A Preliminary Open-Label Trial of Losartan Potassium in Participants with Eosinophilic Esophagitis (EoE) With or Without a Connective Tissue Disorder (CTD)

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Statistical Analysis Plan

CCHMC

A Preliminary Open-Label Trial of Losartan Potassium in Participants with Eosinophilic Esophagitis (EoE) With or Without a Connective Tissue Disorder (CTD)

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Purpose of Study

The primary objective of this proposal is to assess the effect of losartan on the reduction of esophageal eosinophils in participants with eosinophilic esophagitis (EoE) with or without a connective tissue disorder (CTD). Participants aged 5 to 25 years with active EoE will be enrolled and the primary efficacy outcome will be the change in esophageal eosinophil number obtained following a 16-week trial of Losartan. During the screening process, active EoE will be confirmed by research pathologist review (histologic evaluation) of esophageal biopsies obtained during by esophagogastroduodenoscopy (EGD) in subjects with a history consistent with EoE.

Hypotheses

Inhibition of the TGF-β1 pathway could provide a unique means of improving patient care in EoE via the reduction in smooth muscle contraction mediated by the deep-lying mast cells and TGF-β1, as well as reducing pathologic fibrosis and cellular proliferation seen in these patients. Herein we propose that a reduction in TGF-β1 activity via losartan in patients with EoE will lead to reduced eosinophilia, fibrosis, and smooth muscle contractility, which each play a role in the pathogenesis and symptomatology of this disease. Further, the use of a once-a-day oral medication, without the difficulties associated with either steroid medications or dietary modification, could provide improved compliance and quality of life for this

patient population. This represents a preliminary descriptive study in which the impact of losartan therapy in patients with EoE or EoE-CTD is assessed.

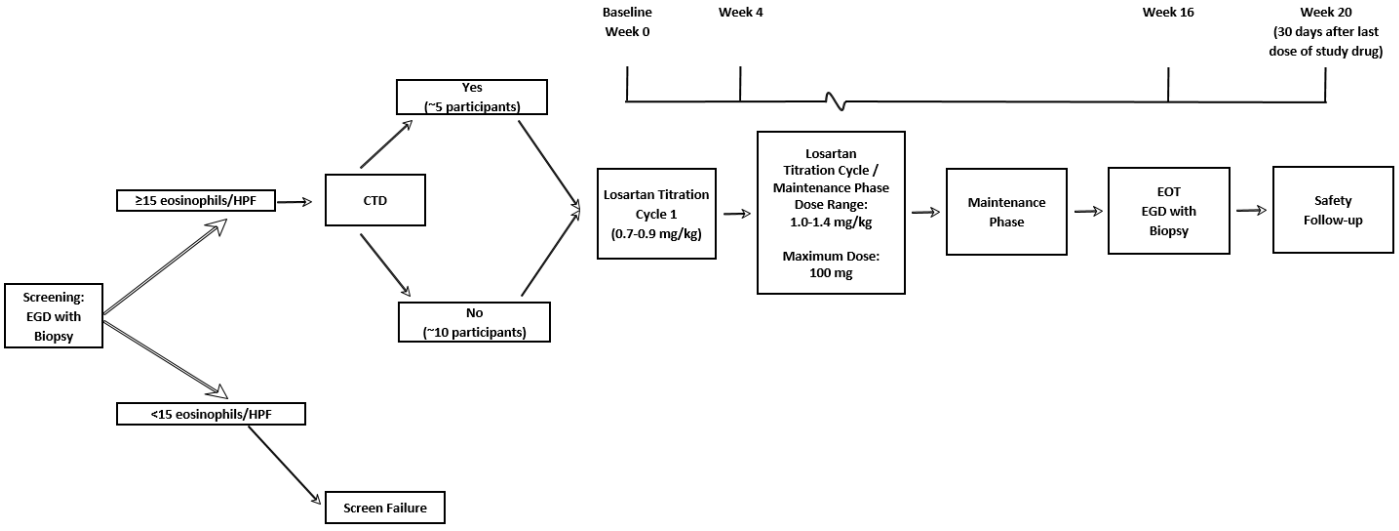
Primary Objective(s)

The primary objective of this open-label pilot study is to assess the effect of losartan on the reduction of esophageal eosinophils in participants with EoE with or without a CTD.

Study design

This is a phase II, open-label trial of losartan in patients diagnosed with EoE with or without a CTD. Prior to entry, patients must meet the current consensus criteria for EoE in which efforts are made to rule out other causes of esophageal eosinophilia. The trial will perform a preliminary assessment of the effect of the maximum tolerated dose of losartan in participants with active EoE as determined by the absence of side effects and by vital signs being within normal limits. The primary endpoint of the study is the reduction in the number of esophageal eosinophils in participants who will receive losartan orally once a day (Figure 1). The primary safety endpoint is descriptive and will require reporting of SAE or Grade 3 and above AEs.

Figure 1 Study Design Flowchart



Analysis Populations

The analysis population for the primary and secondary endpoints will participants who received Losartan and maintained the baseline EoE treatment throughout the course of the study.

Statistical Considerations and Analytical Plan

Overview

Change from baseline for eosinophil counts will be calculated for each participant. The average change from baseline within each group and combining both groups (i.e. EoE and EoE-CTD) will be calculated along with the corresponding two-sided, 95% confidence interval. Additionally, we will perform paired tests which will specifically test whether there was significant change between baseline and study completion. The last observation carried forward (LOCF) method will be used for participants who end the trial early. To assess changes over time for continuous secondary and safety outcomes, a mixed effects model for repeated measures analysis will be used. Unless stated otherwise, all statistical testing will be done at α=0.05.

Variables of Interest

Primary outcome:

1) Change in eosinophils/hpf from baseline to post treatment
2) Rate of SAE / Grade 3 or above AE

Secondary outcomes:

1) Blood (serum) and esophageal TGF-β levels
2) EoE transcriptome
3) Histopathology as assessed by the Histology Scoring System (HSS): Will include the overall score, grade score, stage score, and the inflammatory and structural sub-scores. Scores will be reported as distal, proximal, and maximum
4) Esophageal compliance as measured by EndoFLIP.
5) PROS (Pediatric Eosinophilic Esophagitis Symptom Severity [PEESS], PedsQL Eosinophilic Esophagitis Module [PedsQLTM EoE], EoE Quality of Life Questionnaire, and EEsAI)

Derived Variables.

1) HSS scores. Sum of the feature scores divided by the maximum possible score for the biopsy for grade and stage. The HSS assesses eight histological features: eosinophilic inflammation, basal zone hyperplasia, eosinophilic abscess, eosinophilic surface layering, dilated intercellular spaces, surface epithelial alteration, dyskeratotic epithelial cells, and lamina propria fibres. Each feature is scored on a 4-point scale for severity (grade) or extent (stage) of the abnormality, with 0 representing normal features and 3 denoting most severe or extensive features. A final HSS score (grade or stage) is the sum of the assigned scores for each feature assessed divided by the maximum possible score for that biopsy specimen;7 we defined the HSS total score as the sum of the HSS final scores for grade and stage. Therefore, the HSS total score ranges from 0 to 2 because each

HSS final score ranges from 0 to 1. Inflammatory sub-scores include the following features: eosinophils, abscesses, surface layering, and surface necrosis. Structural sub-scores include basal layer hyperplasia, dilated intercellular spaces, apoptotic epithelia, and lamina propria fibrosis.

2) EndoFLIP –
3) EREFS. In EREFS, five endoscopic features are scored for both the distal and proximal or mid-oesophagus: oedema (0–1), rings (0–3), exudates (0–2), furrows (0–2), and strictures (0–1). We will calculate the EREFS total score by adding the assigned scores for each feature in both the distal and proximal or mid-oesophagus; the EREFS total score ranges from 0 to 18.
4) Adherence. Adherence will be dichotomized into mostly adherent vs not-mostly adherent based on the number of missed doses. The statistician will provide descriptive data to one of the study clinicians to determine what is the appropriate threshold for non-adherence. Once a threshold has been selected, the statistician will provide an adherence classification on each participant.
5) Atopy. Atopy is a multi-faceted disease. Individuals will be defined as atopic if they report a history of asthma, eczema, allergic rhinitis, allergic conjunctivitis, IgE mediating food allergy and/or atopic dermatitis.
6) Time since diagnosis will be calculated as the study date at baseline minus the date at diagnosis.

Prior to any hypothesis testing, all variables will be reviewed for plausibility. Any values which are considered suspect will be reviewed by the clinical team to ensure accuracy. Continuous data will be evaluated for distributional assumptions of normality. Natural log-transformation will be considered for right skewed non-normally distributed data (the most typical skew seen in physiologic data).

Demographics/Patients Characteristics

Demographic, patient characteristics, medication use, and study completion status will be summarized using median [interquartile range] for continuous variables and frequency and percentages for categorical variables. Treatment comparisons will be performed for demographics and patient characteristics using the Wilcoxon Rank sum test for continuous variables and the Fisher's Exact test for categorical variables. All tests will be conducted at α=0.05.

Analysis of Primary Outcome

Primary Objective:

1. To assess the effect of losartan on the reduction of esophageal eosinophils in participants with EoE with or without a CTD.

Change from baseline for eosinophil counts will be calculated for each participant. The average change from baseline within each group and combining both groups (i.e. EoE and EoE-CTD) will be calculated

along with the corresponding two-sided, 95% confidence intervals. If the assumption of normality is not viable for the change from baseline score, we will use a square root transformation on the eosinophil counts prior to calculating the change from baseline and determine if the "change score" attains normality. If this transformation is not successful, the median of the change will be calculated along with the 95% confidence interval for the median using Hodges-Lehmann estimators. Each confidence interval will be visually inspected to determine of zero is a possible value for the true change from baseline (μ). This corresponds to testing the following hypothesis:

H0: $\mu=0$ vs. H1:$\mu\neq0$.

The last observation carried forward (LOCF) method will be used for participants who end the trial early.

Additionally, to evaluate the effect of losartan on the reduction of esophageal eosinophils in participants with EoE (with or without a CTD), we will use paired non-parametric Wilcoxon Rank sum test. The Wilcoxon rank sum paired test will be used given the small sample size and the broad range of eosinophils which could be present in our participants. Analyses will be performed using the whole cohort and stratified by CTD. Statistical significance will be set at $\alpha = 0.05$. This corresponds to testing the following hypothesis:

H0: baseline and study completion are equal vs. H1: baseline and study completion are not equal.

Analyses of Secondary Endpoint(s)/Outcome(s)

To assess changes over time for continuous secondary and safety outcomes, a mixed effects model for repeated measures analysis will be used. The dependent variable will be the change from baseline. The "participant nested within group" term will be considered a random effect. Time (i.e. measurement time) will be included as a continuous effect. Terms in the model will be time, group (i.e., EoE and EoE-CTD), and time-by-group interaction. The baseline value will be used as a covariate in the model. If necessary, to meet the assumptions of the analysis, an appropriate transformation will be used (e.g., square root, log, rank). The hypotheses that will be tested are whether there is a significant trend over time and whether this trend is consistent between the CTD and non-CTD groups (i.e. testing the interaction term). If the trend in not consistent between groups based on the statistical test at $\alpha=0.10$, we will estimate the trend within each of the two groups by including terms in the statistical model that estimates the trend within each group and the hypothesis to be tested within each group is whether this trend is significantly different than zero. We will systematically assess the residuals to determine whether a linear or quadratic model best fits the data. In statistical terms, the hypotheses are provided below.

For testing consistency of the time trend (β) between the CTD and non-CTD group, the hypotheses are:

H0: βEoE-CTD= βEoE (non-CTD) vs. H1: βEoE-CTD$\neq$ βn EoE (non-CTD).

For testing linear trend combining across groups (if there is consistency of the time trend):

H0: $\beta=0$ vs. H1: $\beta\neq0$ where β is the slope of the line across groups. Similar hypotheses will be tested if analysis of the residuals indicates that higher order terms need to be included in the model (e.g. quadratic). If there is strong evidence that the time trend is not consistent between the two groups, we will test the above hypothesis within each of the two groups. In addition, the two-sided, 95% confidence interval for the slopes will be calculated to provide estimates of the slope for future studies.

The percentage of participants for whom peak eosinophil counts are < 15 eosinophil/HPF at Week 16 will be estimated within each CTD group along with a 95% confidence interval.
Safety endpoints that are categorical will be summarized within each group. Further, any SAEs or Grade 3 or above will be reported descriptively.

Software Used (with References) and Specialized Macros (with References)
SAS version 9.4, JMP 14.

This SAP has been reviewed and approved by:

[redacted]

Investigator Date