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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Rare Diseases Clinical Research Network

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

Consortium for Eosinophilic Gastrointestinal Disease Researchers

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Protocol: CEGIR 7803	**Version/Date:** Version 7.0 / June 25, 2018
Site Principal Investigator:	
Title: ***A Preliminary Open-Label Trial of Losartan Potassium in Participants with Eosinophilic Esophagitis (EoE) With or Without a Connective Tissue Disorder (CTD)***	
Study Sponsor: National Center for Advancing Translational Sciences (NCATS) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: *The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please upload the signed document to the DMCC E-regulatory binder, loRA cubby.* Please contact, [redacted] [redacted], Research Project Manager Data Management and Coordinating Center (DMCC) Health Informatics Institute University of South Florida 3650 Spectrum Boulevard Suite 100 Tampa, Florida 33612-9446	
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID. **Site Principal Investigator (Print)**	
Site Principal Investigator (Signature)	**Date**

Protocol Synopsis

Title	A Preliminary Open-Label Trial of Losartan Potassium in Participants with Eosinophilic Esophagitis (EoE) With or Without a Connective Tissue Disorder (CTD).
Short Title	EoE Losartan Trial.
Clinical Phase	Eosinophilic Esophagitis (Phase II).
IND Sponsor/Number	[redacted] / [redacted]
Study Objectives	**Primary Objective:** • The primary objective of this pilot study 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). **Secondary Objectives:** • To investigate the effect of losartan on the levels of transforming growth factor beta (TGF-β) in the blood and esophagus. • To investigate the effect of losartan on the normalization of the EoE transcriptome in participants with EoE with or without a CTD. • To investigate the effect of losartan on general histology of the esophagus, using a histology scoring scale. • To determine the safety of losartan in participants with EoE with or without a CTD. • To investigate the effect of losartan by utilizing patient-reported outcome (PRO) questionnaires to assess for clinical symptom response. • To evaluate the effect of losartan by utilizing the histology scoring system created to express the severity and extent of gastrointestinal abnormalities that often accompany eosinophilic inflammation.
Study Design	Open-Label.

Primary Endpoint(s)	• Change from baseline in the peak esophageal eosinophil count in participants receiving losartan potassium at the end-of-treatment (EOT) visit (or at early withdrawal) as determined by esophagogastroduodenoscopy (EGD) with biopsy and reported as eosinophils per high-power field (eosinophils/HPF). • A primary safety endpoint is also included to monitor for adverse events in the enrolled subjects. Specifically, as a descriptive investigation, any SAE or Grade 3 and above AEs that arise during the conduct of this pilot study will be reported.
Secondary Endpoint(s)	• Change from baseline in the blood (serum) and esophageal TGF-β levels in participants receiving losartan potassium at the EOT visit (or at early withdrawal) as determined by research blood samples and EGD with biopsy, respectively. • Normalization of the EoE transcriptome in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy. • Change from baseline in histopathology in participants receiving losartan potassium at the EOT visit (or at early withdrawal) as determined by EGD with biopsy. • Change from baseline esophageal compliance in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EndoFLIP. • Change from baseline PROS (Pediatric Eosinophilic Esophagitis Symptom Severity [PEESS], PedsQL Eosinophilic Esophagitis Module [PedsQL™ EoE], EoE Quality of Life Questionnaire, and EEsAI) in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EndoFLIP.
Accrual Objective	• It is estimated that 30 patients will need to be screened for a total of approximately 15 participants enrolled in the study. Of the 15 participants enrolled, at least one third will have evidence of EoE with CTD, whereas the remaining participants will have no evidence of a CTD.
Study Duration	• Total duration of treatment is approximately 16 weeks. During the titration phase, participants will be on titrated doses of losartan for 4 weeks. During the maintenance phase, participants will be on a stable dose of treatment for 12 weeks.

Treatment Description	• During the study oral administration of losartan tablets or suspension occurs once a day, at about the same time each day, throughout the treatment period. The dose of the study agent to be administered will depend on the participant's body weight and will be calculated (mg/kg) on the basis of a titration schedule during the treatment (i.e., titration and maintenance) phase, not to exceed the daily dose of 100 mg. The medication dosage for losartan potassium will change throughout this study, titrating to a higher dose.

Inclusion Criteria	1. Written informed consent has been provided by the participant/parent or legal guardian. 2. Participants (of any gender and any race) aged ≥ 5 years to ≤ 25 years at time of screening. 3. The participant has confirmed, active EoE (at Screening or within 12 weeks prior to enrollment) as defined by esophageal mucosal eosinophils ≥ 15 per HPF (400 x magnification) confirmed by the research pathologist AND fits into one of the following categories: a. Participant has not been diagnosed with and does not display phenotypes suggestive of a CTD, **OR** b. Participant has been diagnosed with a CTD (e.g., Marfan syndrome [MFS], Loeys-Dietz syndrome [LDS], Ehlers Danlos syndrome (EDS), hypermobility syndromes). 4. The participant has been on a high dose of proton pump inhibitor (PPI) (at least one dose, once daily) for at least 8 weeks prior to a diagnostic endoscopy of EoE without histologic resolution (i.e., ≥ 15 eosinophils/HPF). Due to the variety of doses and various PPIs available, the dose will be confirmed adequate at the discretion of the site PI. Note: Participants may continue with their acid reflux therapy as long as the dose remains the same throughout the study. 5. Participant must maintain the same diet, swallowed steroid, and PPI therapies throughout the duration of the study. Participants with active EoE on these therapies may be included in the study, as long as they agree to maintain the same diet, swallowed steroid, and PPI therapies. 6. At the time of study entry, a female participant is eligible if she meets one of the following criteria: a. Is of non-childbearing potential (pre-menarchal or surgically sterile with documentation).

	b. Is of childbearing potential with a negative urine pregnancy test (beta human chorionic gonadotropin [β-hCG]) prior to enrollment into the study (i.e., at screening) and at each monthly study visit. Subsequently, these participants must agree to use adequate birth control measures (e.g., condom, oral/injectable/subcutaneous contraceptives, intrauterine device, or sexual abstinence) during the study and for at least one month after the last dose of study drug that will be documented in the source documents.

Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Any past or planned cardiac surgery. 2. An aortic root Z-score ≥ 3.0 on a previous echocardiogram. 3. Intolerance to the study agent (i.e., losartan) such as angioedema, immunoglobulin E (IgE)-mediated allergy, etc. 4. A mean blood pressure measurement (both systolic and diastolic) at screening that is below the 2nd percentile for his/her age as listed in the study staff–generated guideline report for blood pressure ranges for children and adolescents (See section 19), and is currently experiencing hypotension symptoms (1). If the participant is asymptomatic, this will not be considered hypotension. 5. Renal dysfunction with creatinine in excess of the upper normal limit for age 6. Another disorder that causes esophageal eosinophilia (e.g., hypereosinophilic syndrome, Churg-Strauss vasculitis, eosinophilic granuloma, or a parasitic infection). 7. A diagnosis of hepatic insufficiency (e.g. liver failure, history of liver transplantation, or persistent liver transaminase elevation). 8. A history of abnormal gastric or duodenal biopsy or documented gastrointestinal disorders (e.g., Celiac Disease, Crohn's disease or *Helicobacter pylori* infection), not including chronic gastritis, chronic duodenitis, mucosal eosinophilia or other eosinophilic gastrointestinal disorders (EGIDs). 9. Use of anti-IgE monoclonal antibody (mAb), anti–tumor necrosis factor [TNF] mAb, anti–IL-5 agents, or anti–IL-13 within 6 months prior to study entry (i.e., screening). 10. Use of methotrexate, cyclosporine, interferon α, or other systemic immunosuppressive or immunomodulating agents within 3 months prior to the screening visit. 11. A stricture during endoscopy procedure that prevents passage of the endoscope

	12. Taking or is planning to take an angiotensin receptor blocker (ARB), angiotensin-converting enzyme inhibitor (ACEI), beta blocker (BB), or calcium-channel blocker therapy at the screening visit or at any time during the study or has been taking any of these medications for 3 months prior to the screening visit. 13. Taking or is planning to take hydrochlorothiazide, digoxin, warfarin, cimetidine, phenobarbital, rifampin, or fluconazole. 14. Is taking or is planning to take potassium supplements or salt substitutes containing potassium. 15. A female participant who is pregnant or nursing or, if of childbearing potential, is not using a medically accepted, effective method of birth control (e.g., condom, oral/injectable/subcutaneous contraceptive, intrauterine device, or sexual abstinence). 16. Participated/participating in any investigative drug or device study within 30 days prior to study entry. 17. Participated/participating in any investigative biologics study within 3 months prior to study entry. 18. Unable to be confirmed, active EoE (at Screening or within 12 weeks prior to enrollment) as defined by esophageal mucosal eosinophils ≥ 15 per HPF (400 x magnification) confirmed by the research pathologist.
Study Stopping Rules	Study enrollment and treatment will be suspended pending expedited review of all pertinent data after the occurrence of: 1. One death regardless of relationship to the investigational product 2. One nonfatal SAE possibly related to the investigational product 3. Three participants discontinued from study because a particular AE recurred on three occasions and was not mitigated by dosing titration If the study is stopped due to meeting the above criteria, it may not be resumed until all pertinent information is discussed with DAIT NIAID, NIAID Asthma and Allergy DSMB, and the central IRB, and all parties concur with the resumption of the study. Local IRBs will be informed of the study stoppage and the DSMB/central IRB's decision on resumption of the study The study may be terminated by DAIT/NIAID or the NIAID Asthma and Allergy DSMB upon review of any observations, events, or new information that merit such action

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Table of Contents

Glossary of Abbreviations

Abbreviation	Term
AE	Adverse event
ACEI	Angiotensin-converting enzyme inhibitor
ADR	Adverse drug reaction
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ARB	Angiotensin receptor blocker
AST	Aspartate aminotransferase
AT1R	Angiotensin II receptor 1
AT2R	Angiotensin II receptor 2
BB	Beta blocker
B.I.D	Twice (two times) aday
BUN	Blood urea nitrogen
CBC	Complete blood count
CEGIR	Consortium of Gastrointestinal Disease Researchers
CCHM	Cincinnati Children's Hospital Medical Center
C CFR	Code of Federal Regulations
CRF	Case report form
CL	Confidence limit
CTD	Connective tissue disorder
DAIT	Division of Allergy Immunology and Transplantation
DMC	Data Management and Coordinating Center
C	Data safety monitoring board
DSMB	Esophagogastroduodenoscopy
EGD	Eosinophilic gastrointestinal disorders
EGID	Ehlers-Danlos syndrome
EDS	Eosinophilic esophagitis
EoE	Eosinophil diagnostic panel
EDP	Endolumenal functional lumen imaging probe
EndoFLIP	EoE Endoscopic Reference Score
EREF	Epidermal differentiation complex
ED	End of treatment
C	Eosinophilic Esophagitis Activity Index
EOT	Filaggrin
EEsAI	Food and Drug Administration
FLG	Good Clinical Practice
FDA	Gastroesophageal reflux disease
GCP	Gamma-glutamyl transferase
GER	Glomerular filtration rate
D	Human chorionic gonadotropin
GGT	High-power field
GFR	Investigational brochure
hC	
G	
HPF	

IC	International Conference on Harmonization
H	Independent ethics committee
IEC	Immunoglobulin E
IgE	Interleukin
IL	Investigational new drug
IND	Institutional Review Board
IRB	Loeys-Dietz syndrome
LDS	Losartan potassium
Losartan	Monoclonal antibody
mAb	Marfan syndrome
MFS	Mean corpuscular hemoglobin
MCH	Mean corpuscular hemoglobin concentration
MCHC	Mean corpuscular volume
MCV	Milliequivalent
mEq	Milligram
mg	Milliliter
mL	Manual of operations
MOO	Neutralizing antibodies
nAb	National Institute of Allergy and Infectious Diseases
NIAID	National Institute of Diabetes and Digestive and Kidney Diseases
NIDD	National Institutes of Health
K NIH	National Library of Medicine
NLM	Odds ratio
OR	Primary care physician
PCP	Pediatric Eosinophilic Esophagitis Symptom Severity
PEESS	Pediatric Quality of Life Inventory Eosinophilic Esophagitis Module
PedsQL™EoE	Polyethylene terephthalate
PET	Protected health information
PHI	Principal Investigator
PI	Postural orthostatic tachycardia syndrome
POT	Proton pump inhibitor
S PPI	Patient-reported outcome
PRO	Renin-angiotensin system
RAS	Ras homolog gene family, member A
RhoA	Red blood cell
RBC	Red blood cell distribution width
RDW	Serious adverse event
SAE	Serious adverse event report
SAER	Serious adverse reaction
SAR	Mothers against decapentaplegic homolog 2
SMAD2	Single nucleotide polymorphism
SNP	Standard operating procedure
SOP	Transforming growth factor beta
TGF-β	Transforming growth factor beta receptor
TGF-βR	Tumor necrosis factor
TNF	

TSLP	Thymic stromal lymphopoietin
WBC	White blood cell

1 Background and Rationale

1.1 Background and Scientific Rationale

EoE represents a complex clinical entity often requiring coordinated care within the fields of both allergy and gastroenterology. EoE is diagnostically defined by revised consensus criteria that in brief require an esophageal-specific level of ≥ 15 eosinophils per HPF, as obtained from 2 to 4 biopsies from either the proximal or distal esophagus, that is not mitigated by the use of PPIs (2). Further, features such as response to diet may also be used in establishing the diagnosis. Symptomatically, patients with EoE often complain of dysphagia, vomiting, reflux, food aversions, and frank food impactions (2-4). These patients commonly have diagnoses associated with atopy and other allergic disorders such as IgE-mediated food allergy, asthma, allergic rhinoconjunctivitis, and urticaria. Current treatment modalities center around the use of swallowed steroids therapies with either fluticasone propionate or budesonide, oral steroids, and a variety of restrictive diets that rely upon the use of allergy test results, the empiric avoidance of common food allergens, or the elimination of all antigenic proteins via the use of elemental formulas (5-13). Though all of these treatments are effective, they require continual use or the disease will recrudesce in the majority of patients. For the use of steroids, there is the risk of obesity, adrenal suppression, osteopenia, and steroid-induced diabetes and cataracts, whereas the dietary restrictions can require significant familial or personal commitment to severe dietary restrictions that are life changing.

In evaluating the fundamental underlying processes of EoE, through the use of a several methodologies, genes such as eotaxin 3 (*CCL26*), IL 13 (*IL13*), and thymic stromal lymphopoietin (*TSLP*) have been directly associated with the disease pathogenesis (14-18). In addition to these genes, TGF-□ has been of interest due to evidence of patients with EoE having increased TGF-β1, which has been localized to eosinophils within the inflamed and fibrotic esophageal lamina propria, along with deeper-lying mast cells that are physically intertwined with esophageal smooth muscle cells (19-22). After treatment with TGF-β1 *in vitro*, cultured esophageal smooth muscle cells physically fixed in collagen contracted in size by nearly 50%. This suggests a potential clinical link to dysphagia and food impaction, related to the production and release of TGF-β1 and other mediators from these close-lying mast cells, in addition to TGF-β1's potential long-term impact on fibrosis and stricture formation (22, 23). TGF-β1 is associated with the direct regulation of fibrosis (24, 25) and can promote the generation of periostin that leads to eotaxin 3–mediated eosinophil recruitment and tissue remodeling (26), which is likely further enhanced by the additional cascade of periostin-induced activation of TGF-β1 from its latent to active form (27). In addition to being expressed by both eosinophils and mast cells, TGF-β1 is expressed by T regulatory cells, which are dysregulated in both patients with EoE and in animal models of EoE (28, 29). In experimental models of parasite infection of mice, mice with defective TGF-β1 signaling mount only a limited reactive mastocytosis while retaining a high parasitic worm burden relative to controls, suspected to be due to low expression of IL-9 after infection (30). In addition, the

esophagi of patients with EoE overexpress IL-9, a cytokine that was originally isolated as a potent mast cell growth factor, which is potentially driven by TGF-β1 overexpression which would support the reactive mastocytosis seen in EoE patients (7, 22,31-36).

Excess TGF-β1 expression has been associated with connective tissue disorders CTDs such as Duchenne muscular dystrophy and MFS, and these disorders are often associated with dysphagia; however, to date there have been only two reports associating other CTDs (scleroderma, polymyositis, and dermatomyositis) with non-esophageal EGIDs in 7 patients (37-40). Unusually for 6 of these patients, an unexpected and concomitant increase in mast cells was also noted in the gastrointestinal biopsies (39). It has now become apparent, through work performed here at Cincinnati Children's Hospital Medical Center (CCHMC) and at John Hopkins Hospital, that there is a subset of EoE related to CTDs (41, 42). We have identified a cohort of 34 individuals with EoE and with varied CTD syndromes associated with TGF-□ pathway dysregulation inclusive of MFS, EDS or benign hypermobility syndrome (formerly EDS type III (43)). The elevated prevalence of patients with CTD within the EoE population represents a greater than 8-fold higher risk of EoE in patients with CTD in comparison to the general population based upon analyses of our hospital-wide de-identified i2b2 patient dataset (odds ratio [OR] = 8.0, confidence limit [CL] 4.4-14.5; $\chi^2_1 = 66.0$; P< 0.001 unpublished observations). We also examined our well-defined EGID Research Registry (n = 1,008 patients with EoE) for evidence of CTDs. In this EoE group, there was a 3.4% prevalence of CTDs. These results did not appear to be driven by patient selection bias, as 2.2% of EoE patients residing in the immediate catchment area of our hospital (n = 267) also had a CTD. Beyond our work, EoE has also been noted in several patients with another CTD; LDS is associated with the TGF-β receptors 1 & 2 (TGF-βR1 & TGF-βR2) but was not identified within our EoE-CTD cohort, which had limited numbers of patients with LDS. Further, in a mouse model of LDS, nearly every mouse appeared to have evidence of EoE (See informal reference for LDS associated with EoE; YouTube Video Conference: http://www.youtube.com/watch?v=WLPNcfExOLU).

This relationship between CTDs and EoE extends beyond just this subset of patients with EoE in that the physiological disruption of the TGF-□ pathway in the patients with EoE-CTD also appears to play a causal role in the larger set of patients with EoE without demonstrable CTD features. This current and ongoing work demonstrates through a variety of avenues this central role for TGF-□ in the genetics and pathophysiology of EoE in general, regardless of the presence of CTD features, as summarized in Figure 1.

1.2 Figure 1: Molecular and cellular mechanisms involved in eosinophilic esophagitis pathogenesis

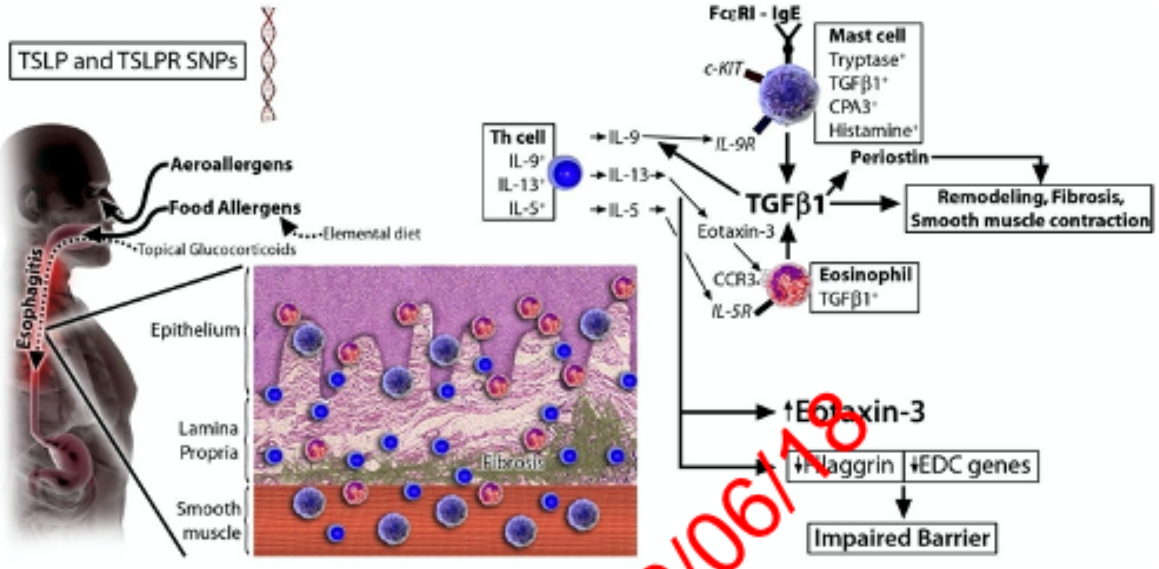

Figure 1: A proposed model to explain the molecular and cellular mechanisms involved in eosinophilic esophagitis (EoE) pathogenesis, TGF-□1–associated pathology, eosinophil recruitment, and treatments. Aeroallergen and food-allergen sensitization have been implicated in EoE pathogenesis. Elemental diet, glucocorticoids, and anti–IL-5 treatments have been noted to improve the microscopic features of EoE, acting on the disease pathogenesis at different levels. Hyperplastic epithelial cells of the esophagus overexpress eotaxin 3, likely in response to IL-13. Eotaxin 3 overexpression promotes chemoattraction of CCR3+ eosinophils. EoE disease susceptibility is genetically defined by strong association to single-nucleotide polymorphisms (SNPs) in the *TSLP* gene with a lesser contribution from a SNP in the filaggrin (*FLG*) gene. TGF-□1 and Th2 cytokines promote IL-9 generation, which promotes mastocytosis. Th2 cytokines (e.g., IL-13 and IL-4) drive eotaxin 3 production, as well as synergize with TGF-□1 to promote Th9 cells, which drive mastocytosis, thereby resulting in a positive feedback loop as mast cells and eosinophils produce TGF-□1. TGF-□1 has been shown to induce several processes that promote EoE disease pathogenesis, including fibrosis, smooth muscle contractility, and periostin expression, which further promotes tissue remodeling, while reducing epidermal differentiation complex (EDC) genes and regulating eotaxin-mediated eosinophil adhesion and migration (Taken from Abonia et al., (23, 44)).

The mast cells directly infiltrate the esophageal smooth muscle (ESM) in patients with EoE and generate TGF-β1 (22). *In vitro* TGF-β1 pathway activation induces phospholamban in human esophageal smooth muscle cells and myofibroblast leading to contraction (45). As such, TGF-β1 may directly and adversely impact esophageal motility and symptoms of dysphagia. *In toto*, this information suggests that the 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 deeper lying mast cells and TGF-β1, as well as reducing pathologic fibrosis and cellular proliferation seen in these patients. Evidence supporting the blockade of TGF-β1 activity outside of EoE has also been demonstrated in two mouse models of CTDs, MFS (fibrillin 1 knockout mice, *Fbn1*$^{C1039G/+}$) and Duchenne muscular dystrophy (dystrophin knockout mice, *mdx*) (46, 47). In the MFS mouse model, using neutralizing antibodies (nAb) against TGF-β1 resulted in normalization of aortic root architecture, as well as the reduced phosphorylation and nuclear translocation of the mothers against decapentaplegic homolog 2 (SMAD2) transcription factor that is part of TGF-β1 pathway signaling (46, 48). Further, in *mdx* mice, the use of nAb significantly attenuated the fibrosis in a mouse model that recapitulates the pathologic findings commonly seen in patients with Duchenne muscular dystrophy

(47). In human studies to date, one potential therapy for patients with EoE would be the use of a humanized nAb against TGF-β1, which is currently being evaluated in preliminary clinical trials (fresolimumab, GC1008) and has been targeted for the treatment of various cancers, idiopathic pulmonary fibrosis, focal segmental glomerulosclerosis, and scleroderma (ClinicalTrials.gov as of 02/16/2012).

EoE also represents a chronic disorder whose enduring complications often appear in late adolescence and includes symptoms of dysphagia associated with repetitive episodes of near-food impaction, along with the physical findings of esophageal rigidity and strictures (19, 49-51). In essence, the TGF-□1 pathway can support the formation of a rigid cellular and fibrotic connective tissue expansion, combined with ineffective muscular contraction that creates an unforgiving esophageal architecture that is unsuitable for the easy passage of food which worsens in the absence of effective therapy (19-22, 51, 52). Consistent with this model of esophageal dysfunction, one of our CEGIR Investigators, Dr. Ikuo Hirano, has quantitatively demonstrated reduced esophageal compliance using *in vivo* analyses of adult EoE patients with endolumenal functional lumen imaging probe (EndoFLIP) (53, 54). Further, worsening esophageal compliance was associated with an increased risk of food impaction (55). This procedure may therefore provide a unique physiologic and quantitative marker of esophageal dysfunction with clinical utility in addition to what is currently available via examination superficial esophageal biopsies specimens. Indeed, while several studies have demonstrated biopsy-proven control of eosinophilic inflammation via the use of common therapies for EoE, there has often been a disconnect between the biopsy report and patient-reported symptoms. The combination of histologic evaluation of esophageal biopsies, direct assessment of TGF-□1 markers, measurement of esophageal compliance, and correlation with clinical instruments that quantify symptoms, esophageal function, and quality of life would be unique in an EoE clinical trial. Further, combining these measures in a therapeutic intervention trial aimed at reducing the activity of pro-fibrotic factors such as TGF-β1 via the use of losartan, could provide powerful insights into disease mechanisms and elucidate potential novel therapeutic intervention strategies. As such, the TGF-□1 pathway represents an ideal target for therapeutic intervention.

1.3 Rationale for Selection of Investigational Product or Intervention

The same mice studies noted above also evaluated the use of an angiotensin II receptor 1 (AT1R) antagonist losartan, a commonly used anti-hypertensive medication that reduces TGF-β1 activity in other non-CTD disease models due to the extensive cross-talk between the shared elements of the TGF-β1 and AT1R pathways (56). Activation of either TGF-β1R or AT1R results in the phosphorylation of SMAD2/3, leading to transcription of several products inclusive of TGF-β1 itself; further activation of either pathway leads to activation of the ras homolog gene family, member A (RhoA) and contraction of vascular smooth muscle fibers (56). Similar to treatment with nAb, the use of losartan in the *Fbn1*$^{C1030G/+}$ and *mdx* mouse models normalized the aortic root architecture, reduced SMAD2 phosphorylation and translocation, and attenuated evidence of fibrosis in their respective models (46, 47). Additional theoretical advantages of the use of AT1R inhibition via the use of losartan are the potential beneficial effects obtained by biasing angiotensin-based signaling towards the angiotensin II receptor 2 (AT2R). AT2R receptor

signaling appears to anti-inflammatory effects, and the use of renin-angiotensin system (RAS) blockade in humans with MFS has already been attempted (57, 58). In these studies beneficial effects having been noted with the use of angiotensin-converting enzyme inhibitors (ACEI) and ARBs, which have demonstrated reduced aortic root diameter in a small, randomized trial and retrospective analysis, respectively (59, 60). Some controversy has been noted; there has been a potential association between excess AT2R signaling, as would be expected with the use of an ARB like losartan, and the cystic medial degeneration that is seen in aortic aneurysm. This is based upon an *in vitro* study utilizing samples from patients with MFS with end-stage aneurysms, which demonstrated vascular smooth muscle cell apoptosis, which was worsened with ARB use (61). However, Habashi *et al.* later demonstrated worsening aortic root dilation in double knockout mice lack both the fibrillin and *AT2R* genes, implying a further beneficial effect of AT2R beyond the anti-inflammatory effects noted above as opposed to the hypothetical, perilous effect implied by the *in vitro* study of patients with MFS (62).

1.4 Preclinical Experience

Herein we propose that reducing TGF-β1 activity with losartan in patients with EoE will lead to reductions in the eosinophilia, fibrosis, and smooth muscle contractility that each play a role in the pathogenesis and symptomatology of this disease. While the use of losartan has not been studied to date in either pediatric or adult EoE, it does represent a medication with support of mechanistic data which suggest it as a suitable target for pharmacologic intervention. TGF-β1 expression is increased in the esophageal biopsies of EoE patients relative to normal controls, and in CTD patient populations there is an increased risk of EoE (MFS, EDS, LDS, etc.) (19, 21-23, 41, 42, 45, 63). As losartan is already used in both pediatric and adult populations for hypertension and proteinuria, it represents a unique medication to potentially treat EoE across a broad range of ages. 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. We hypothesize that we will see beneficial effects in terms of esophageal pathology within the study period, as the beneficial effect upon aortic root dilation was noted within 36 weeks with the use of the ACEI (enalapril (60)), and within 12 months with the use of an ARB (losartan (59)). This proposal represents the first exploratory prospective study to evaluate the effects of an ARB in EoE, while simultaneously differentiating between EoE vs. EoE-CTD. Though some controversy exists with the use of an ARB vs. ACEI in patients with MFS, both of these drug classes have excellent safety profiles, and both classes are currently in use clinically for these patients. In addition, losartan has been in use for treatment of hypertension for over a decade, and there are clinical trials utilizing losartan long-term in the MFS population without evidence of unusual AEs reported to date. Therefore, we do not have an expectation of AEs that would uniquely be present with the EoE patient population. It is our hope and expectation that this study will demonstrate the clinical value of losartan without evidence of adverse effects.

1.5 Treatment with Losartan

Although losartan is most commonly used for treatment of systemic hypertension, it is also routinely used without significant AEs for treatment of normotensive patients with proteinuria (64, 65). Losartan has a negligible effect on resting blood pressure and heart rate in healthy volunteers (66-68).

Losartan is generally well-tolerated for the treatment of hypertension and proteinuria in adult and pediatric patients. However, the current prescribing information for losartan does not provide information regarding the use in patients less than five-years-old (69). The use of losartan is approved and indicated for hypertensive pediatric patients ≥6 of age with a typical starting dose of 0.7 mg/kg once daily. Currently, the losartan prescribing information (09/2014 Revision) indicates that the adverse event profile for pediatric patients mirrors that of adult patients (69). The approval described in the prescribing information was most likely based in part based upon the outcome of the use of losartan in 177 pediatric patients with hypertension aged between 6-18 years old. While the prescribing information does indeed indicate that 177 patients were consented into the study, two patients were actually re-randomized in the study, for a genuine total of 175 pediatric patients (70). Further, while this report describes patients aged between 6-16 years old, 4 patients less than 6-years-old were enrolled in the study [See Table 1, Ref. (70)]. In spite of the conflict between the prescribing information and the Merck study, losartan remains in regular use for the treatment of both hypertension and proteinuria in pediatric patients. The AEs described in the study are consistent with those described in other studies, and in the current protocol, we intend to monitor the common AEs noted in this and other studies that have evaluated losartan [See Study Stopping Rules, See section 10.5].

The use of losartan in 18 pediatric subjects with Marfan's syndrome significantly slowed the rate of progressive aortic-root dilation and supports our proposal to treat subjects as young as 5 years of age. In the small Marfan's study, no adverse events or side effects were documented among subjects while receiving ARB therapy (59).

In pediatric patients, the largest randomized control trial to date evaluated losartan vs. amlodipine/placebo in 411 subjects with proteinuria (with or without hypertension), who were 1-17 years old at the time of entry (71). Of these subjects, 33 were less than six-years old and were treated with losartan alone over the course of 12 weeks [See Table 1, Ref. (71)]. Adverse events (AE) were followed for all subjects in the study, with n=7 (4.6%) having a serious adverse event (SAE) for those being treated with losartan [See Table 2, Ref. (71)]. None of these SAEs were associated with drug. Eight (5.3%) drug-related AE were noted. No patients in the study were discontinued due to a drug-related AE [See Table 2, Ref. (71)]. While renal dysfunction [reduced glomerular filtration rate (GFR)] was noted for two of the patients treated with losartan, this was no different than the amlodipine/placebo group, and losartan was not stopped. In addition, hypotension was noted for one patient treated with losartan but did not result in stopping of the drug. Dosing of losartan is identical for that proposed for the current study, although a maximum limit is not provided, whereas a maximum of 100 mg is planned for the current study.

In a separate one-year longitudinal study of 52 subjects with proteinuria treated with losartan, the reasons for discontinuation were noted and described. These subjects were aged between 3.73 to 17.99 years. The reasons for discontinuation included elevated serum creatinine/potassium, blurred vision, syncope / dizziness, unexplained fatigue, ineffectiveness, or resolution of proteinuria. No SAEs were mentioned in the article (64).

In a similar study by the same authors, 45 patients aged between 3.7-17.9 years old with hypertension treated with losartan, a single SAE associated with the use of losartan was noted (65). However, this occurred in a patient being simultaneously treated with an angiotensin converting enzyme inhibitor (ACEI) and losartan. This patient had pre-existing renal insufficiency and history of borderline high serum potassium, and had septic shock, acute oliguria, and severe hyperkalemia (K^+ 7.6 mEq/L)(65). As a consequence, the authors no longer allow the simultaneous use of an ARB or ACEI in patients, especially if there is evidence of renal insufficiency.

As this current study proposal was originally designed, renal insufficiency was excluded via evaluation of serum creatinine (exclusion criteria 5) and the use of common antihypertension medications ACE, ARB, β-blockers is disallowed in exclusion criteria 12.

1.6 Clinical Studies

Losartan has been in use for treatment of hypertension for over a decade, and there are clinical trials utilizing losartan long-term in the MFS population without evidence of unusual AEs reported to date. Thus, we do not have an expectation of AEs that would uniquely be present within the EoE patient population. It is our hope and expectation that this study will demonstrate the clinical value of losartan without evidence of adverse effects.

2 Study Hypotheses/Objectives

1. 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.

2. 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.

2.3 Secondary Objective(s)

- To evaluate the effect of losartan on the levels of TGF-β and markers of pathway activity in the blood and esophagus.
- To investigate the effect of losartan on the normalization of the EoE transcriptome in participants with EoE with or without a CTD.
- To evaluate the effect of losartan on general histology of the esophagus, using a histology scoring scale.
- To determine the safety of losartan in participants with EoE with or without a CTD.
- To evaluate the effect of losartan by utilizing PRO questionnaires to assess for clinical symptom response.
- To evaluate the effect of losartan by utilizing the histology scoring system created to express the severity and extent of gastrointestinal abnormalities that often accompany eosinophilic inflammation.
- To evaluate the effect of losartan on esophageal compliance through the use of EndoFLIP.

3 Study Design

3.1 Description of 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 2). The primary safety endpoint is descriptive, and will require reporting of SAE or Grade 3 and above AEs.

3.2 Figure 2: Study design

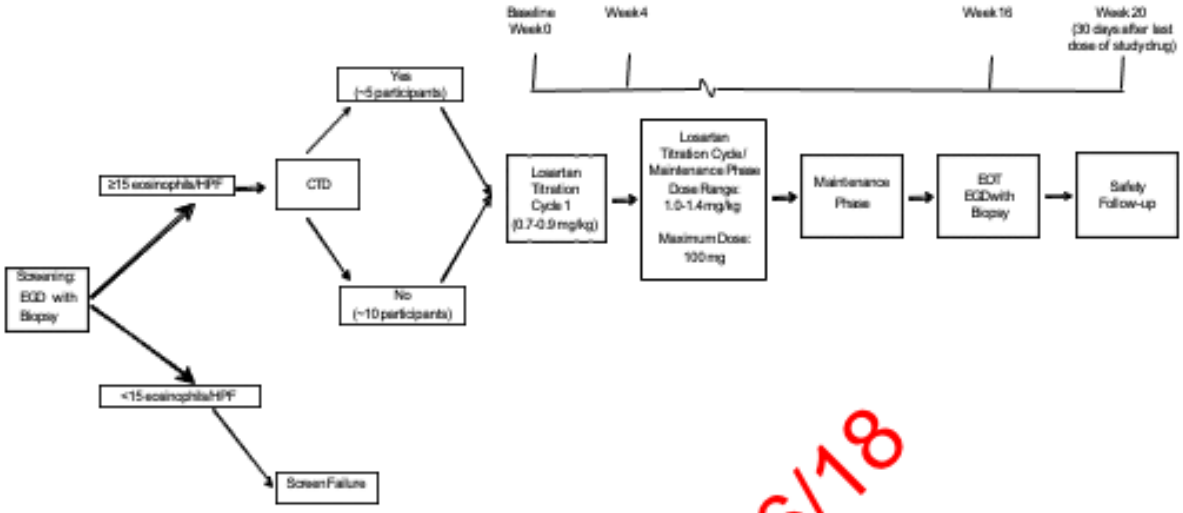

Figure 2: Schematic of study design demonstrating the flow of participants through different stages of the protocol

3. Primary Endpoint(s)/Outcome(s)

- Change from baseline in the peak esophageal eosinophil count in participants receiving losartan potassium at the end-of-treatment (EOT) visit (or at early withdrawal) as determined by esophagogastroduodenoscopy (EGD) with biopsy and reported as eosinophils per high-power field (eosinophils/HPF).
- A primary safety endpoint is also included to monitor for adverse events in the enrolled subjects. Specifically, as a descriptive investigation, any SAE or Grade 3 and above AEs that arise during the conduct of this pilot study will be reported.

3.4 Secondary Endpoint(s)/Outcome(s)

- Change from baseline in the blood (serum) and esophageal TGF-β levels in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by research blood samples and EGD with biopsy, respectively.
- Normalization of the EoE transcriptome in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy.
- Change from baseline in histopathology in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy.
- Change from baseline esophageal compliance in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EndoFLIP.
- Change from baseline PROS (Pediatric Eosinophilic Esophagitis Symptom Severity [PEESS], PedsQL Eosinophilic Esophagitis Module [PedsQL™ EoE], EoE Quality of Life Questionnaire, and EEsAI) in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EndoFLIP.

3.5 Exploratory Endpoint(s)/Outcome(s)

Serum and esophageal tissue will be collected from all participants at screening and at the EOT visit (or at early withdrawal) for the determination of TGF-β levels and eosinophils. Exploratory laboratory tests will be analyzed by the research personnel in the [redacted].

Participants are not required to fast prior to the blood draw.

The total volume of blood collected for exploratory laboratory tests will be approximately 17.5 mL per visit (screening and EOT/early withdrawal). At any time during the study, the amount of blood collected from the participants will not exceed 5% of the estimated total blood volume over a 24-hour period per institutional policies. All blood and specimen collection and handling will be done in accordance with the institution's laboratory and infection control policies.

4 Selection of Participants and Clinical Sites/Laboratories

1. Rationale for Study Population

Participants aged 5 to 25 years with active EoE with or without a CTD will be included in this study. Participants of any gender and any race will be included.

2. Inclusion Criteria

Participants must meet all of the following inclusion criteria to be eligible for participation in this study.

1. Written informed consent has been provided by the participant /parent or legal guardian.
2. Participants (of any gender and any race) aged ≥ 5 years to ≤ 25 years at time of screening.
3. The participant has confirmed, active EoE (within 12 weeks prior to enrollment) as defined by esophageal mucosal eosinophils ≥ 15 per HPF (400 x magnification) confirmed by the research pathologist AND fits into one of the following categories:
    a. Participant has not been diagnosed with and does not display phenotypes suggestive of a CTD, or
    b. Participant has been diagnosed with a CTD (e.g., MFS, LDS, EDS, or hypermobility syndromes).
4. The participant has been on a high dose of PPI (at least one dose, once daily) for at least 8 weeks prior to a diagnostic endoscopy of EoE without histologic resolution (i.e., ≥ 15 eosinophils/HPF). Due to the variety of doses and various PPIs available, the dose will be confirmed adequate at the discretion of the site PI.
   Note: Participants may continue with their acid reflux therapy as long as the dose remains the same throughout the study.
5. Participant must maintain the same diet, swallowed steroid, and PPI therapies throughout the duration of the study. Participants with active EoE on these therapies may be included in the study, as long as they agree to maintain the same diet, swallowed steroid, and PPI therapies.
6. At the time of study entry, a female participant is eligible if she meets one of the following criteria:

a. Is of non-childbearing potential (pre-menarchal or surgically sterile with documentation);
b. Is of childbearing potential with a negative urine pregnancy (β-hCG) prior to enrollment into the study (i.e., at screening) and at each study visit. Subsequently, these participants must agree to use adequate birth control measures (e.g., condom, oral / injectable / subcutaneous contraceptives, intrauterine device, or sexual abstinence) during the study and for at least one month after the last dose of study drug, which will be documented in the source documents.

3. Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants :

1. Any past or planned cardiac surgery.
2. An aortic root Z-score ≥ 3.0 on a previous echocardiogram.
3. Intolerance to the study agent (i.e., losartan) such as angioedema, immunoglobulin E(IgE)-mediated allergy, etc.
4. A mean blood pressure measurement (both systolic and diastolic) at screening that is below the 2nd percentile for pediatric participants and below the 5th percentile for adult participants as listed in the study staff–generated guideline report for blood pressure ranges for children and adolescents (Refer to Manual of Operations (MOO)),
5. Renal dysfunction with creatinine in excess of the upper normal limit for age.
6. Another disorder that causes esophageal eosinophilia (e.g., hypereosinophilic syndrome, Churg Strauss vasculitis, eosinophilic granuloma, or a parasitic infection).
7. A diagnosis of hepatic insufficiency (e.g. liver failure, history of liver transplantation, or persistent liver transaminase elevation).
8. A history of abnormal gastric or duodenal biopsy or documented gastrointestinal disorders (e.g., Celiac Disease, Crohn's disease or *Helicobacter pylori* infection), not including chronic gastritis, chronic duodenitis, mucosal eosinophilia or other eosinophilic gastrointestinal disorders (EGIDs).
9. Use of anti-IgE mAb, anti–TNF mAb, anti–IL-5 agents, or anti–IL-13 within 6 months prior to study entry (i.e., screening).
10. Use of methotrexate, cyclosporine, interferon α, or other systemic immunosuppressive or immunomodulating agents within 3 months prior to the screening visit.
11. A stricture during endoscopy procedure that prevents passage of the endoscope.
12. Taking or is planning to take an ARB, ACEI, BB, or calcium-channel blocker therapy at the screening visit or at any time during the study or has been taking any of these medications for 3 months prior to the screening visit.
13. Taking or is planning to take hydrochlorothiazide, digoxin, warfarin, cimetidine, phenobarbital, rifampin, or fluconazole.

14. Is taking or is planning to take potassium supplements or salt substitutes containing potassium.
15. A female participant who is pregnant or nursing or, if of childbearing potential, is not using a medically accepted, effective method of birth control (e.g., condom, oral/injectable/subcutaneous contraceptive, intrauterine device, or sexual abstinence).
16. Participated/participating in any investigative drug or device study within 30 days prior to study entry.
17. Participated/participating in any investigative biologics study within 3 months prior to study entry.
18. Unable to be confirmed, active EoE (at Screening or within 12 weeks prior to enrollment) as defined by esophageal mucosal eosinophils ≥ 15 per HPF (400 x magnification) confirmed by the research pathologist.

Of note, patients with esophageal eosinophilia can run a gamut of diagnoses inclusive of gastroesophageal reflux disease (GERD), erosive esophagitis, Barrett's esophagitis, and EoE. The principle means of distinguishing between these diagnoses is primarily dependent upon their symptomatic presentation along with endoscopic and pathologic findings. Patients included in this study will directly meet the consensus criteria consistent with EoE prior to entry. Indeed, the utilization of these criteria has begun to demonstrate significant differences between patients with GERD versus those with proton-pump inhibitor responsive esophageal eosinophilia (PPI-REE) and EoE. EoE has been distinguished from GERD on microarray studies (14), and has been found to be nearly identical to PPI-REE when assessed via the eosinophil diagnostic panel (EDP)(72). Currently esophageal pH monitoring is not used as standard of care in the diagnosis of EoE given the requirements for the placement and tolerance of a nasogastric tube for 24 hours. As such, the use of pH monitoring is not planned to assess patients in this study. The patient's use of a PPI will not be changed as a consequence of enrollment into the study, and would be maintained unchanged during the trial.

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of Investigational Product or Intervention from Investigator Brochure and/or Package Insert

The following information of sections 5.1-5.2 were directly excerpted from the Teva Pharmaceuticals USA, Inc., Losartan Potassium, Prescribing Information and is based on previous clinical trial data:

Hypertension

Losartan potassium has been evaluated for safety in more than 3300 adult patients treated for essential hypertension and 4058 patients/subjects overall. Over 1200 patients were treated for over 6 months and more than 800 for over one year. In general, treatment with losartan potassium was well-tolerated. The overall incidence of adverse experiences reported with losartan potassium was similar to placebo.

In controlled clinical trials, discontinuation of therapy due to clinical adverse experiences was required in 2.3 percent of patients treated with losartan potassium and 3.7 percent of patients given placebo.

The following table of adverse events is based on four 6 to 12 week, placebo-controlled trials involving over 1000 patients on various doses (10 to 150 mg) of losartan and over 300 patients given placebo [(See Table 5, Ref. (69)]. All doses of losartan are grouped because none of the adverse events appeared to have a dose-related frequency. The adverse experiences reported in ≥1% of patients treated with losartan potassium and more commonly than placebo:

Musculoskeletal: Muscle cramp, back pain, leg pain;

Nervous System/Psychiatric: Dizziness;

Respiratory: Nasal congestion, upper respiratory infection, sinusitis.

The following adverse events were also reported at a rate of 1% or greater in patients treated with losartan, but were as, or more frequent, in the placebo group: asthenia/fatigue, edema/swelling, abdominal pain, chest pain, nausea, headache, pharyngitis, diarrhea, dyspepsia, myalgia, insomnia, cough, sinus disorder.

Adverse events occurred at about the same rates in men and women, older and younger patients, and Black and non-Black patients.

A patient with known hypersensitivity to aspirin and penicillin, when treated with losartan potassium, was withdrawn from study due to swelling of the lips and eyelids and facial rash, reported as angioedema, which returned to normal 5 days after therapywas discontinued.

Superficial peeling of palms and hemolysis were reported in one subject.
In addition to the adverse events above, potentially important events that occurred in at least two patients/subjects exposed to losartan or other adverse events that occurred in < 1% of patients in clinical studies are listed below. It cannot be determined whether these

events were causally related to losartan:

Body as a Whole: facial edema, fever, orthostatic effects, syncope;

Cardiovascular: angina pectoris, second degree AV block, CVA, hypotension, myocardial infarction, arrhythmias including atrial fibrillation, palpitation, sinus bradycardia, tachycardia, ventricular tachycardia, ventricular fibrillation;

Digestive: anorexia, constipation, dental pain, dry mouth, flatulence, gastritis, vomiting;

Hematologic: anemia;

Metabolic: gout;

Musculoskeletal: arm pain, hip pain, joint swelling, knee pain, musculoskeletal pain, shoulder pain, stiffness, arthralgia, arthritis, fibromyalgia, muscle weakness;

Nervous System/Psychiatric: anxiety, anxiety disorder, ataxia, confusion, depression, dream abnormality, hypesthesia, decreased libido, memory impairment, migraine, nervousness, paresthesia, peripheral neuropathy, panic disorder, sleep disorder, somnolence, tremor, vertigo;

Respiratory: dyspnea, bronchitis, pharyngeal discomfort, epistaxis, rhinitis, respiratory congestion;
Skin: alopecia, dermatitis, dry skin, ecchymosis, erythema, flushing, photosensitivity, pruritus, rash, sweating, urticaria;
Special Senses: blurred vision, burning/stinging in the eye, conjunctivitis, taste perversion, tinnitus, decrease in visual acuity;
Urogenital: impotence, nocturia, urinary frequency, urinary tract infection.

Persistent dry cough (with an incidence of a few percent) has been associated with ACE-inhibitor use and in practice can be a cause of discontinuation of ACE-inhibitor therapy. Two prospective, parallel-group, double-blind, randomized, controlled trials were conducted to assess the effects of losartan on the incidence of cough in hypertensive patients who had experienced cough while receiving ACE-inhibitor therapy. Patients who had typical ACE-inhibitor cough when challenged with lisinopril, whose cough disappeared on placebo, were randomized to losartan 50 mg, lisinopril 20 mg, or either placebo (one study, n = 97) or 25 mg hydrochlorothiazide (n = 135). The double-blind treatment period lasted up to 8 weeks. The incidence of cough is shown below (Table 1).

5.1.1 Table 1: Cough incidence

Study 1*	HCTZ	Losartan	Lisinopril
Cough	25%	17%	69%
Study 2†	**Placebo**	**Losartan**	**Lisinopril**
Cough	35%	29%	62%

*Demographics = (89% Caucasian, 64% female)
†Demographics = (90% Caucasian, 51% female)

These studies demonstrate that the incidence of cough associated with losartan therapy, in a population that all had cough associated with ACE-inhibitor therapy, is similar to that associated with hydrochlorothiazide or placebo therapy.

Cases of cough, including positive re-challenges, have been reported with the use of losartan in postmarketing experience.

Pediatric Patients

No relevant differences between the adverse experience profile for pediatric patients and that previously reported for adult patients were identified.

Hypertensive Patients With Left Ventricular Hypertrophy

In the LIFE study, adverse events with losartan potassium were similar to those reported previously for patients with hypertension.

Nephropathy in Type 2 Diabetic Patients

In the RENAAL study involving 1513 patients treated with losartan potassium or placebo, the overall incidences of reported adverse experiences were similar for the two groups. Losartan potassium was generally well tolerated as evidenced by a similar incidence of discontinuations due to side effects compared to placebo (19% for losartan potassium, 24% for placebo). The adverse experiences, regardless of drug relationship, reported with an incidence of ≥ 4% of patients treated with losartan potassium and occurring more commonly than placebo, on a background of conventional antihypertensive therapy, are shown below:

Body as a Whole: Asthenia/Fatigue, Chest Pain, Fever, Infection, Influenza-like disease, Trauma;

Cardiovascular Hypotension, Orthostatic hypotension;

Digestive: Diarrhea, Dyspepsia, Gastritis;

Endocrine: Diabetic neuropathy, Diabetic vascular disease;

Eyes, Ears, Nose and Throat: Cataract, Sinusitis;

Hematologic: Anemia;

Metabolic and Nutrition: Hyperkalemia, Hypoglycemia, Weight gain;

Musculoskeletal: Back pain, Leg pain, Knee pain, Muscular weakness;

Nervous System: Hypesthesia;

Respiratory: Bronchitis, Cough;

Skin: Cellulitis;

Urogenital: Urinary tract infection.

5.2 Risks of Investigational Product or Intervention cited in Medical Literature

Postmarketing Experience

The following additional adverse reactions have been reported in postmarketing experience:

Digestive: Hepatitis (reported rarely).

General Disorders and Administration Site Conditions: Malaise.

Hematologic: Thrombocytopenia (reported rarely).

Hypersensitivity: Angioedema, including swelling of the larynx and glottis, causing airway obstruction and/or swelling of the face, lips, pharynx, and/or tongue has been reported rarely in patients treated with losartan; some of these patients previously experienced angioedema with other drugs including ACE-inhibitors. Vasculitis, including Henoch-Schönlein purpura, has been reported.

Anaphylactic reactions have been reported.

Metabolic and Nutrition: Hyperkalemia, hyponatremia have been reported with losartan.

Musculoskeletal: Rare cases of rhabdomyolysis have been reported in patients receiving angiotensin II receptor blockers.

Nervous system disorders: Dysgeusia.

Respiratory: Dry cough (see above).

Skin: Erythroderma.

Laboratory Test Findings

In controlled clinical trials, clinically important changes in standard laboratory parameters were rarely associated with administration of losartan potassium.

Creatinine, Blood Urea Nitrogen: Minor increases in blood urea nitrogen (BUN) or serum creatinine were observed in less than 0.1 percent of patients with essential hypertension treated with losartan potassium alone.

Hemoglobin and Hematocrit: Small decreases in hemoglobin and hematocrit (mean decreases of approximately 0.11 grams percent and 0.09 volume percent, respectively) occurred frequently in patients treated with losartan potassium alone but were rarely of clinical importance. No patients were discontinued due to anemia.

Liver Function Tests: Occasional elevations of liver enzymes and/or serum bilirubin have occurred. In patients with essential hypertension treated with losartan potassium alone, one patient (< 0.1%) was discontinued due to these laboratory adverse experiences.

Overdosage

Significant lethality was observed in mice and rats after oral administration of 1000 mg/kg and 2000 mg/kg, respectively, about 44 and 170 times the maximum recommended human dose on a mg/m2 basis.

Limited data are available in regard to overdosage in humans. The most likely manifestation of overdosage would be hypotension and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation. If symptomatic hypotension should occur, supportive treatment should be instituted.

Neither losartan nor its active metabolite can be removed by hemodialysis.

Drugs that act directly on the renin-angiotensin system can cause fetal and neonatal morbidity and death when administered to pregnant women. Several dozen cases have been reported in the world literature in patients who were taking angiotensin converting enzyme inhibitors. When pregnancy is detected, losartan potassium should be discontinued as soon as possible.

The use of drugs that act directly on the renin-angiotensin system during the second and third trimesters of pregnancy has been associated with fetal and neonatal injury, including hypotension, neonatal skull hypoplasia, anuria, reversible or irreversible renal failure, and death. Oligohydramnios has also been reported, presumably resulting from decreased fetal renal function; oligohydramnios in this setting has been associated with fetal limb contractures, craniofacial deformation, and hypoplastic lung development. Prematurity, intrauterine growth retardation, and patent ductus arteriosus have also been reported, although it is not clear whether these occurrences were due to exposure to the drug.

These adverse effects do not appear to have resulted from intrauterine drug exposure that has been limited to the first trimester.

3. Reducing Risks of Losartan Use

To minimize the potential risks of losartan, the starting doses of the study agent will be relatively low and will be up-titrated carefully (See section 6.1.2). Participants will be followed closely for adverse drug reactions (ADRs) and will only be up-titrated if the current study agent dose is tolerated. In addition, clinical labs (i.e., complete blood count [CBC] with differential and chemistry) will be measured to monitor serum creatinine, potassium, hemoglobin, hematocrit, AST, and ALT; any abnormalities in laboratory values that are determined to be AEs will be evaluated and managed by the Investigator.

Pregnancy or planned pregnancy during the trial is exclusionary as losartan exposure represents a high-risk problem for the fetus if a mother is inadvertently exposed to losartan during pregnancy. The use of losartan would not merit potential benefit in EoE, given the significant risk of frank mortality and morbidity to the fetus associated with these exposures. At the time of informed consent and throughout the study, female participants of childbearing potential and their legal guardians will be educated and notified about the potential risk of the study agent. The risk will also be described in the consent and the assent forms. Birth control measures will be required for female participants of childbearing potential.

4. Risks of Study Procedures

1. Blood Draw

Risks associated with venous blood draw may include moderate pain or stinging sensation at the site of blood draw. To decrease the pain or stinging sensation, participants can request the option of EMLA or Spray and Stretch Spray to be applied prior to the venipuncture. Other risks are rare but may include excessive bleeding, fainting or feeling light-headed, hematoma, or infection. A trained physician or personnel skilled in phlebotomy will perform the blood draw. In order to minimize discomforts, and rather than performing separate blood draws, attempts will be made to obtain blood via placement of an intravenous line while the participant is under anesthesia and prior to the endoscopy, if and when possible. The amount of blood drawn will follow the NIH Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center (5/2012) For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. For adults the amount shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

2. Study Questionnaires

If the participant is between the ages of 5 and 18 years old, he/she, as well as the parent or legal guardian, will be asked to complete up to two quality-of-life questionnaires. Participants between the ages of 18 and 25 years

will also complete these questionnaires, though their parents or legal guardians will not. There are no foreseeable physical discomforts or significant risks related to answering the patient- or parent-proxy-reported outcomes or study questionnaires. However, some questions may be difficult and may make the participants uncomfortable to answer. The participants may also feel inconvenienced to complete the questionnaires. Participants may refuse to answer any questions asked on the survey. The participant questionnaires typically take approximately 15 to 20 minutes to complete. All participants will be given ample time to complete the survey.

3. Esophageal Biopsies

Additional endoscopy procedures will not be performed because of participation in the study. However, esophageal biopsies (with up to four from the proximal and distal esophagus) will be taken for research at the time of the endoscopy that is done as part of clinical management. The use of four esophageal biopsies provides a diagnostic probability of 98% for EoE (73). It is considered standard of care for patients with active EoE to have an upper gastrointestinal endoscopy every three to six months to monitor the disease, or to evaluate changes in therapy (74).

Endoscopy is a well-established procedure. Few patients have unexpected or serious complications. The risks associated with collecting additional esophageal biopsies for research at the time of the endoscopy include: bleeding at the site of tissue (biopsy) collection, and a small chance of perforation (hole) of the stomach, duodenum, or esophagus. Perforation is the most severe gastrointestinal complication, but generally it is self-resolving and poses no life-threatening risk. Additional biopsies will only be collected if the endoscopist feels it is appropriate to do so. The participant may have an adverse reaction to the anesthetic or medication used for sedation, which may cause apnea (not breathing), bradycardia, excessive sweating, hypotension (low blood pressure), laryngospasm (spasm of the larynx), or respiratory depression (difficulty breathing). The overall risk is less than 1 out of 1,000 people (75). To minimize the risks of endoscopy, the procedure will be carried out or supervised by a skilled endoscopist.

4. Privacy and Confidentiality

Although the study staff will do its best to adhere to strict privacy guidelines, the possible risk of a breach of confidentiality does exist. Every effort will be made to maintain the confidentiality of the participant's medical and research information. The investigator will take all necessary measures to keep all of the participant's personal information private and confidential. Electronic study records will be protected with electronic safeguards (e.g., computer passwords, restricted access privileges). Paper-based PHI will be secured in locked cabinets. Access to study records will be limited only to research staff. For this study, the research study staff will collect and utilize data obtained from the participant's medical and research records and observations, including laboratory tests. This information, along with the study survey forms, will be used by the Sponsor and

Investigator as part of the study data. The participants will be assigned and identified by a participant study identification number. The link to the participant's study identifications and their names and identifiers will be kept by the PI and/or the research staff in a secure location. Because the participant's PHI will be collected and used for the study, the research staff will obtain a signed HIPAA authorization and informed consent from the participant and/or legal guardian. The research staff will perform informed consent and all study procedures in a private setting away from the public. In addition, the research staff will only collect the minimum amount of personal information about the participant necessary for the research study.

The PI, members of the study's research team, assigned study monitor(s), DMCC, on-site medical staff, Federal and other regulatory agencies (including the Food and Drug Administration and DAIT/NIAID), CCHMC IRB and Office of Research Compliance and Regulatory Affairs, and DSMB will have access to the participant's medical and research records related to this study. The data from the study may be published; however, the participant will not be identified by name. There may be unknown or unforeseen risks associated with study participation.

5.5 Potential Benefits

Participants who participate in this study are not guaranteed a direct medical benefit; however, losartan has the potential to improve EoE symptoms, pathology, and compliance. By reducing the TGF-β1 activity in patients with EoE, losartan may 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.

6 Investigational Agent / Device / Intervention

6.1 Investigational Agents / Devices / Interventions

6.1.1 Investigational Agent #1

Losartan potassium generic formulation. Formulation, Packaging, and Labeling

Losartan is to be supplied in bottles containing units of 25-mg 50-mg tablets and bottles that will be prepared for suspension form (200 ml for 2.5 mg/mL) at the site pharmacies. Tablets are used to generate suspensions as described in Section 6.1.4 Administration and Preparation of Losartan.

The dose of the study agent to be administered to the pediatric participants (5 yrs. to < 18 yrs.) will depend on the participant's body weight and will be calculated (mg/kg) on the basis of a titration schedule during the treatment (i.e., titration and maintenance) phase, not to exceed the daily dose of 100 mg. The dose of the study agent to be administered to the adult participants (≥ 18 yrs) will start at 50 mg, not to exceed the daily dose of

100 mg. The study agent is to be delivered orally once a day at approximately the same time each day. This daily dose can be divided into twice a day if participant has trouble tolerating a once daily dose. The medication dosage for losartan potassium will change throughout this study, titrating to a higher dose (see Titration Cycle 2: Up Titration).

6.1.2 Losartan Titration Description

Dose Rationale

The dose ranges used for the losartan trial for a pediatric hypertension have been between 0.05 to 2.5 mg/kg/day, with higher doses improving blood pressure in these patients (70). This trial represented one of three successful anti-hypertensive drug trials out of a total six studied in Benjamin *et al.*, 2008, that evaluated the appropriate outcome endpoints and dose ranges in pediatric populations. The authors speculated that the higher doses utilized and the availability of a pediatric formulation were potentially responsible for the successful completion of those three trials as these represented features in common among the successful drug trials (76). Further, the dose used in the losartan trial for hypertension exceeds the upper planned limit of ~1.4 mg/kg in pediatric patients for the proposed EoE Losartan trial, and has previously been used in the study of losartan in patients with Marfan's Syndrome (59). In this small cohort study of Marfan's patients, the rate of change in the aortic root diameter was significantly decreased in patients treated with losartan who had previously failed other medical therapy (59). A lower dose is of further importance in this study, as there is the risk of postural orthostatic tachycardia syndrome in patients with EDS. The adult dose ranges are derived from well-established ranges described in the prescribing information available for losartan.

Enrollment - Initiation of Study Agent: Titration Cycle 1

For enrolled participants, approximately 0.7-0.9 mg/kg will be given on days 1-21 for enrollees less than 18 years of age and 50 mg for those 18 and older as per dosing commonly recommended for hypertension in pediatric and adult patients. These dosing instructions represent a common starting dosing for pediatric and adult patients undergoing treatment for hypertension. A daily maximum dose of 100 mg per day will be allowed for all participants regardless of age as this study is designed to minimize risks associated with hypotension in patients agreeing to enroll in this study.

Of note, in review of patients enrolled into the CCHMC EGID research database, 5% of all EoE patients have a weight which exceeds 90 kg. When limited to those EoE patients who are 18 and older, this percentage climbs to 8%. This indicates that the pool of patients is small and only a few patients would be at risk for the maximum dose planned for this clinical trial at the outset of their enrollment. Review of blood pressures for 6 subjects enrolled in the trial to date reveals that the lowest systolic and diastolic blood pressure percentiles were 27th and 21st percentile respectively following initiation of losartan.

The participants will be instructed to call the study coordinator or the study physician if any Adverse Drug Reactions (ADRs) occur. On day 6, 7, or 8, after each dosing visit, the study coordinator will contact each participant (or parent/legal guardian) and verify the absence of ADRs.

After each participant has taken the study agent for at least 21 days but less than 28 days (7-day window), they will return to the clinic for a visit. During that visit the research staff will verify the absence of ADRs. If no reported ADRs the participants will then proceed to Cycle 2 (see below).

Up-titration: Titration Cycle 2

Up-titration will proceed according to the schedule outlined in Table 2. The dose of study agent will be increased as tolerated to a dose of approximately ~1.4 mg/kg, not to exceed 100 mg. The maximum dose will continue through the maintenance phase. For each cycle, after participants have taken the agent for at least 21 days but less than 28 days (7-day window), the study coordinator will verify the absence of ADRs.

6.1.3 Table 2: Losartan titration schedule

Duration of Dosing for each Titration Cycle	Initiation Titration Cycle 1	Up-Titration Titration Cycle 2	Down-titration Increment (See Section 6.4)
21-28 days	0.7-0.9 mg/kg	1 - 1.4 mg/kg (maximum 100 mg)	1st: twice-a-day dosing* 2nd: 0.2 mg/kg/d

*e.g. For a dose of 50 mg once a day associated with significant hypotension, the patient would first be down-titrated to 25 mg twice a day. If this were insufficient to control symptoms, the dose would be reduced by 0.2 mg/kg/d until the subject is asymptomatic.

6.1.4 Administration and Preparation of Losartan

Losartan tablets (25 and 50 mg) will be used for patients not requiring a suspension. Bottles of ten 50 mg tablets that will be used to prepared suspension form (200 ml for 2.5 mg/mL) at the site pharmacies will be used in this trial.

Preparation of Suspension

Preparation of suspension will follow the directions located in the Product Insert (Sections 5.1-5.2) and in the Pharmacy Manual. Each bottle sent to the site will contain ten 50 mg tablets. Once prepared at the site pharmacy the suspension will be stored at 2 to 8 degrees Celsius and must be discarded after 4 weeks from date of preparation.

2. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR§312.62), the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

Study agent will be dispensed through the site Investigational Drug Service per its standard operating procedures (SOPs). All study agent administered to each participant will be recorded on the Drug Accountability Form maintained by the Investigational Pharmacy.

Tablet/Suspension Dispensing: The participant (or parent/legal guardian) will receive a medication. Participant specifies study agent in the form of tablets or suspension will be dispensed by the pharmacy every 3 to 4 weeks. Unused study medication will be returned and retained until study completion.

At the end of the study, the study monitor will be responsible for study agent reconciliation. All unused study agent will only be disposed and discarded after authorization from NIAID per guidelines outline in the Pharmacy Manual. All study documents located in the Investigational Pharmacy should be moved to the investigator binder at the end of the study to ensure that all study documentation is maintained in one location.

3. Assessment of Participant Compliance with Investigational Agent

The study coordinator will perform medication accountability and calculate the level of compliance.

4. Toxicity Prevention and Management

If a participant develops certain ADRs such as fatigue or dizziness, the study agent can be changed to night-time administration or twice-a-day administration at the discretion of the PI. If drug reactions persist or abnormalities are noted in the safety lab work, the dose of the study agent will be decreased by the down-titration increments listed in Table 2. The participant will be withdrawn from the study if serum creatinine, serum potassium, AST, or ALT are increased such that they would be classified as a moderate or more severe AE according to the schema in the Common Terminology Criteria for AEs (CTCAE) (77). For serum creatinine, this value is greater than 1.5 times the upper limits of the age-matched normal value; for potassium, this value is 5.6-6.0 mEq/L in a non-hemolyzed sample; and for AST and ALT, this value is greater than 2.5 times the upper limit of the age-matched normal value. Abnormal laboratory values for serum creatinine, serum potassium, AST, and ALT will always be measured twice to re-examine for error.

If the participant's blood pressure (systolic and diastolic) falls below the 2nd percentile for age listed in the study staff-generated guideline report for blood pressure ranges for children and adolescents (See section 19) and has concurrent symptoms of hypotension, the participant's dosage will be decreased by the down-titration increments listed in Table 2. If the participant is asymptomatic, this will not be considered hypotension. Participants will be re-evaluated after down-titration of the study agent at the PI's discretion. Unlike the situation with heart-rhythm abnormalities associated with withdrawal of beta-blockers (78), there is no similar literature noted for ARB medications and no taper of losartan is planned following completion of the study.

5. Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

- Occurrence of an AE that, in the opinion of the investigator, requires the participant's permanent withdrawal from the study agent. In the event of withdrawal from the study agent due to the occurrence of a SAE, the Investigator and Sponsor must be notified within 24 hours (See section 11.3). A participant withdrawn secondary to an ongoing SAE that is considered possibly or probably related to the study agent must be followed clinically until resolution or stabilization of the SAE.
- Insufficient therapeutic effect requiring alternative or additional therapy for the treatment of EoE, including the need for "rescue" systemic or swallowed inhaled corticosteroid therapy, in addition to the study agent, for the treatment of the current EoE. Criteria for withdrawal due to lack of efficacy will rely on the best clinical judgment of the PI that the participant requires a new therapeutic intervention due to the clinical state of his/her EoE. Examples would include: worsening of the presenting predominant symptom of EoE, the emergence of a new additional symptom of EoE that is at least moderate in intensity and is due to EoE, or the need for new systemic treatment (e.g., oral corticosteroids). In contrast, small dietary modifications or modest increases in symptom frequency without increased severity would not constitute withdrawal criteria.
- Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

7 Other Medications

7.1 Concomitant Medications

Prior medications, such as acid reflux therapies (including PPIs with or without histamine H2 receptor antagonists) or swallowed corticosteroids (e.g. swallowed fluticasone), may be continued as concomitant medications during the study provided that the dose of the medication remains unchanged during the study period.

7.2 Table 3: Prohibited medications and washout time

Medication	Washout Time Prior to Screening Visit (i.e., Study Entry)
Anti-immunoglobulin E(IgE) mAb	6 months
Methotrexate, cyclosporine, interferon α	3 months
Anti-tumor necrosis factor (TNF)mAb	6 months
Other systemic immunosuppressive or immunomodulatory agents	3 months
Angiotensin receptor blocker (ARB)	3 months
Angiotensin-converting enzyme inhibitor (ACEI)	3 months
Beta blocker (BB) therapy	3 months
Calcium-channel blocker	3 months
Other investigative biologics	3 months
Omalizumab	6 months
Anti–IL-5 agents	6 months
Anti–IL-13 agents	6 months
Other investigative drugs or device	1 month

Note: Any changes to the participant's asthma or allergy therapies and other concomitant medications during the study will be documented in the appropriate source documents and case report forms (CRFs).

7.3 Rescue Medications

No specific rescue medications are planned for this study

8 Study Procedures

1. Visit 1 Screening / Baseline Visit (Week -12 to 0)

Recruitment

The research study will be explained in lay terms to each potential research participant. Participants will be recruited from the gastroenterology, allergy, or other clinics at each study site when potential participants with EoE and CTD may be seen. Participants who receive care from other institutions that express interest in participating may be enrolled in the study. The PI and the study staff team will work with the participant's primary care physician to explain the study objectives and requirements for the study. If the participant's primary care physician agrees that the participant would be a good candidate for the study, study staff will contact the participant and carry out all recruitment and screening procedures. In addition, potential participants will be identified through the EGID database (CCHMC IRB studies #2008-0090 and 2008-0098), as well as other databases and resources at each study site. Research staff will pre-screen or identify potentially eligible participants by conducting a review of existing medical and/or research records and IRB-approved recruitment materials.

The clinical trial information will be entered into a databank on www.clinicaltrials.gov maintained by the U.S. National Library of Medicine (NLM) at the National Institutes of Health (NIH) or other websites.

The purpose of the screening period is to confirm eligibility to participate in the study. The screening visit should occur at a maximum of 12 weeks prior to the enrollment visit. The research staff member will perform the informed consent process according to Section 19. After obtaining informed consent, the following study assessments/procedures should be performed and documented up to 12 weeks prior to the enrollment (Week 0) visit or during the enrollment visit:

- Eligibility (inclusion and exclusion criteria)
- Medical, surgical and diet history
- Conduct vitals (blood pressure, heart rate, respiratory rate, temperature), height, and weight
- Demographics (age, gender, race)
- Physical exam performed by a study clinician
- Clinical laboratory samples, including CBC with differential, chemistry, and IgE
- Research laboratory samples (See Table 4)
- Urine pregnancy test for females of childbearing potential
- Concomitant medications and therapies
- For those patients who meet the base screening inclusion/exclusion criteria, patients will be considered for evaluation of esophageal compliance via the use of EndoFLIP. EndoFLIP is a SOC evaluation for esophageal dysmotility, done at the same time as their endoscopy. Patients will undergo consent to retrieve EndoFLIP datasets obtained prior to entry and during this study to assess esophageal compliance.

Note: If the participant is on a current dose of acid reflux therapy (e.g., PPIs and histamine H2 receptor antagonists) or swallowed corticosteroid (e.g. swallowed fluticasone), the participant is permitted to continue the acid reflux therapy or swallowed corticosteroid as long as the dose of the medication remains the same throughout the study. If the participant has had an EGD and biopsies performed at CCHMC or at an outside institution prior to the screening visit, the results of the EGD may be used to determine eligibility if it falls within the 12-week window prior to enrollment. These participants will have all other screening procedures completed at the in-office, screening visit. EGDs performed at an outside institution may be used as baseline if the corresponding pathology report and slides are available and reviewed by the CCHMC research pathologist to determine eligibility. In addition, EoE Endoscopic Reference Score (EREF) will be obtained endoscopies performed at the participating institutions.

Some participants who have been seen at CCHMC or at other institutions may have had research samples, tissue and blood, collected at the time of their EGD for further analysis to occur in the [redacted] or in the [redacted]. If so, permission to utilize the participants' samples for this study will be obtained at the time of informed consent, and will be transferred to the respective labs under a material transfer agreement. Screening physicals will be performed by trained physicians using the Villefranche criteria, Beighton, and Ghent criteria (79, 80). These are simply guidelines, and a physician can still have the capability to provide a participant with a diagnosis of a CTD if their scores do not fall within the abnormal limits.

8.2 Visit 2 Enrollment / Titration Cycle 1 / (Week 0)

All eligible participants will be enrolled into the study at Week 0 if they meet all eligibility criteria and have completed the screening procedures. Screening and enrollment visits may be combined when deemed necessary. If the screening and enrollment visit are combined, the study drug will not be dispensed until all inclusion and exclusion criteria have been met and all screening visit procedures have been performed. This includes the review of all laboratory tests performed during the screening portion of the visit. If a participant is screened and enrolled within a 5-day time frame, study procedures that would be repeated will only be performed once. The enrollment visit also marks the beginning of the Treatment Phase and Titration Cycle 1. At this visit, staff members will:

- Reassess eligibility (inclusion and exclusion criteria)
- Conduct vitals (blood pressure, heart rate, respiratory rate, temperature), height, and weight
- ☐ Administer patient- and parent-reported outcome questionnaires
- ☐ Urine pregnancy test in females of childbearing potential
- ☐ Assess and inquire about AEs
- ☐ Assess concomitant medications and/or therapies
- ☐ Assess participant compliance with diet
- ☐ Dispense study agent with instructions

- Dispense diet and medication log

The PI or the sub-investigator will determine the initial dose of study agent per the titration schedule in Sections 6.1.2 - 0. The participant's weight obtained at this visit will be used to calculate the initial dose of losartan. The Investigational Pharmacy will dispense the appropriate dose and amount of the study agent per the Manual of Operations (MOO). The participant will be instructed to administer the study agent orally once daily, at approximately the same time each day, with or without food, for at least 21 days but less than 28 days. The participant will also be instructed to record the start and stop date of the study agent and each dose in the study diary. The participants will be instructed to call the PI and/or study coordinator if they have any questions, concerns, adverse events, or changes in medication.

8.3 Telephone Calls (Week 1)

On Days 6, 7, or 8 of Titration Cycles 1 (Week 1) after the start of the titrated doses, the study coordinator will call the participant for the following purposes:

- Verify the absence of ADRs
- Verify the absence of AEs
- Inquire about changes to concomitant medications/therapies and diet
- To answer any participant's questions and concerns

ADRs and changes to concomitant medications should be recorded in the participant's source documents. If the participant experiences ADRs, the PI should be consulted, and the appropriate procedures should be followed to adjust the study agent administration and down-titration, as needed and at the discretion of the PI (See sections 6.1.2 - 0).

8.4 Visit 3 Up-titration / Titration Cycle 2 / (Week 4)

Up-titration (beginning of Maintenance Phase; Week 4) will be conducted in the clinic. The study visit should occur after the participant has taken the study agent for at least 21 days but less than 28 days (7-day window). The following study procedures should be performed:

- Conduct vitals (blood pressure, heart rate, respiratory rate), height, and weight
- Physical exam
- Administer patient- and parent-reported outcome questionnaires
- Clinical laboratory samples, including CBC with differential, chemistry
- Urine pregnancy test in females of childbearing potential
- Assess AEs
- Assess changes to concomitant medication/therapies (to ensure participant has not taken any prohibited medication)

- Assess participant compliance with diet (to ensure no diet change)
- Sample / Diary Collection
  - Collect diet and medication log
    - Collect study agent bottle and unused study agent
- Medication accountability
  - Determine participant's compliance with study agent during Titration Cycle 1

- Dispense
  - Dispense study agent with instructions
  - Dispense diet and medication log

The participant's weight obtained at this visit will be used to determine the study agent dose for Titration Cycle 2. Up-titration should only occur if the participant has verified the absence of ADRs. The maximum tolerated dose for the study agent will not exceed 100 mg per day. The dose will be determined at this visit (Week 4) and will continue throughout the maintenance phase.

8.5 Titration Cycle Telephone Calls (Week 5)

On Days 6, 7, or 8 of Titration Cycles 2 (Week 5) after the start of the titrated doses, the study coordinator will call the participant for the following purposes:

- Verify the absence of ADRs
- Verify the absence of AEs
- Inquire about changes to concomitant medications/therapies and diet
- To answer any participant's questions and concerns

ADRs and changes to concomitant medications should be recorded in the participant's source documents. If the participant experiences ADRs, the PI should be consulted, and the appropriate procedures should be followed to adjust the study agent administration and down-titration, as needed and at the discretion of the PI (See sections 6.1.2 - 0).

8.6 Visit 4 and 5 Maintenance 1 and 2 (Weeks 8 and 12)

The following procedures will be performed during the maintenance phase:

- Conduct vitals (blood pressure, heart rate, respiratory rate, temperature), height, and weight
- Urine pregnancy test in females of childbearing potential
- Clinical laboratory samples, including CBC with differential, chemistry
- Administer patient- and parent-reported outcome questionnaires in person or through email or regular mail
- Assess AEs

- Assess changes to concomitant medication/therapies
- Assess participant compliance with diet
- Collect diet and medication log
- Collect study agent bottle and unused study agent dispensed at the previous study visit
- Dispense study agent supply with instructions
- Dispense diet and medication log
- Determine participant's compliance with study agent

The study staff will provide the participant with the monthly supply of the maintenance study agent, along with the study diary, and instructions. The study staff will instruct the participant to stop taking the study agent dispensed at the previous visit and to start taking the new supply of study agent upon receipt.

The visit window for Week 8 and 12 is ± 7 days; participants should be supplied with enough study agent to ensure continual administration of the study agent throughout the maintenance phase leading into the EOT Visit (Week 16).

8.7 Monthly Telephone Calls (Maintenance Week 9 and 13)

On Days 6, 7, or 8 of Maintenance (Week 9 and 13) the study coordinator will call the participant for the following purposes:

- Verify the absence of ADRs
- Verify the absence of AEs
- Inquire about changes to concomitant medications/therapies and diet
- To answer any participant's questions and concerns

ADRs and changes to concomitant medications should be recorded in the participant's source documents. If the participant experiences ADRs, the PI should be consulted, and the appropriate procedures should be followed to adjust the study agent administration and down-titration, as needed and at the discretion of the PI (See sections 6.1.2 - 0).

8.8 Visit 6 End-of-Treatment (EOT) or Early Withdrawal (Week 16 ± 7 days) Visit

For participants who have completed the maintenance phase or who have withdrawn early from the study, the following procedures will be performed in the clinic:

- Conduct vitals (blood pressure, heart rate, respiratory rate, temperature), height, and weight
- Physical exam
- Administer patient- and parent-reported outcome questionnaires
- Clinical laboratory samples, including CBC with differential, chemistry, and IgE
- Research laboratory samples

- Urine pregnancy test in females of childbearing potential
- Assess AEs
- Assess changes to concomitant medication/therapies
- Obtain esophageal biopsies including biopsies taken for clinical purposes and up to 4 biopsies from the distal esophagus for research purposes obtained from standard of care (SOC) endoscopy
- Assess participant compliance with diet
- Collect diet and medication log
- Collect study agent bottle and unused study agent
- Determine participant's compliance with the study agent

The EOT visit may be divided into two appointments, if necessary, in order to allow for all necessary procedures to be performed. Participants who have completed the maintenance phase should be instructed not to take the study agent on the day of this visit; the last dose of study agent should be taken the day prior to the EOT visit. If a participant withdraws from the study before completing the maintenance phase, the EOT/early withdrawal visit, including EGD and biopsy, should be conducted within one week of the participant's last dose of the study agent.

8.9 Visit 7 Follow-Up Call/Visit (Week 20 ± 7 days)

The research personnel will conduct a follow-up safety telephone call to the participant 30 ± 7 days after the last dose of the study agent. Females of childbearing potential will have their urine pregnancy test conducted in the clinic. The call/visit will consist of the following procedures:

- Administer patient- and parent reported outcome questionnaires
- Assess AEs
- Assess concomitant medications
- Assess diet and concomitant medication
- Urine pregnancy test in females of childbearing potential

10. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit. Unscheduled visits will take place for an unexpected pregnancy or any complication or AE/SAE that requires an extra visit. The procedures to be performed at the unscheduled visits will be at the discretion of the PI. These visits will be documented in the source document and the unscheduled visit case report.

11. Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows for each scheduled visit are ±7 days of the week indicated on Table 4.

8.12 Table 4: Study Design and Schedule of Assessments

PHASE	PRE-TREAMENT PHASE	Titration Phase[b,c]		TREATMENT PHASE Maintenance Phase				EOT or Early Withdrawal	FOLLOW-UP PHASE
Visit Number	1	2	2a	3	3a	4	5	6	7
Visit Description	Screening	Enrollment Baseline Titration Cycle 1	Week 1 Visit Check BP/ AEs	Visit 3 Up-titration Titration Cycle 2	Phone call	Maintenance Month 2	Maintenance Month 3	EOT Visit	Safety Phone call
Week	-12 to 0	0	1	4	5	8	12	16	20
Perform informed consent/assent	X								
Assess eligibility (inclusion/exclusion criteria)	X	X							
Obtain medical, surgical, dietary history, & demographics	X								
Vitals (height, weight, blood pressure, heart rate, respiratory rate, temperature)	X	X		X		X	X	X	
Physical exam	X			X				X	
SOCEGD with biopsies[f]	X[a]							X	
EREFS	X							X	
EndoFLIP[g]	X							X	
PedsQL™ EoE Module[h]		X		X		X	X	X	X
PEESSV 2.0[h]		X		X		X	X	X	X
EESal		X		X		X	X	X	X
EoE-QoL- Adult		X		X		X	X	X	X
Participant Intake Form		X							
Clinical laboratory samples	X			X		X	X	X	
Research laboratory samples	X[d]							X[d]	
Urine pregnancy test[i]	X	X		X		X	X	X	X
Assess AEs		X	X	X	X	X	X	X	X
Assess concomitant medications and/or therapies	X	X	X	X	X	X	X	X	X
Dispense study drug, drug diary, and instructions		X		X		X	X		
Determine compliance with study drug				X		X	X	X	
Collect unused study drug				X		X	X	X	

[a] First EGD used to determine eligibility may be conducted within 12 weeks prior to enrollment visit.
[b] At each monthly or titration visit, the participant must have been on previously dispensed dose of study drug for at least 21 days but no more than 28 days
[c] Clinical labs: CBC with differentials, chemistry panel, and IgE (first and last visit only).
[d] Research labs: mRNA in blood and tissue, TGF-β in blood and tissue.
[e] In addition to assessing diet and concomitant medication compliance at each scheduled visit, research personnel will also conduct one telephone call per month to assess compliance during the maintenance phase. During the titration phase, diet and concomitant medication compliance will be conducted during scheduled phone calls (Visits 2a and 3a).
[f] Participants who withdraw from the study before completing the maintenance phase should complete the EOT/early withdrawal visit, including SOCEGD and biopsy, within two weeks of the last dose of study drug.
[g] EndoFLIP is a SOC evaluation for esophageal dysmotility, done at the same time as their endoscopy.
[h] The data gathered from these questionnaires will be correlated with several biomarkers of interest that have been associated with EoE. Each visit window (for visits 1-7) is ±7 days.
[i] Urine pregnancy tests will be performed in females of childbearing potential on a monthly basis at each visit (except phone calls) and will be performed in the clinic

9 Biospecimen Storage / Laboratory Evaluations

Serum and esophageal tissue will be collected from all participants at screening and at the EOT visit (or at early withdrawal) for the determination of TGF-β levels and eosinophils. Exploratory laboratory tests will be analyzed by the research personnel in the [redacted] as well as in the Aceves laboratory in the Division of Rheumatology, Allergy, and Immunology at Rady Children's Hospital. These assays will include but are not limited to fibrosis markers, collagen deposition, assessment of TGF-β levels, and assessment of the esophageal transcriptome via the EDP. Participants are not required to fast prior to the blood draw.

The total volume of blood collected as per the NIH Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center (5/2012) For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. For adults the amount shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

All blood and specimen collection and handling will be done in accordance with the institution's laboratory and infection control policies. It is currently planned to retain samples indefinitely following completion of the study.

10 Criteria for Participant and Study Completion and Premature Study Termination

1. Study Completion

Before a study can be considered completed or terminated, the investigator must have the following data and materials:

- Clinical laboratory findings, clinical data, and all special test results from screening through the EOT visit (to 30 days after the last dose of study agent)
- CRFs properly completed by appropriate study personnel and reviewed and approved by the investigator
- Complete Drug Accountability records (study agent inventory log and, if applicable, an inventory of clinical supplies)
- Copies of protocol amendment(s) and IRB approval/notification if appropriate
- ☐ A summary of the study prepared by the PI (an IRB/independent ethics committee [IEC] summary letter is acceptable)

2. Participant Stopping Rules and Withdrawal Criteria

Participants may choose to discontinue from the study at any time, for any reason, specified or unspecified, and without prejudice. The reason for withdrawal, if specified, should be recorded on the source documents and CRF. Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.

2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participanthave failed).
3. The participant dies.
4. The Investigator or Sponsor no longer believes that participation is in the best interest of the participant.
5. Participant becomes pregnant.
6. The participant develops an SAE related to therapy in during the study.
7. If a particular AE were to recur on 3 occasions, and was not mitigated by dosing protocols.
8. Participant develops severe complications from their EoE (for instance, esophagealstrictures).

3. Participant Replacement

Enrolled participants who receive the study agent and prematurely discontinue/withdraw from the study will be replaced if they do not reach Week 16 of the study, including the EOT visit EGD. Additional participants will be enrolled in the same manner as all other participants. Participant numbers will not be re-used. Any enrolled participant will be included in the intent-to-treat analysis.

4. Follow-up after Early Study Withdrawal

Participants who withdraw prematurely from the study should have all EOT procedures (including a required urine β-hCG pregnancy test in all female participants of childbearing potential) performed at the time of withdrawal and should have a safety follow-up phone call 30 days after the last dose of the study agent.

5. Study Stopping Rules

Study enrollment and treatment will be suspended pending expedited review of all pertinent data after the occurrence of:

1. One death regardless of relationship to the investigational product
2. One nonfatal SAEpossibly related to the investigational product
3. Three participants discontinued from study because a particular AE recurred on three occasions, and was not mitigated by dosing titration

If the study is stopped due to meeting the above criteria, it may not be resumed until all pertinent information is discussed with DAIT NIAID, NIAID Asthma and Allergy DSMB, and the central IRB, and all parties concur with the resumption of the study. Local IRBs will be informed of the study stoppage and the DSMB/central IRB's decision on resumption of the study

The study may be terminated by DAIT/NIAID or the NIAID Asthma and Allergy DSMB upon review of any observations, events, or new information that merit such action

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

10.6 Site Termination Criteria

The study can be terminated or stopped at the site for reasons including but not limited to:

1. Investigator or sponsor request to withdraw from study participation
2. Serious and/or persistent noncompliance by the investigator with the protocol, the Form Food and Drug Administration (FDA) 1572, or other local applicable regulatory guidelines in conducting the study
3. IRB or DSMB decision to terminate or suspend approval for the investigation or the investigator

11 Safety Monitoring and Reporting

1. Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 11.7, *Reporting of Serious Adverse Events and Adverse Events*) to the sponsor. Appropriate notifications will also be made to site PIs, Institutional Review Boards (IRBs), and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21 CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI)'s CTCAE, Version 4 (referred to herein as the NCI-CTCAE manual). http://ctep.cancer.gov/reporting/ctc.html.

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved consent forms.

2. Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. The NIAID/DAIT appointed Data Safety Monitoring Board (DSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance annually. The DSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate reports – detailed by

severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed on a monthly basis by the research team and NIAID Medical Monitor. A separate report detailing protocol compliance will also be available from the DMCC for site and NIAID Medical Monitor review on a monthly basis. The research team and NIAID Medical Monitor will then evaluate whether the protocol or informed consent document requires revision based on the reports.

11.3 Definitions

11.3.1 Adverse Event

Any untoward or unfavorable medical occurrence associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research (modified from the definition of AEs in the 1996 International Conference on Harmonization [ICH] E-6 Guidelines for Good Clinical Practice [GCP]) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2.

Study Therapy Regimen

Any event occurring within 30 days of end of treatment with study agent will be considered a possible adverse event and evaluated further for any relationship.

Study Mandated Procedures:

Any event occurring within one week of a mandated study procedure will be considered a possible adverse event of these procedures and will be evaluated further for any relationship. These procedures include: study agent, phlebotomy and esophageal biopsies

Blood Draws

- Fainting / vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2cm

Esophageal Research Biopsies

- Bleeding at biopsy site estimated to be less than 5 ml
- Perforation

Lab Results

- Any result that is listed as clinically significate by the site or protocol PI, the independent medical monitor and/or the NIAID Medical Monitor.

2. **Suspected Adverse Reaction (SAR)**

A suspected adverse reaction is any AE for which there is a reasonable possibility that the investigational drug (or investigational study therapy regimen) caused the AE. For the purposes of safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any AE caused by a drug (21 CFR312.32[a]).

The investigator must assess the relationship of any AE to the use of the study agent, on the basis of the available information, using the guidelines listed in Table 6: *Attribution of Adverse Events*.

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

3. **Unexpected Adverse Event**

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity, or rate of occurrence that has been observed or if it is not consistent with the risk information described in the general investigational plan or elsewhere in the investigational new drug (IND) application.

"Unexpected" also refers to AEs or suspected adverse reactions that are mentioned in the package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR312.32[a]).

4. **Serious Adverse Event (SAE)**

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator, sponsor or DAIT/NIAID], it results in any of the following outcomes (21 CFR312.32(a)):

ICH Guide (E6) for GCP, 1996:

Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.

A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.

Inpatient hospitalization or prolongation of existing hospitalization.

Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

Congenital anomaly or birth defect.

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

Elective hospitalizations or hospital admissions for the purpose of conducting protocol-mandated procedures are not to be reported as an SAEunless hospitalization is prolonged due to complications.

11.4 Grading and Attributing Adverse Events

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4 (CTCAE). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the principal investigator and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (Table 5):

11.4.1 Table 5: Grading Criteria. The severity of each AEwill be categorized using the following criteria:

Grade 1	□	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
	□	Awareness of sign, symptom, or event but easily tolerated; requires no special treatment and does not interfere with the participant's daily activities
Grade 2	□	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*
	□	Discomfort enough to cause interference with usual activity and may warrant intervention
Grade 3	□	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**
	□	Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention
Grade 4	□	Life-threatening consequences; urgent intervention indicated
	□	Places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death)

Grade 5 • Death related to AE

Activities of Daily Living (ADL)

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

The study site will grade the severity of AEs experienced by the study participants according to the criteria set forth in the NCI-CTCAE manual (Version 4), which provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE manual has been reviewed by [redacted] and has been deemed appropriate for the participant population to be studied in this protocol.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

11.5 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE. Final determination of attribution for safety reporting will be determined by DAIT/NIAID, and sponsor. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 6.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

11.5.1 Table 6: Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
Unrelated Category		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
Related Categories		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

6. Collecting and Recording of Adverse Events

1. Collection Period

AEs will be collected from the time after the participant signs informed consent/assent (Screening or Enrollment study visit) and will end after the cessation of the study agent (Week 20 [30 days after the last dose of the study agent]).

2. Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant
- Interviewing the participant (e.g., using a checklist, structured questioning, diary, etc.)
- Receiving an unsolicited complaint from the participant
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE, as defined in Section 11.3.

11.6.3 Recording Adverse Events

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

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

11.7 Reporting of Serious Adverse Events and Adverse Events - RDCRN Adverse Event Data Management System (AEDAMS)

The site investigator will report by entry into the Adverse Event Data Management System (AEDAMS) all serious adverse events (See section 11.3.4, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event. Notification is by e-mail.

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs and the NIAID Medical Monitor of any reported adverse events via email.

Serious adverse events: The NIAID appointed Medical Monitor (MM) determines causality (unrelated, possibly related, definitely related) of the adverse event. The MM and sponsor, [redacted], may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MM beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MM monthly or on an as needed basis but no less than biannually. Local site investigators are also required to fulfill all reporting requirements of the central IRB.

The DMCC will post aggregate reports of all reported adverse events for site investigators and IRBs.

[redacted] or:

[redacted]

3333 Burnet Avenue / ML7028
Cincinnati, Ohio 45229-3039

[redacted]

Contact Information for NIAID Medical Monitor:

[redacted]
Allergy, Asthma and Airway Biology Branch, DAIT, NIAID
Department of Health and Human Services
National Institutes of Health
5601 Fishers Lane, Room 6B54 MSC 9827
Bethesda, Maryland 20892-9827
[redacted]

The SAE should be reported to the Sponsor within 24 hours with as much of the above information as available at that time. All initial and follow-up SAEs should be reported to the Sponsor.

Occasionally, SAEs that occur during a study are unresolved at the time that the participant's study participation ends. SAEs are to be followed to resolution; until they resolve, disappear, or become stable. It is important for the investigator to realize that SAEs may occur in a study participant after the study follow-up period is complete, but that these SAEs still merit reporting in the AEDAMS. The investigator, together with the sponsor and NIAID Medical Monitor, will try to determine whether the SAE is related to the study agent.

All other (suspected) reportable AEs must be reported to the SDCRN by entering into the AEDAMS within **20 working days** of the notification of the event or of the site becoming aware of the event.

Central IRB requirements, and any GCR oversight committee, remain the responsibility of the treating physician. Reporting to the FDA is the responsibility of [redacted] (sponsor).

1. **Reporting to Health Authority**
   All necessary personnel will be entered in the DMCC Adverse Event Notification System to receive email notification when AEs requiring 24-hour are submitted by the site investigator and assessed by the Study Sponsor and DAIT/NIAID Medical Monitor.

2. **Annual Reporting**
   The sponsor will include in the annual study report to health authorities all AEs classified as:

   - Serious, expected, suspected adverse reactions (See section 11.3.2, *Suspected Adverse Reaction* and Section 11.3.3, *Unexpected Adverse Event*)
   - Serious and not a suspected adverse reaction (See section 11.3.2, *Suspected Adverse Reaction*)
   - Pregnancies

   Note that all AEs (not just those requiring 24-hour reporting) will be reported in the annual FDA report.

   The DAIT/NIAID will receive a copy of the annual study report submitted by the sponsor to the health authorities.

The sponsor shall summarize safety data at the end of the study and periodically throughout the study for IND regulatory filings, the data safety monitoring board (DSMB) and for the Medical Monitor. These reports will meet the needs of the DSMB and the Medical Monitor and may include (but are not limited to) masked summaries and listings of AEs, SAEs, changes in vital signs, lab results, events requiring discontinuation of a study-mandated procedure for individual subjects, protocol deviations, and/or events listed as study stopping rules.

3. **Expedited Safety Reporting**

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction (SUSAR)** (See section 11.3.2. *Suspected Adverse Reaction* and section 11.3.3. *Unexpected Adverse Event* and 21 CFR312.32[c][1]i).

The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the AE, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury)
2. One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates that those events occur more frequently in the drug treatment group than in a concurrent or historical control group

Certain SAEs occur commonly in this study population and will not be considered as a SUSAR unless there is evidence to suggest a causal relationship to the study drug. These events will be captured in the study database but will not be reported as expedited safety reports:

- Food impaction
- Esophageal dilation

Category 2: Any findings from studies that suggests a significant human risk

The sponsor shall report any findings from other epidemiological studies, analyses of AEs within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-

related change in the protocol, informed consent, investigator brochure or package insert, or other aspects of the overall conduct of the study.

The sponsor shall notify the FDA and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

11.7.4 Reporting of Adverse Events to IRBs/IECs

All investigators shall report AEs, including expedited reports, in a timely fashion to the central IRB in accordance with applicable regulations and guidelines. All FDA safety reports shall be distributed by [redacted] or his designee to all participating institutions for site IRB/IEC submission.

11.8 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study participant as all participants will be receiving drug during this study. A pregnant participant shall be instructed to stop taking study medication immediately, as there are known risks to the fetus as a consequence of the use of losartan potassium. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant shall continue until the conclusion of the pregnancy. The investigator shall report all pregnancies to the DAIT/NIAID within 1 business day of becoming aware of the event using the pregnancy paper CRF. All pregnancies identified during the study shall be followed to conclusion, and the outcome of each must be reported. The pregnancy paper CRF shall be updated and submitted to the DAIT/NIAID when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study participant.

- Information requested about the delivery shall include:
  - Gestational age at delivery
  - Birth weight, length, and head circumference
  - Gender
  - Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities

An SAE shall be submitted to the DAIT/NIAID and Study Sponsor ([redacted]) using the SAE reporting procedures described above for all pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion.

9. Reporting of Other Safety Information

An investigator shall promptly notify the central IRB, as well as the DAIT/NIAID, Study Sponsor [redacted]) when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an AE.

10. Review of Safety Information

1. Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive IND annual reports from the sponsor [redacted] compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate CRFs.

In addition, the DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the DMCC (See sections 11.7, *Reporting of Serious Adverse Events and Adverse Events*, and 11.8, *Pregnancy Reporting*).

2. DSMB Review

The Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. The DSMB will be informed of an Expedited Safety Report within 10 days.

***1. Ad hoc* DSMB Reviews**

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID and sponsor. In addition, the following events will trigger an *ad hoc* comprehensive DSMB Safety Review:

- Any death that occurs in the study, which is possibly or definitely related to study treatment regimen.
- The occurrence of a Grade 3 or higher related and unexpected SAE in 1 or more of the study participants who have received a study treatment.
- The occurrence of Grade 2 or higher related to the study agent in 2 or more of the study participants who have received study therapy

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

11.11 Temporary Suspension of Enrollment / Drug Administration for *Ad Hoc* DSMB Safety Review

(See section 10.2, *Participant Stopping Rules and Withdrawal Criteria*)

A temporary halt in enrollment and/or drug administration will be implemented if an *ad hoc* DSMB safety review is required. In this context, no new participants would be consented until the safety review is completed. No additional procedures would be planned during the review, save for those necessary for regular patient care.

12 Statistical Considerations and Analytical Plan

1. 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. 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.

2. Outcomes

1. Primary Outcomes

- Change from baseline in the peak esophageal eosinophil count in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy and reported as eosinophils/HPF

2. Secondary Outcomes

- Change from baseline in the blood (serum) and esophageal TGF-β levels in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by research blood samples and EGD with biopsy, respectively.
- Normalization of the EoE transcriptome in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy.
- Change from baseline in histopathology in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EGD with biopsy.
- Change from baseline esophageal compliance in participants receiving losartan at the EOT visit (or at early withdrawal) as determined by EndoFLIP.
- Change from baseline PROS (Pediatric Eosinophilic Esophagitis Symptom Severity [PEESS], PedsQL Eosinophilic Esophagitis Module [PedsQL™ EoE], EoE Quality of Life Questionnaire, and EEsAI) in participants receiving losartan at the EOT visit (or at early withdrawal).

3. Measures to Minimize Bias

All eligible participants will be assigned to take the losartan. The investigator or designee physician will calculate the losartan dose according to the titration schedule on the basis of the participant's weight. The investigational pharmacist will dispense and prepare the study agent for administration. The DAIT/NIAID, investigator, study staff, and participant will have knowledge of the dose.

4. Analysis Plan

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:

H_0: μ=0 vs. H_1:μ≠0.

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. 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 α=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:

H_0: $\beta_{EoE\text{-}CTD} = \beta_{EoE\ (non\text{-}CTD)}$ vs. H_1: $\beta_{EoE\text{-}CTD} \neq \beta_{EoE\ (non\text{-}CTD)}$.

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

H_0: $\beta=0$ vs. H_1: $\beta\neq0$

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.

All analyses will be performed using SAS version 9.2 or later (Cary, NC).

12.5 Sample Size Considerations

Five patients with EoE-CTD and 10 patients with EoE will be recruited into the study. Since this represents a pilot study to evaluate whether the treatment affects endpoints, the sample size was not selected on the basis of power considerations for statistical testing. The estimates of treatment effect from this study will be used to power future studies. Based on historical data from the [redacted], it is estimated that the standard deviation of the change from baseline at 12 weeks based on the square root transformation of the eosinophil/HPF values will be approximately 5. Table 7 provides the width of the two-sided 95% confidence interval for the change from baseline in eosinophil/HPF for various scenarios of sample size and standard deviation.

12.5.1 Table 7: Standard Deviation and Confidence Interval Widths

Sample Size	Standard Deviation (square root transform)	Width of Confidence Interval (square root transform)
15 (both groups combined)	5	±2.8
	10	±5.5
10 (EoE)	5	±3.6
	10	±7.2
5 (EoE-CTD)	5	±6.2
	10	±12.4

For binary endpoints (e.g. percentage of participates for whom peak eosinophil counts are < 15 eosinophil/HPF), the following table provides the width of the two-sided 95% confidence interval for the binary endpoint given various scenarios of response rates (Table 8).

12.5.2 Table 8: 95% Two-sided Score Confidence Interval

Sample Size	Response Rate	Estimated Confidence Interval
15 (both groups combined)	80%	55% to 93%
	60%	36% to 80%
	40%	23% to 64%
10 (EoE)	80%	49% to 94%
	60%	31% to 83%
	40%	17% to 69%
5 (EoE-CTD)	80%	38% to 96%
	60%	23% to 88%
	40%	12% to 77%

See Reference (81)

13 Identification and Access to Source Data

1. **Source Data**

Source documents and source data are considered to be the original documentation in which participant information, visits, consultations, examinations, and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations, and other activities during a clinical trial. This will include instruments to evaluate participant symptoms, quality of life, and histologic score of esophageal biopsy samples.

2. **Access to Source Data**

The site investigators and site staff will make all source data available to the DAIT/NIAID, the Sponsor, DSMB, and IRB as is needed. Authorized representatives as noted above are legally and ethically bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

14 Protocol Deviations

1. Protocol Deviation Definitions

Protocol Deviation

The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation)

A Protocol Violation is a deviation from the IRB-approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local, or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety, or well-being or the completeness, accuracy, or reliability of the study data.

2. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation (major or minor) has occurred, the study staff will a) notify the site Principal Investigator, b) notify the DMCC and c) will complete a Protocol Deviation form. The Protocol Deviation form will document at a minimum the date PD occurred, the date PD identified, a description of event, whether the deviation resulted in SAE/AE, the signature of PI, report to central IRB, and documentation of a corrective action plan. The DMCC and DAIT/NIAID may request discussion with the PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will complete and sign the Protocol Deviation form and submit it to the DMCC and to the central IRB, per IRB regulations. Major protocol deviations will be reported to the DSMB by the NIAID Medical Monitor at the Medical Monitor's discretion.

15 Ethical Considerations and Compliance with Good Clinical Practice (Quality Control and Quality Assurance)

1. Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the Cincinnati Children's Hospital Medical Center IRB (CCHMC IRB), which will serve as the central IRB for the study. Any amendments to the protocol or to the consent materials will also be approved by the CCHMC IRB before they are implemented.

The investigator will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable country and local-specific regulatory requirements prior to initiating the study. The investigator is responsible for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential participants/parents or legal guardians (e.g. advertisements or information that supports or supplements the informed consent) are reviewed and approved by the IRB. The investigator agrees to allow the IRB direct access to all relevant documents. The IRB must be constituted in accordance with all applicable regulatory requirements. The investigator will provide the IRB with relevant document(s)/data that are needed for approval of the study.

2. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the delegation log will review the consent and answer questions. The consent designee must be listed on the delegation log, have knowledge of the study and received training (from the local IRB, PI, or study coordinator) in the consent process The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

This study will be conducted in compliance with ICH E6 GCP: Consolidated Guidelines pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, participants will give their written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. If the participant is unable to provide written informed consent, the participant's legally acceptable representative may provide written consent as approved by institutional

specific guidelines. The informed consent document must be signed and dated by the participant, or the participant's legally authorized representative, prior to study participation. A copy of the informed consent document must be provided to the participant or the participant's legally authorized representative. Signed consent forms must remain in the participant's study file and be available for verification by the monitor, IRB, and/or regulatory authorities at any time. If participant's legally acceptable representative provides written consent, participants will also give their written assent to participate in the study as approved by institutional specific obtaining assent.

The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

15.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing protected health information (PHI) to the study sponsor or their representatives.

Although the study staff will do its best to adhere to strict privacy guidelines, the possible risk of a breach of confidentiality does exist. Every effort will be made to maintain the confidentiality of the participant's medical and research information. The investigator will take all necessary measures to keep all of the participant's personal information private and confidential. Electronic study records will be protected with electronic safeguards (e.g., computer passwords, restricted access privileges). Paper-based PHI will be secured in locked cabinets. Access to study records will be limited only to research staff. For this study, the research study staff will collect and utilize data obtained from the participant's medical and research records and observations, including laboratory tests. This information, along with the study survey forms, will be used by the Sponsor and Investigator as part of the study data. The participants will be assigned and identified by a participant study identification number. The link to the participant's study ID's and their names and identifiers will be kept by the PI and/or the research staff in a secure location. Because the participant's PHI will be collected and used for the study, the research staff will obtain a signed HIPAA authorization and informed consent from the participant and/or legal guardian. The research staff will perform informed consent and all study procedures in a private setting away from the public. In addition, the research staff will only collect the minimum amount of personal information about the participant necessary for the research study.

The PI, members of the study's research team, assigned study monitor(s), CCHMC medical staff, federal and other regulatory agencies (including the FDA), CCHMC IRB, and Office of Research Compliance and Regulatory Affairs, and DSMB will be have access to the participant's medical and research records related to this study. The data from the study may be published; however, the participant will not be identified by name.

15.4 Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify the participant in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless the participant has consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if the participant consents to the disclosure, including for their medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research participants.

The certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the U.S. Department of Health and Human Services and/or the National Institutes of Health, which is finding this project or for information that must be disclosed in order to meet the requirements of the Federal Food and Drug Administration (FDA). The Certificate of Confidentiality does not prevent a participant from voluntarily releasing information about themselves or their involvement in this research, If a participant wants to research information released to an insurer, medical care provider, or any other person not connected with the research, the participant must provide consent to allow the researchers to release it.

Even with the Certificate of Confidentiality, the investigators continue to have ethical obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person. The Certificate of Confidentiality will also not be used to prevent disclosure as required by federal, state, or local law, such as reports of child abuse and neglect, or harm to self or others.

16 Investigator Requirements

16.1 Protocol Adherence

The investigator must adhere to the protocol as detailed in this document and agree that the Sponsor and the IRB must approve any change to the protocol. The investigator will be responsible for enrolling only those participants who have met the protocol screening and study entry criteria.

2. Case Report Forms

The CRFs will be used for the recording of all information and study data as specified by this protocol. The CRFs must be completed by the research personnel. The principal investigator is responsible for ensuring that accurate CRFs are completed in a timely manner. Collected data will be entered into online electronic case report forms. Electronic case report forms will be developed in collaboration with the Data Management and Coordinating Center that contain the requisite data fields.

3. Source Document Maintenance

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, study progress notes, e-mail correspondence, computer printouts, clinical laboratory data, and recorded data from automated instruments. All source documents produced in this study will be maintained by the investigator and made available for inspection by regulatory authorities. The original signed dated informed consent form for each participating participant shall be filed with records kept by the investigator and a copy given to the participant. The patient outcome questionnaires will be recorded directly on the paper forms and will be considered as source data.

4. Inspection of Records

Data generated by this study must be available for inspection by any regulatory authorities and the IRB as appropriate. At a participant's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Participant medical information obtained during the course of this study is confidential and disclosure to third parties other than those noted above is prohibited.

5. Retention of Records

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

6. Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.

- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

7. Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

8. Data Entry

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

9. Laboratory Data Flow

The DMCC will provide laboratories with on-line forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. Online forms exist to verify specimen receipt, report specimen issues and submit test results for specimens. The preferred method to exchange data electronically is through the Specimen Management System Web Service. The Web Service allows laboratories to obtain specimen shipment information, receive individual specimens or specimen shipments, report specimen issues and communicate specimen aliquots in a secure manner (test result submission is planned). The DMCC will also support uploading of files electronically. All transactions are logged and validated for both methods.

10. Study Completion

Before a study can be considered completed or terminated, the investigator must have the following data and materials:

- Clinical laboratory findings, clinical data, and all special test results from screening through the EOT visit (to 30 days after the last dose of study agent).
- CRFs properly completed by appropriate study personnel and reviewed and approved by the investigator.
- Copies of protocol amendment(s) and IRB approval/notification if appropriate.
- A summary of the study prepared by the principal investigator (an IRB/IEC summary letter is acceptable).

11. Audits and Inspections

The principal investigator will permit study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to CRFs and source data/documents.

12. Institutional Review Board Approval

This protocol, the informed consent document, and all relevant supporting data must be submitted to the IRB for approval. IRB approval of the protocol, informed consent document, study materials and any advertisement (if applicable) used to recruit study participants must be obtained before the study may be initiated. This study will utilize a centralized IRB, and the IRB at Cincinnati Children's Hospital Medical Center will serve as the central IRB for the study.

The principal investigator (PI) is responsible for keeping the IRB advised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The PI is also responsible for notifying the IRB of any unanticipated AEs that occur during the study in accordance with local IRB policies. Recent guidance from the USA FDA suggests that the following AEs should be reported to the IRB/IEC as "unanticipated problems:"

- Any AE that, even without detailed analysis, represents a serious unexpected AE that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- A series of AEs that, on analysis, is both unanticipated and a problem for the study. There would be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and were significant to the rights and welfare of participants.
- An AE that is described or addressed in the IB/package insert, protocol, or informed consent documents, or is expected to occur in study participants at an anticipated rate (e.g., expected progression of disease, occurrence of events consistent with background rate in participant population), but occurs at a greater frequency or at greater severity than expected.
- Any other AE that would cause the sponsor to modify the investigator brochure, study protocol, or informed consent form, or would prompt other action by the IRB to assure protection of human participants.

It will be the responsibility of the investigator to assure that the essential documents are available at the investigator site. Any or all of these documents may be subject to, and should be available for, audit by CCHMC or Sponsor auditor and inspection by the regulatory authorities as defined in the monitoring plan.

17 Publication Policy

The CEGIR policy on the publication of study results will apply to this trial. The data generated in this clinical study are the exclusive property of the Sponsor and PI and are confidential. Written approval from the Sponsor is required prior to disclosing any information related to this clinical study. Authorship on the primary publication of the results from this study will be determined by the Sponsor and PI and will be based on contributions to study design, enrollment, data analysis, and interpretation of results.

18 References

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19 Appendices

1) 7803 Female Systolic Blood Pressures Age 5 - 17 Years

2) 7803 Female Diastolic Blood Pressures Age 5 - 17 Years

3) 7803 Male Systolic Blood Pressures Age 5 - 17 Years

4) 7803 Male Diastolic Blood Pressures Age 5 - 17 Years

5) 7803 Adult Blood Pressures ≤18 Years

19.1 Systolic Blood Pressures for Females Age 5 – 17 Years (Updated)

				Systolic Height Percentile								
Gender	Age (Y)	Age (M)	BP Percentile	1st	2nd	5th	10th	25th	50th	75th	90th	95th
Female	5	60	2	68	69	70	71	72	74	75	77	78
Female	6	72	2	70	70	71	72	74	75	77	78	79
Female	7	84	2	71	71	72	73	75	76	78	79	80
Female	8	96	2	71	72	73	74	75	77	79	81	82
Female	9	108	2	71	72	73	74	76	79	81	83	84
Female	10	120	2	73	74	75	76	78	81	83	85	86
Female	11	132	2	75	76	78	79	81	84	85	86	86
Female	12	144	2	79	80	81	83	85	86	86	86	86
Female	13	156	2	82	83	84	85	86	87	87	87	88
Female	14	168	2	84	85	86	86	87	87	88	89	89
Female	15	180	2	85	85	86	87	88	88	89	90	90
Female	16	192	2	86	86	87	88	89	90	91	91	91
Female	17	204	2	87	87	88	89	91	91	92	92	93

19.2 Diastolic Blood Pressures for Females Age 5 – 17 Years (Updated)

				Diastolic Height Percentile								
Gender	Age (Y)	Age (M)	BP Percentile	1st	2nd	5th	10th	25th	50th	75th	90th	95th
Female	5	60	2	31	31	31	32	32	33	33	34	34
Female	6	72	2	31	31	32	32	32	33	33	33	33
Female	7	84	2	31	31	31	31	31	32	32	32	33
Female	8	96	2	29	30	30	30	31	32	34	35	36
Female	9	108	2	31	32	32	33	33	34	35	36	37
Female	10	120	2	32	33	33	33	34	35	35	36	37
Female	11	132	2	33	33	33	33	34	34	35	37	38
Female	12	144	2	33	33	33	33	34	35	36	37	37
Female	13	156	2	35	35	35	35	36	36	37	37	37
Female	14	168	2	39	39	39	39	39	40	41	42	43
Female	15	180	2	43	43	43	43	43	45	46	47	47
Female	16	192	2	44	44	44	43	44	46	48	48	49
Female	17	204	2	44	44	43	42	44	47	48	48	48

19.3 Systolic Blood Pressures for Males Age 5 – 17 Years (Updated)

				Systolic Height Percentile								
Gender	Age (Y)	Age (M)	BP Percentile	1st	2nd	5th	10th	25th	50th	75th	90th	95th
Male	5	60	2	70	71	71	72	73	75	76	77	78
Male	6	72	2	71	72	73	74	75	76	77	78	79
Male	7	84	2	72	73	74	75	76	77	78	80	80
Male	8	96	2	73	74	75	76	77	79	80	81	82
Male	9	108	2	75	76	77	78	79	81	82	83	84
Male	10	120	2	77	78	79	80	81	83	84	85	85
Male	11	132	2	79	80	81	82	83	84	85	86	87
Male	12	144	2	81	82	83	83	85	86	87	88	89
Male	13	156	2	83	83	84	85	86	87	89	90	91
Male	14	168	2	83	84	84	85	87	89	91	92	92
Male	15	180	2	84	84	86	87	89	91	92	93	94
Male	16	192	2	86	87	88	90	92	94	94	95	95
Male	17	204	2	88	89	91	93	95	96	97	97	98

19.4 Diastolic Blood Pressures for Males Age 5 – 17 Years (Updated)

				Diastolic Height Percentile								
Gender	Age (Y)	Age (M)	BP Percentile	1st	2nd	5th	10th	25th	50th	75th	90th	95th
Male	5	60	2	28	28	29	29	30	31	32	33	33
Male	6	72	2	30	30	31	31	32	33	34	34	34
Male	7	84	2	31	31	31	32	32	33	34	34	34
Male	8	96	2	30	30	31	31	32	33	34	35	36
Male	9	108	2	29	30	31	31	33	35	36	37	38
Male	10	120	2	30	30	32	33	34	36	38	39	40
Male	11	132	2	30	31	32	33	35	36	38	38	38
Male	12	144	2	30	31	32	33	34	36	36	36	35
Male	13	156	2	31	31	32	33	34	34	34	34	34
Male	14	168	2	33	33	34	34	35	35	36	37	38
Male	15	180	2	34	35	36	36	37	39	40	41	42
Male	16	192	2	36	37	38	39	41	42	43	44	44
Male	17	204	2	38	39	40	41	43	44	45	46	46

19.5 Adult Blood Pressures for Males and Females ≥18 Years

	Systolic and Diastolic Blood Pressure 5th Percentile	
	Systolic Min	Diastolic Min
Female	105	60
Male	105	60