Baseline (eosinophils/mm ²)	1083.33 ± 747.65	0.78	923.71 ± 545.77
P Value**	0.0025		0.82
Post-treatment (eosinophils/mm ²)	617.37 ± 518.78	0.19	974.19 ± 700.7
Eosinophil load distal esophagus (EPX staining)			
Baseline (eosinophils/mm ²)	1220.66 ± 732.39	0.86	1137.32 ± 494.13
P Value**	0.0281		0.48
Post-treatment (eosinophils/mm ²)	807.51 ± 383.8	0.43	1000 ± 557.51
Secondary			
Extracellular EPX deposits			
Baseline (score)	2.12 ± 0.36	0.39	2.00 ± 0.45
P Value**	0.12		0.74
Post-treatment (score)	1.89 ± 0.47	0.80	1.94 ± 0.44
Tryptase + cells (mast cell)			
Baseline (cells/mm ²)	311.03 ± 201.88	0.49	251.17 ± 146.71
P Value**	0.83		0.48
Post-treatment (cells/mm ²)	321.6 ± 104.46	0.24	274.65 ± 185.45
CD3+ cells			
Baseline (cells/mm ²)	754.69 ± 193.67	0.78	774.65 ± 192.49
P Value**	0.53		0.83
Post-treatment (cells/mm ²)	793.43 ± 278.17	0.94	760.56 ± 149.06
IL-13 + CD3+ cells			
Baseline (cells/mm ²)	727.7 ± 186.62	0.74	734.74 ± 192.49
P Value**	0.48		0.98
Post-treatment (cells/mm ²)	769.95 ± 279.34	0.98	737.09 ± 146.71
CRTH2 + CD3+/CRTH2-CD3+/-			
Baseline (ratio)	16.87 ± 14.79	0.16	7.70 ± 7.03
P Value**	0.76		0.11
Post-treatment (ratio)	13.14 ± 8.97	0.80	17.27 ± 15.33
CRTH2 expression by epithelial cells			
Baseline (score)	1.13 ± 0.32	0.94	1.08 ± 0.39
P Value**	0.19		0.21
Post-treatment (score)	1.36 ± 0.48	0.70	1.29 ± 0.37
Thymic stromal lymphopoietin expression by epithelial cells			
Baseline (score)	1.29 ± 0.24	0.53	1.34 ± 0.21
P Value**	0.11		0.84
Post-treatment (score)	1.42 ± 0.30	0.30	1.32 ± 0.30
Transforming growth factor-β1 expression by epithelial cells			
Baseline (score)	1.57 ± 0.77	0.22	1.80 ± 0.46

Table 2 (continued)

End points	OC000459 (<i>n</i> = 14)	<i>P</i> Value*	Placebo (<i>n</i> = 12)
<i>P</i> Value**	0.0162		0.68
Post-treatment (score)	2.02 ± 0.47	0.19	1.70 ± 0.74
Extracellular tenascin C deposits			
Baseline (score)	2.20 ± 0.68	0.22	1.76 ± 0.74
<i>P</i> Value**	0.09		0.73
Post-treatment (score)	1.71 ± 0.99	0.74	1.85 ± 0.82
Blood eosinophil count			
Baseline (per mm ³)	0.58 ± 0.26	0.06	0.41 ± 0.23
<i>P</i> Value**	0.0193		0.88
Post-treatment (per mm ³)	0.43 ± 0.14	0.51	0.40 ± 0.15
Serum total IgE			
Baseline (kU/l)	158.77 ± 160.53	0.94	442.67 ± 614.12
<i>P</i> Value**	0.30		0.61
Post-treatment (kU/l)	149.38 ± 136.43	0.57	499.45 ± 682.68
Serum CRP			
Baseline (mg/l)	3.31 ± 4.67	0.62	1.64 ± 1.39
<i>P</i> Value**	0.10		0.29
Post-treatment (mg/l)	1.24 ± 1.34	0.66	1.28 ± 0.87

CRTH2, chemoattractant receptor expressed on Th2 cells; EPX, eosinophil peroxidase.

Mean values ± SD.

*Wilcoxon test.

***t*-test (on after–before difference; paired samples).

whole-blood IC₅₀ (35 ng/ml) (33), illustrating that a sufficient systemic bioavailability was achieved.

Safety

Peroral application of the CRTH2 antagonist OC000459 was well tolerated. One serious adverse event occurred in the OC000459 group – an acute appendicitis during the follow-up period, 37 days after the last intake of the study medication – which was assessed as not being related to the study medication. A total of 15 minor adverse events were registered, of which six occurred with OC000459 and nine with placebo. One single adverse event, a temporary episode of dizziness in a patient receiving placebo, was possibly related to the study.

Discussion

Swallowed topical corticosteroids have proven efficacy in bringing into remission both adult and pediatric patients suffering from active EoE (18–26). This type of medication has now been established as the first-line drug treatment for active EoE (4–6, 12). However, EoE is a chronic inflammatory disease (1, 39) requiring long-term management (6, 12, 40). Unfortunately, topical corticosteroids have shown only limited efficacy as maintenance treatment for EoE (27) and, as long as more than one staple food must be excluded indefinitely, elimination diets are not valid alternatives (41).

For Th2-type inflammatory disorders, CRTH2 blockade is a novel therapeutic approach that targets CRTH2-expressing leukocytes, such as Th2 lymphocytes, eosinophils, and basophils. CRTH2 antagonists interfere with the prostaglandin

pathway, blocking the PGD₂ receptor, consecutively preventing the activation and the recruitment of the CRTH2-expressing inflammatory cells (33). OC000459 is a selective and orally bioavailable CRTH2 antagonist and therefore a promising new drug for the treatment of allergic diseases (35). This is the first placebo-controlled, double-blind trial analyzing efficacy and safety of a CRTH2 blockade in patients with active EoE.

Data from this trial demonstrate that in these patients having severe, corticosteroid-dependent or corticosteroid-refractory EoE, an 8-week treatment with the CRTH2 antagonist OC000459 led to a modest, but significant, reduction in the eosinophilic inflammation of the esophageal tissue. As expected, the placebo did not reduce eosinophil infiltration. In parallel with the alleviation of the eosinophilic inflammation, a significant relief of symptoms occurred. In addition, the degree of endoscopic abnormalities seen in active EoE likewise showed a trend toward improvement under the CRTH2 blockade. Taken together, with CRTH2 blockade by OC000459, histologic abnormalities as well as symptoms significantly improved.

However, the following reasons may explain the limited overall efficacy of OC000459: (i) OC000459 is a new investigational drug. We enrolled therefore only patients having a severe and refractory EoE in this study. Selecting these ‘toughest-to-treat’ patients might have had a negative impact on the efficacy of the drug. (ii) The decision to use OC000459 in a daily dose of 200 mg was based on the experience with this compound in the treatment of asthma, but not on a dose-finding study. We achieved reasonable plasma levels but we did not determine tissue concentrations of this compound. It can therefore be speculated that a higher dose

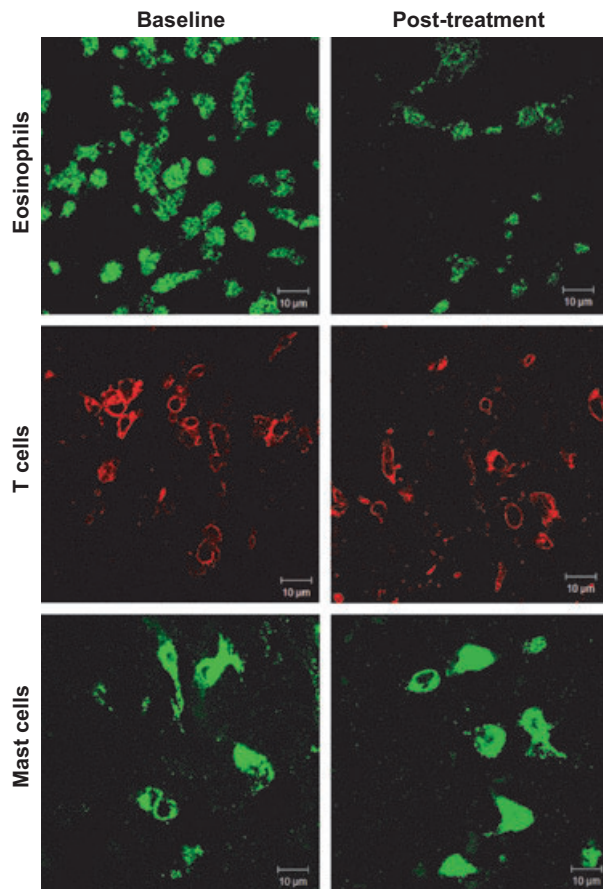


Figure 3 Inflammatory cell infiltration of the epithelial layer of the esophagus in patients with eosinophilic esophagitis before and after therapy, assessed by immunofluorescence. Specimens were stained with anti-eosinophil peroxidase (eosinophils), anti-CD3 (T cells), and antitryptase (mast cell) antibody. Bars, 10 μ m.

of OC000459 might have shown more impressive results. (iii) OC000459 exerted a slightly more pronounced anti-eosinophil effect in the proximal than in the distal, acid-exposed esophagus. By comparing the effect of an OC000456 monotherapy with a combination of OC000459 with a PPI (esomeprazole) in a limited number of patients, superior results were seen than with OC000459 alone. It is possible therefore that acid reflux may contribute to tissue eosinophilia in the distal esophagus (5) and a combination of CRTH2 antagonist with a PPI may be required to provide substantial reduction in the total eosinophil load. However, the number of patients in this subgroup analysis was rather small ($n = 3$), and these preliminary findings call for additional studies with larger groups of patients. (iv) OC000459 could not block sufficiently the CRTH2 receptor in the esophageal tissue. The results of our immunostainings show that, after treatment, CRTH2 expression by epithelial cells and T cells did not decrease when compared with the levels before CRTH2 antagonist administration. (v) OC000459 likely impairs the recruitment of eosinophils into the esophageal tissue, but does not induce apoptosis of those already

Table 3 Effect of treatment on clinical manifestations of eosinophilic esophagitis (EoE)

	OC000459 ($n = 14$)	<i>P</i> Value**	Placebo ($n = 12$)
Patient-reported outcomes (PRO)			
Total PRO Score*			
Baseline (points)	16.50 $\pm$ 6.05	0.96	16.55 $\pm$ 6.71
<i>P</i> Value***	0.0154		0.0144
Post-treatment (points)	10.79 $\pm$ 6.52	0.68	9.73 $\pm$ 8.16
Dysphagia (assessed by visual dysphagia questionnaire)			
Baseline (points)	12.64 $\pm$ 4.13	0.47	13.36 $\pm$ 5.87
<i>P</i> Value***	0.0437		0.0105
Post-treatment (points)	8.71 $\pm$ 6.11	0.65	8.09 $\pm$ 7.35
Chest pain frequency			
Baseline (points)	1.29 $\pm$ 1.27	0.71	1.09 $\pm$ 1.14
<i>P</i> Value***	0.0682		0.0455
Post-treatment (points)	0.79 $\pm$ 1.12	0.60	0.45 $\pm$ 0.69
Chest pain severity			
Baseline (points)	1.14 $\pm$ 1.10	0.62	0.91 $\pm$ 0.94
<i>P</i> Value***	0.0261		0.22
Post-treatment (points)	0.57 $\pm$ 0.76	0.88	0.55 $\pm$ 0.82
Chest pain duration			
Baseline (points)	1.43 $\pm$ 1.34	0.67	1.18 $\pm$ 1.25
<i>P</i> Value***	0.0186		0.0519
Post-treatment (points)	0.71 $\pm$ 0.99	0.81	0.64 $\pm$ 1.03
Physician-reported outcomes			
Physician's global assessment of EoE activity*			
Baseline (cm)	7.13 $\pm$ 1.22	1	6.74 $\pm$ 2.00
<i>P</i> Value***	0.0350		0.35
Post-treatment (cm)	5.18 $\pm$ 2.79	0.59	5.80 $\pm$ 2.52
Global assessment of endoscopic appearance*			
Baseline (cm)	6.42 $\pm$ 1.49	0.0536	5.63 $\pm$ 1.71
<i>P</i> Value***	0.27		0.85
Post-treatment (cm)	6.06 $\pm$ 1.79	0.44	5.57 $\pm$ 2.20

Mean values $\pm$ SD.

*Definitions and method of assessment, see Method section.

**Wilcoxon two-sample test on scores.

****t*-test (on after-before difference; paired samples).

present there. The potential of OC000459 to maintain a previously achieved remission might therefore be greater than its ability to bring an active inflammation into remission.

Remodeling of the esophagus is a hallmark of untreated EoE, and endoscopic, histologic as well as molecular features of this process have been described (1, 4–6, 12, 14–16). Of note, the deposition of extracellular matrix proteins, including its associated subepithelial fibrosis, was reversible in EoE patients receiving topical corticosteroid therapy (15, 16, 25). Under OC000459 treatment, the fibrosis-associated marker, TGF- β 1, expressed by epithelial cells increased, whereas the number of tenascin C deposits was reduced, suggesting that it is not only the TGF- β 1 generated by the epithelial cells

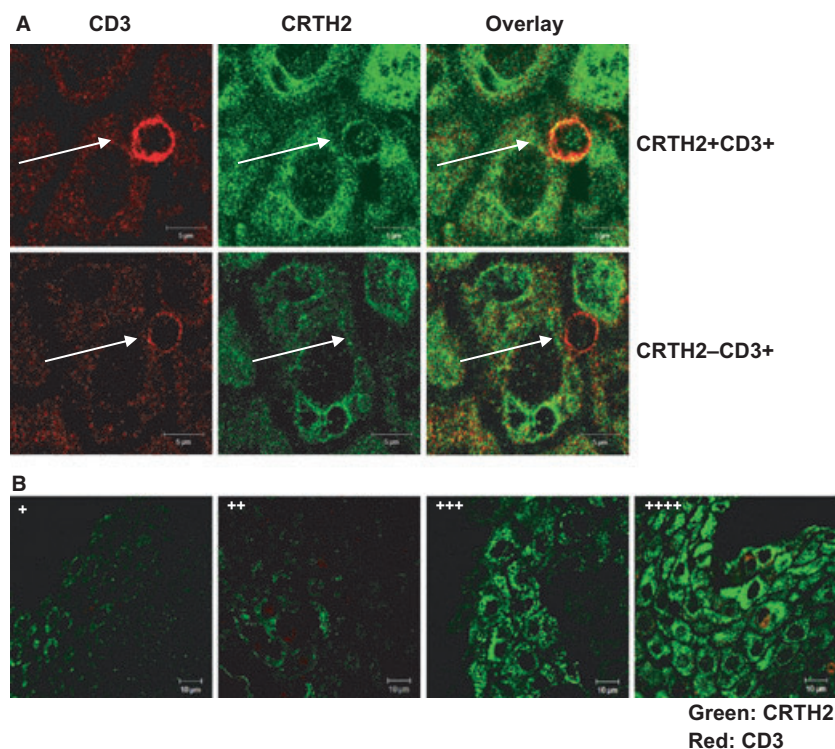


Figure 4 CRTH2 expression of T cells and epithelial cells. (A) Specimens were stained with anti-CRTH2 and anti-CD3 antibodies. Esophageal tissues contained both CRTH2-positive (upper panels) and CRTH2-negative T cells (lower panels). Bars, 5 μ m. (B) Illustration of the semi-quantitative scale used for the evaluation of

CRTH2 expression by epithelial cells. Staining intensity of the epithelium was assessed as weak (+), moderate (++), strong (+++), or very strong (++++). Bars, 10 μ m.

that regulates the amount of extracellular matrix protein deposition in the esophageal remodeling processes. Whether or not OC000459 therapy may alter this disabling process thus requires further evaluation.

Thymic stromal lymphopoietin is produced by epithelial cells at barrier surfaces, such as the intestine, airways and skin, and is known to preferentially drive Th2 inflammatory responses (42). Recently, TSLP was also reported to up-regulate surface adhesion molecules and chemokine release by eosinophils expressing the TSLPR/IL-7R α receptor complex (43), as well as to stimulate the formation of eosinophil extracellular DNA traps (44). In this study, we observed increased TSLP expression by esophageal epithelial cells in EoE. On the other hand, no reduction in TSLP expression by the esophageal epithelium under a CRTH2 blockade was seen. Even comparing the expression in patients having a significant reduction in the eosinophilic inflammation with nonresponders did not reveal a difference (data not presented).

OC000459 is a new investigational drug and substantial safety concerns had to be considered during the planning and conducting this clinical trial. In concurrence with previous reports (36), no drug-related relevant adverse events were recorded and the tolerability of the drug was favorable, comparable with that of placebo.

In summary, this trial demonstrates that an 8-week treatment with the CRTH2 antagonist OC000459 is well tolerated

and modestly effective in achieving inflammation resolution and symptom improvement in adult patients with severe and active EoE. Limitations of this study include that no information regarding the property of OC000459 as maintenance medication is provided and that the increased efficacy of a combination treatment of OC000459 with PPI is not thoroughly evaluated. Considering the excellent safety profile of the compound, these questions should be addressed in future clinical trials.

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Authors' contributions

The study protocol was designed by AS, CB, MP, RP, MH, MP, and HUS. AS performed the gastroenterological; CB the histologic; HUS, SH, and MS the immunological examinations. LPC performed the pharmacodynamic analyses. JS was responsible for the monitoring of the study. All investi-

gators contributed to gathering of data, reviewed manuscript drafts, and approved the final manuscript. AS and HUS wrote the paper.

Conflict of interest

Alex Straumann and Christian Bussmann received research support for the clinical trial from the sponsor. Mike Perkins, Lisa Pearce Collins, Roy Pettipher, Michael Hunter, and Jan Steiner are employed by Oxagen Ltd. The remaining authors disclose no conflicts of interest.

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