TEXAN ALLERGY & SINUS CENTER MOUNTAIN CEDAR INTRA-LYMPHATIC IMMUNOTHERAPY STUDY (TX-SMILE)

Protocol Number: TASC-ILIT-MC-2018

National Clinical Trial (NCT) Identified Number: TBD

Sponsor-Investigator: Christopher Thompson, MD

Institution: Texan Allergy & Sinus Center, Inc.

IRB of Record: IntegReview IRB

Version Number: 2.0

19 December 2018

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.1	Administrative changes	IND and NCT numbers added, clarifications made to working of exclusion criteria.
2.2	Additional information regarding use of glycerinated extracts	Provide further consideration of the suitability of the diluent in relation to the lymphatic system
2.3	Additional information about risk of pre-procedural anxiety added	Pre-procedural anxiety reported by some subjects before initiation of treatment.
3	Moved Safety Objective to Primary and Efficacy Object to Secondary	Based on stage of development and prior studies of ILIT comparative safety was prioritized over a priori testing of efficacy.
6	Lot and expiration information for study materials added following production of study IP	Expiration of immunotherapy vials is based on the components, concentration, and production date of the patient-specific vials.
8.2	Pre-specified stopping rules added	Clarify that enrollment and treatment will be put on hold in the event that a serious, treatment-emergent AE or WAO Grade 3 allergic reaction occurs.

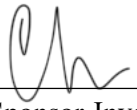
Table of Contents

STATEMENT OF COMPLIANCE

This trial will be carried out in accordance with International Conference on Harmonization Guideline for Good Clinical Practice (ICH GCP) and the following: United States (US) Code of Federal Regulations (CFR) applicable to clinical studies 45 CFR 46, 21 CFR Part 50 and 21 CFR Part 56, and 21 CFR Part 312, as applicable. This trial involves the use of a commercially-available, FDA approved allergenic extract that is lawfully marketing in the United States. It is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the product labeling, promotion or commercialization of the drug.

Protocols, informed consent form(s), recruitment materials, and all participant materials will be submitted for Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

I agree to personally supervise the conduct of this study.



Sponsor-Investigator Signature

12/20/18

Date

Christopher Thompson

Sponsor-Investigator Name

MD

Title

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Sponsor-Investigator:	Christopher Thompson, MD
Study Title:	**Te**X**a**n Allergy & **S**inus Center **M**ountain Cedar **I**ntra-**L**ymphatic Immunoth**E**rapy Study (**TX-SMILE)**
Study Description:	TX-SMILE is an investigator-initiated, multi-center, randomized, double-blind, parallel-group, placebo-controlled study to evaluate the safety, tolerability, and efficacy of an alternative injection site and associated adjustments to dosing and treatment regimen for allergen immunotherapy with a commercially-available, FDA-approved allergenic extract (ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) for the immunotherapy treatment of allergic rhinitis and conjunctivitis due to pollen from the conifer Mountain Cedar (*Juniperus ashei*). The purpose of this study is to evaluate the effect of a three-dose treatment series of ultrasonography-guided, intra-lymphatic immunotherapy injections over a two-month period prior to the start of the 2018-2019 Mountain Cedar allergy season in central Texas. Patients will be evaluated for safety and tolerability during the treatment period and for efficacy during the 2018-2019 Texas Mountain Cedar pollen season.
Study Number:	TASC-ILIT-MC-2018
IND Number:	18-643
NCT Number:	NCT03682965
General Study Design:	TX-SMILE is an investigator-initiated, multi-center, randomized, double-blind, parallel-group, placebo-controlled study to evaluate the safety, tolerability, and efficacy of intra-lymphatic administration of an approved allergenic extract for the immunotherapy treatment of allergic rhinitis and conjunctivitis due to pollen from the conifer Mountain Cedar (Mountain Cedar pollinosis).
Background:	More than 40 million people in the United States (approximately 14% of adults and 13% of children) and more than 400 million people worldwide experience allergic rhinitis (AR). AR can substantially reduce patients' quality of life and can impair many aspects of physical and social well-being, including reduced school and work performance. Furthermore, patients with AR experience significant out-of-pocket medication costs and make nearly twice as many visits to health care providers as matched controls. The disease manifestations of AR include both nasal and non-nasal symptoms. The primary nasal symptoms are nasal itching, sneezing, rhinorrhea, and congestion, while the major non-nasal symptoms include itchy watery, red, and/or swollen eyes, earache, headache, cough and irritation of the palate. AR is also an independent risk factor for the development of asthma and it is reported that 38% of American patients with AR have asthma and up to 78% of American asthma patients have AR.

	For regulatory purposes, allergic rhinitis is differentiated into two related disorders, seasonal AR and perennial AR. The pathophysiology of SAR and PAR are similar in terms of the chemical mediators involved and clinical manifestations, with the differences between the two primarily based on the cause and duration of disease. Although there are many treatments available, including over-the-counter remedies for reduction of symptoms of AR, allergen immunotherapy (AIT) is the only treatment option that can provide significant improvements in allergic symptoms and reduce the need for pharmacotherapy. Since it was introduced in 1911, AIT has been clinically shown to provide long-term benefits, including symptomatic disease remission and a reduction in allergic disease progression from rhinitis to asthma. The World Health Organization recognizes allergen immunotherapy as the only allergy treatment for AR that currently treats the underlying disease rather than just the disease symptoms. Conventional AIT involves a dose-escalation series of thirty (30) to seventy (70) subcutaneous injections of over period of three (3) to five (5) years, leading to relief of allergy symptoms by inducing tolerance to the allergen, reducing or eliminating the natural immune response. However, this regimen presents barriers to patient adherence, including the time commitment required to complete therapy. Thus, fewer than 5% patients with serious AR symptoms that could benefit from immunotherapy receive AIT treatment. A variety of allergen extracts are FDA-approved for immunotherapy based on individualized subcutaneous dosing (subcutaneous immunotherapy; SCIT) determined by the treating clinician based on patient sensitivity, clinical response, and tolerance to the extract during the early phases of an injection regimen. In 2008, Senti and colleagues first reported on an alternative treatment regimen for AIT involving the administration of allergenic extract directly into the lymph nodes, the proposed site of action for SCIT. This route is called Intra-lymphatic Immunotherapy (ILIT) and since that initial clinical trial, several additional randomized controlled trials of ILIT have been conducted and generally demonstrate more rapid tolerization at lower doses than conventional SCIT but with similar safety and tolerability.
Objectives:	1. To evaluate the systemic safety of ILIT for Mountain Cedar pollinosis relative to intranodal injections of placebo control based on the proportion of subjects receiving allergenic extract versus the proportion of subjects receiving placebo: a. experiencing anaphylaxis, or b. treated with epinephrine, or c. Experiencing any other treatment-emergent, serious adverse event (SAE) within 60 minutes of the procedure. 2. To evaluate the safety profile of ILIT versus placebo using a standardized scoring system for systemic AEs of interest (AEIs) up to 60 minutes post-procedure 3. To evaluate the efficacy of ILIT relative to placebo during the 2018-2019 Texas Mountain Cedar allergy season as assessed by the daily

	total combined score (TCS), a composite of the Daily Symptoms Score (DSS) and Daily Medication Score (DMS). 4. To evaluate the tolerability of ILIT using a patient-reported outcome measure 5. To assess the patients' self-reported satisfaction with treatment at the end-of-study. 6. To assess the induction of tolerance to the causative allergen using allergen-specific serum IgE testing
Endpoints:	Primary Efficacy Endpoint: 1. Average daily TCS during the 2018-2019 Mountain Cedar pollen season Secondary Efficacy Endpoints: 1. Proportion of days during pollen season for which active patients experience a lower TCS than placebo patients 2. Patient reported pain or discomfort immediately after and 30 minutes post ILIT procedure 3. Patient-reported treatment satisfaction at the end of the study 4. Reduction in skin reactivity assessed using allergenic-specific serum IgE testing from pre-treatment to post-treatment (the end-of-study visit) Exploratory Endpoints: 1. Use of rescue inhalers by patients with stable asthma Safety Endpoints: 1. Proportion of subjects experiencing a serious, treatment-emergent AE up to 60 minutes after an intranodal injection procedure 2. Proportion of subjects experiencing systemic reactions to ILIT relative to placebo and severity of reactions relative to placebo as assessed by the Total Safety Score (TSS) 3. Proportion of subjects reporting local injection site reactions relative to placebo and severity of local reactions relative to placebo, as assessed by the TSS
Study Population:	This study will include up to 40 screened and 32 enrolled adult patients aged 18 or older with seasonal allergic rhinitis (SAR) and conjunctivitis due to pollen from the conifer Mountain Cedar (*Juniperus ashei)*.
Key Inclusion Criteria:	1. Both male and female adult patients with a history of SAR with bothersome symptoms due to Mountain Cedar pollinosis confirmed using the Modified Quantitate Test (MQT; defined as a wheal greater than or equal to 3 millimeters larger than the diluent control) [a] 2. Patients must be willing to provide written, informed consent 3. Patients must be willing and able to comply with study procedures 4. Women of childbearing potential must agree to use an acceptable form of contraception during the trial
Key Exclusion Criteria:	1. Patients less than 18 years of age 2. Clinically-significant chronic sinusitis, as determined by the investigator

	3. History of systemic allergic reaction, e.g. anaphylaxis with cardiorespiratory symptoms, generalized urticaria, or severe facial angioedema. 4. Participation in another clinical trial or use of an experimental medication within 30 days of enrollment 5. Medically significant co-morbidities that, in the opinion of the investigator, place the subject at increased risk during the study or may confound the interpretation of results, including but not limited to: a. Autoimmune diseases, other than AR, stable allergic asthma[c], eczema and food sensitivities b. Pulmonary or respiratory diseases other than stable asthma c. Cancer other than basal cell carcinoma d. Coronary artery disease or hypertension treated with beta-blockers e. Clinically significant impairment of renal or hepatic function f. Clinically-significant lab abnormalities 6. Use of concomitant medications that, in the opinion of the investigator, may reduce the effectiveness of rescue treatments for anaphylaxis (e.g. beta-blockers) or alter the immune response to AIT (e.g., immunosuppressants, systemic corticosteroids) 7. Previously completed SCIT or SLIT for Mountain Cedar pollinosis, that in the opinion of the investigator would interfere with the assessment or treatment of the patient[b] 8. Inability to access suitable lymph nodes for ILIT injections 9. Plans to leave the area for a significant period of the upcoming Mountain Cedar pollen season 10. Pregnant or lactating females
Study Phase and Stage of Development	Mountain Cedar pollen allergenic extract (ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) is an FDA-approved, commercially available product. This investigation is a phase 2 proof-of-concept study to evaluate an alternative injection site location (inguinal lymph node versus subcutaneous injection into the upper aspect of the arm) with an associated reduction in the extract dose and number of injections.
Sites/ Facilities Enrolling Participants:	Up to sixteen (16) centers in the United States, that are affiliated with Texan Allergy & Sinus Center will recruit patients. Enrolled subjects will receive treatment at one of three (3) TASC ambulatory surgical centers in San Antonio, Dallas, and Austin, Texas.
Study IRB of Record	IntegReview IRB 3815 S. Capital of Texas Hwy, Suite 320 Austin, TX 78704 IRB Organization (IORG) number: IORG0000689
Description of Study Intervention:	After informed consent subjects will be randomized 1:1 to receive a series of three (3) injections (0.1 mL) of the allergenic extract (Mountain Cedar Pollen, ALK-Abelló, Inc., Port Washington, NY 11050; US

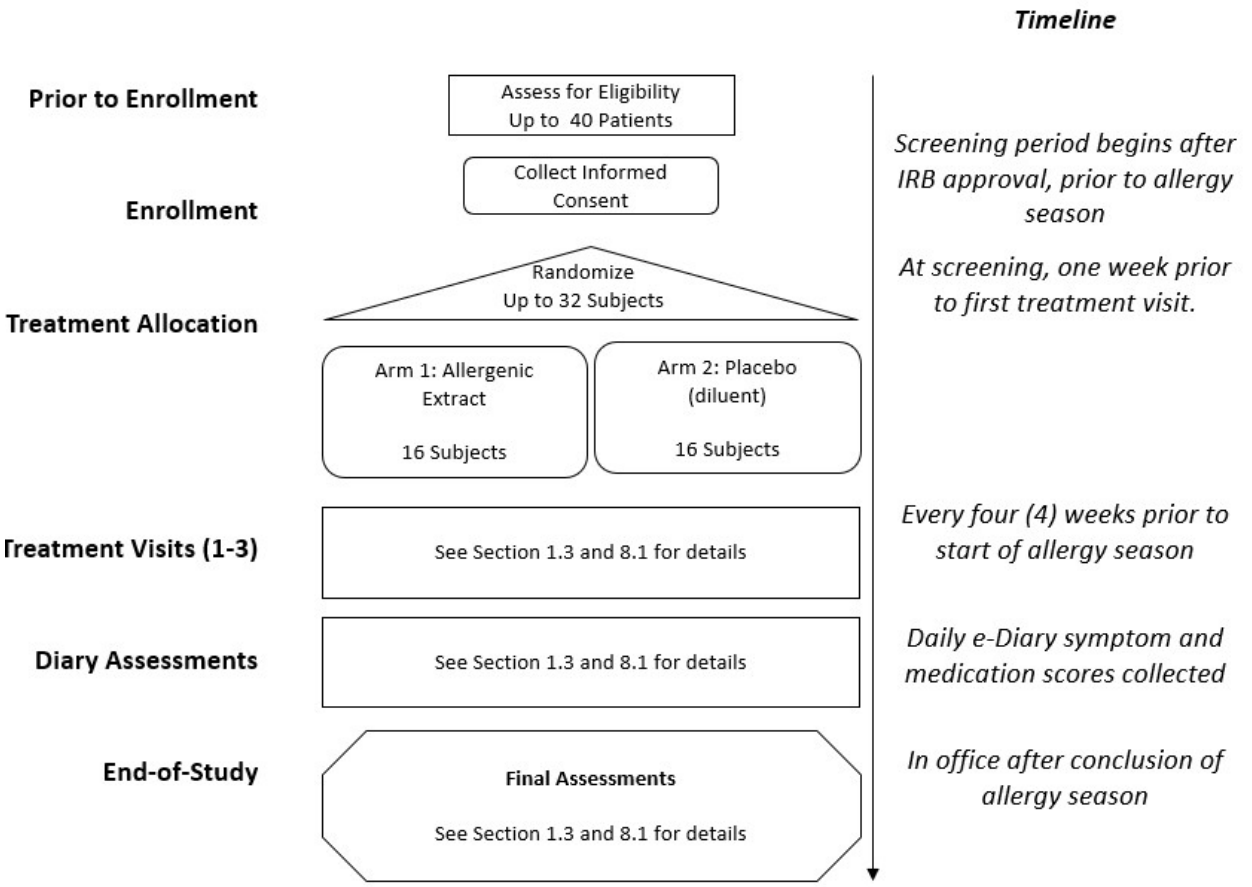
	Government License No. 1256) or diluent as placebo control (sterile saline solution containing 0.4% phenol as a preservative; ALK-Abelló, Inc., Port Washington, NY) every four weeks into a superficial inguinal lymph node through guidance via ultrasonography using a 1-mL hypodermic syringe with a 25-gauge or smaller needle.
Study Duration:	The treatment period will be up to three (3) months, followed by three (3) months of follow-up beginning prior to the start of the 2018-2019 Texas Mountain Cedar allergy season and concluding at the end of the season. The total study duration will therefore be from approximately September 2018 thru August 2019 for completion of data analysis. Study results will be reported within 12 months of study completion.
Participant Duration:	Patients will receive a series of three ILIT injections four weeks apart prior to the start of the 2018-2019 Texas Mountain Cedar allergy season and will be followed monthly for up to four months. The end-of-study visit will be scheduled to take place after the pollen season has ended, as determined by the daily pollen count measures recorded at each participating center. The total study duration for individual participants will be less than seven months.

[a] Patients that are hypersensitive to Mountain Cedar allergenic extract as defined in section 5.2 (a wheal for allergenic extract greater than that for the positive control), the initial during skin testing are clinically the most likely to benefit from AIT and will therefore be allowed to enroll. Starting doses for these patients are reduced in accordance with section 6.2.

[b] Patients that previously began but did not complete adequate AIT treatment for Mountain Cedar allergy and meet the other inclusion and exclusion criteria will be eligible at the discretion of the investigator.

[c] Patients with asthma will require documentation that their asthma is well controlled using ACT score and/or normal spirometry to be eligible. Patients with poorly controlled asthma are defined as those with frequent use of short-acting beta agonists (SABA), night-time awakenings, reduction in normal activity levels per the National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma, or have any history of a life-threatening asthma attack, hospitalization or who have sought clinical intervention for asthma including use of oral corticosteroids within the past three (3) months, or have a lung function measured by FEV1 of less than or equal to 70% of the predicted value while on their usual asthma medication.

1.2 SCHEMA AND TIMELINE



1.3 SCHEDULE OF EVENTS

		Treatment			Follow-up	
Procedures	Screening Visit	Treatment Visit 1[c]	Treatment Visit 2	Treatment Visit 3	Patient Diary	End-of-Study Visit 4
	Day -21 to Day 1	Day 1	Day 28 +/-3	Day 56 +/-6	Approx. Dec 2018 to March 2019	March – April 2019
Informed consent	X					
Demographics	X					
Medical history	X					
Physical exam	X					X
Modified Quantitative Test	X					
Allergen-specific Serum IgE	X					X
Vital signs [a]	X	X	X	X		
Urine pregnancy test [b]	X	X	X	X		
Eligibility Assessment	X					
Randomization	X					
Intralymphatic Injection (allergenic extract or placebo)		X	X	X		
Pain and discomfort (NRS-11)		X	X	X		
Adverse Event review	X	X	X	X		X
Total Safety Score		X	X	X		
DSS and DMS					X	
Review patient diary data						X
Treatment satisfaction						X
Concomitant Medication Review	X	X	X	X	X	X
Complete Case Report Forms (CRFs)	X	X	X	X	X	X

[a] Vital signs at treatment visits to be performed before injection and every fifteen minutes post-injection for sixty (60) minutes.
[b] Urine pregnancy testing only for women of childbearing potential.
[c] Screening and Treatment Visit 1 may be combined in some circumstances. In such cases, procedures specific to both should be completed.

2 INTRODUCTION

2.1 STUDY RATIONALE

Allergic Rhinitis

More than 40 million people in the United States (approximately 14% of adults and 13% of children) and more than 400 million people worldwide experience allergic rhinitis (AR). AR can substantially reduce patients' quality of life and can impair many aspects of physical and social well-being, including reduced school and work performance. Furthermore, patients with AR experience significant out-of-pocket medication costs and make nearly twice as many visits to health care providers as matched controls. The disease manifestations of AR include both nasal and non-nasal symptoms. The primary nasal symptoms are nasal itching, sneezing, rhinorrhea, and congestion, while the major non-nasal symptoms include itchy watery, red, and/or swollen eyes, earache, headache, cough and irritation of the palate. AR is also an independent risk factor for the development of asthma and it is reported that 38% of American patients with AR have asthma and up to 78% of American asthma patients have AR (Skoner D. 2001). Of clinical concern is the "atopic march" or progression of IgE-mediated sensitivity over time from childhood eczema, to food allergies, allergic rhinitis and conjunctivitis, to asthma. The prevalence of allergies has been rising, and there is a need for increased use of effective disease-modifying treatments.

Allergic rhinitis is clinically differentiated into two related disorders, seasonal AR and perennial AR. The pathophysiology of SAR and PAR are similar in terms of the chemical mediators involved and clinical manifestations, with the differences between the two primarily based on the cause and duration of disease. SAR can be identified by the appearance of symptoms that correlate with periods of pollen exposure. In contrast, PAR symptoms are commonly triggered by indoor aeroallergens and are not usually affected by seasonality. SAR accounts for about 20% of AR cases and PAR for about 40% of cases, with the remainder of patients having a mixed cause (Skoner D, 2001).

In south and central Texas, pollen from the tree *Juniperus ashei*, commonly called Mountain Cedar, is an important cause of SAR. The mountain cedar pollinates heavily during the months of December, January, and February and somewhat less so in November and March, depending on yearly weather conditions. In South central Texas during the winter months, the pollen from the Mountain Cedar is the only pollen present in significant amounts. Further, Mountain Cedar pollen counts are higher than those typically observed with other seasonal pollens, making it an excellent research model with which to evaluate the efficacy of treatments of pollen-induced AR (Ratner et al., 1994).

Allergen Immunotherapy

Although there are many treatments available, including over-the-counter remedies for reduction of symptoms of AR, the World Health Organization currently recognizes allergen immunotherapy (AIT) as the only disease-modifying treatment for AR (Bousquet et al., 1998). Since it was introduced in 1911, AIT has been clinically shown to provide long-term benefits, including symptomatic disease remission and a reduction in allergic disease progression from rhinitis to asthma. Conventional AIT involves a dose-escalation series of thirty (30) to seventy (70) subcutaneous injections of over period of three (3) to five (5) years, leading to relief of allergy symptoms by inducing tolerance to the allergen, reducing or eliminating the natural immune response. However, this regimen presents barriers to patient adherence, including the time commitment required to complete therapy. Thus, fewer than 5% patients with serious AR symptoms that could benefit from immunotherapy receive AIT treatment (Canonica et al., 2011; Canonica and Durham. 2018).

A variety of allergen extracts for aeroallergens and venoms are FDA-approved for immunotherapy based on individualized subcutaneous dosing (subcutaneous immunotherapy; SCIT) determined by the treating

clinician based on patient sensitivity, clinical response, and tolerance to the extract during the early phases of an injection regimen. Pence and colleagues (Pence et al., 1976) first established the safety and effectiveness of SCIT for treatment of SAR caused pollen from the conifer Mountain Cedar in a forty (40) subject, randomized, double-blind, placebo-controlled study. Additional studies have provided detailed immunological assessment of the effects of SCIT for Mountain Cedar allergy

In addition to SCIT, oral immunotherapy (OIT) has been investigated for both aeroallergens and food allergens. In the last several years, these investigations have led to the approval and adoption of sub-lingual immunotherapy (SLIT), in the form of oral tablets. Both SLIT and SCIT provide demonstrated benefits in the form of long-term tolerization to allergens, but both have different short-term safety profiles. SCIT may be less tolerated by patients and parents than SLIT due to the length of the treatment and invasive nature of the injections, side-effects, in the form of acute hypersensitivity are possible, but typically easily controlled in the clinical setting. SLIT, which typically requires up to one year of daily dosing is more palatable but can be associated with the development or worsening of Eosinophilic Esophagitis and has the potential for less obvious and less easily treated hypersensitivity reactions (James and Bernstein, 2017). Both modalities provide the potential for long-term benefit, and evidence supports higher doses lead to better tolerization, but with both SCIT and SLIT, doses are limited because of local and systemic allergic side effects caused by tissue-resident IgE-bearing mast cells and basophils (Calderon et al., 2011). Thus, there remains a need for a method of immunotherapy that can more rapidly induce tolerance.

In 2008, Senti and colleagues published the results of a clinical trial examining an alternative treatment regimen for AIT involving the administration of allergenic extract directly into the lymph nodes. This route is called Intra-lymphatic Immunotherapy (ILIT) and since that seminal clinical trial, several additional randomized, controlled trials of ILIT have been conducted. These trials generally demonstrate more rapid tolerization at lower doses than conventional SCIT but with similar safety and tolerability. Because ILIT offers the potential benefits associated with other types of AIT, but with fewer injects given over a shorter period-of-time, it may provide an alternative treatment modality that enables treatment patients that might otherwise benefit from AIT.

2.2 BACKGROUND

Most allergies are IgE-mediated hypersensitivity reactions. The allergic response is commonly directed against environmental proteins (allergens) and can manifest as a localized reaction within a specific organ system or systemically. These reactions produce to AR, allergic asthma, food allergies, atopic dermatitis and other skin irritation, and anaphylaxis. Although the causes of allergies are complex, the sequence generally begins with the priming of allergen-specific (CD4+) T-helper 2 (TH2) cells, which then results in the production of TH2 cytokines, such as interleukin-4 (IL-4) and interleukin-13 (IL-13), which cause allergen-specific IgE production through down-stream effects by B cells. IgE then circulates and binds to and sensitizes tissue-resident mast cells (MCs) and basophils by binding the high-affinity cell surface receptor for IgE (Larché et al. 2006). Large populations of MCs are found in tissues exposed to the external environment, including the dermis, and the intestinal and airway mucosa. They are frequently located proximal to blood vessels and nerves, which enables MCs to function as a sentinel in early host defense (Marshall JS. 2004). Mature MCs are long-lived in these tissues, while they are not typically detected in blood and are rare in parenchymal tissues, including the lymph nodes (Liu et al., 2013).

The interaction of IgE with high-affinity receptors on MCs by an allergen leads to receptor cross-linking and degranulation. This process releases cellular granules containing histamine, prostaglandins and cysteinyl leukotrienes, chemokines and other cytokines, which together mediate the immediate phase of the allergic reaction. IgE also binds to receptors on the surface of dendritic cells (DCs) and monocytes, as well as CD23 on the surface of B cells (Trapani and Smith, 2002). This process increases the uptake of

allergen by these antigen-presenting cells (APCs) and the subsequent presentation of allergen-derived peptides to specific CD4+ T cells, which drives the late phase of the allergic reaction (Akdis and Akdis, 2014).

Allergen-specific Immunotherapy (AIT) has been used for more than 100 years to induce active immunity to the offending antigen. This process involves the careful introduction of the offending allergen to lymphoid tissue via sub-cutaneous, intradermal, or sublingual administration. Antigen presentation at increasing doses induces immune tolerance through an IgG-mediated response, which includes induction of allergen-specific antibodies (including IgA), production of a functional allergen-specific regulatory T-cell population and is associated with increased production of interleukin-10 (Larché et al., 2006; Nouri-Aria et al., 2005)

It is hypothesized that within the specialized lymph node compartment, there is an interaction between B cells and T cells following the binding of injected allergen to the cell-surface B-cell receptor. Following processing within the B cell, allergen-derived peptides are presented via the Major Histocompatibility Complex (MHC) class II pocket to allergen-specific CD4+ T-helper cells. This B cell induced activation of T cells, enables T-helper cell-licensed class switching and affinity maturation of IgG (Hylander et al. 2016). Cumulatively, these responses are thought to lead to tolerization and the subsequent reduction of allergen-specific IgE-mediated activation of mast cells and basophils (Wilson et al., 2001). However, the ability for AIT to induce a suitable immune response requires frequent, low-dose administration of the allergen over a long period of time.

Physiologically, antigens are concentrated from superficial tissues exposed to the environment via the lymphatic circulation into lymph nodes, which provide an environment that maintains mature, naive lymphocytes, and supports the presentation of antigens to B cells and T cells, after which they migrate to the germinal centers of the lymph nodes, which support the clonal expansion and maturation of T cells and B cells. After maturation, those lymphocytes can return to the circulation and peripheral lymphoid tissues in the skin and mucosa where they can perform their antigen-specific immune surveillance function (Angel et al., 2009).

To explain the lengthy treatment regimen that is required to induce allergen immunotherapeutic responses from SCIT, Kündig and colleagues at the Institute of Experimental Immunology, University Hospital, Zurich proposed a “geographical concept” of immunogenicity, which is briefly summarized as follows.

> *Immune responses are induced in secondary lymphoid organs, where professional antigen-presenting cells present antigenic epitopes to lymphocytes, and where T and B-cell interactions take place. If antigens stay outside organized lymphoid tissues, then even large amounts of antigens can be ignored by the immune system (see Zinkernagel, 2000 and Zinkernagel et al 1997). Therefore, antigen localization is an important regulator of the immune response. Accordingly, direct administration of antigens into lymph nodes should be a more efficient route of immunization (Martinez-Gomez et al. 2009).*

Alternatively, this could be explained as follows, since T cells and B cells can recognize a vast array of antigens through the combinatorial production of receptors during lymphocyte development, an adaptive immune response requires exposure of an antigen to millions of T cells and B cells, before the antigen encounters one of the few (perhaps only) cells that will have, specific, high-affinity receptors for that antigen. The lymphatic system drains antigens into lymph nodes to facilitate this interaction. However, it has been shown that oligopeptides and proteins (and other substances with low *in vivo* stability) are not effectively concentrated in the lymph nodes with less than one one-thousandth of the typical sub-cutaneous injected dose detectable in distal lymphoid organs. This would explain why the relative

potency of conventional AIT to stimulate an adaptive immune response is so low. Following on earlier work that showed greater immune response to various peptide and DNA-based vaccines following Intralymphatic injection in animals, they proposed testing this route of administration (ILIT) using commercially available allergenic extracts.

Mountain Cedar (*Juniperus ashei*) is not a member of the cedar family, rather it is a small, non-sprouting, evergreen tree of the cypress-juniper family with its center of abundance within the Hill Country of central Texas. Mountain Cedar pollinates during the winter months, with the peak season occurring from December to March. The pollen can travel long distances on the strong northern wind fronts prevalent during the Texas's winter season, which may be due in part to the fact that Mountain Cedar pollen has a higher carbohydrate content than many other aeroallergens. Pollen studies have shown that it has been common throughout the region for at least 16,000 years. In that past fifty years, the range and abundance of Mountain Cedar have been increasing, due to both human and natural factors, including the elimination of naturally occurring fires through forestry management, overgrazing, by cattle, soil erosion, drought, and an increase in atmospheric carbon dioxide, among other factors (Biology and Ecology of Ashe Juniper by Smeins and Fuhlendorf).

Mountain Cedar has been clinically recognized as an important seasonal allergen in Texas, Northern Mexico, and New Mexico for nearly 100 years (Ramirez D. 1984). Mountain Cedar pollen remains a major seasonal aeroallergen in south and central Texas with pollen counts often recorded above 10,000 per cubic meter. Despite this difference, mechanistic studies have shown that Mountain Cedar allergies share similar immunological features to other allergen-induced, IgE-mediated atopic diseases (Parker et al., 1989). For patients prone to atopic diseases, it takes relative few seasonal exposures to develop a reaction to Mountain Cedar pollen. Even patients not otherwise disposed to atopic disease may develop sensitivity to Mountain Cedar after repeated seasonal exposure in areas where Mountain Cedar is common (Ramirez D. 1984), making it a nuisance to natives and non-natives alike. Due to the absence of other, confounding outdoor aeroallergens during the winter months, Mountain Cedar SAR has been often used as a model for development of new SAR therapeutics (Ratner et al. 1994).

Although several ILIT trials of outdoor aeroallergens have been conducted, this study would be the first ILIT trial conducted for the treatment SAR caused by MC pollen.

Summary of Previous Intralymphatic Inoculation Animal Studies

There have been numerous animal studies demonstrating improved T cell response to antigens delivered via direct injection into the lymph nodes. In 1983, Juillard and Bubbers reported that of fourteen (14) dogs treated with ILIT for canine atopic dermatitis eight (8) dogs went into complete remission for between two (2) months and up to two (2) years of follow-up. This reflected an initial treatment success rate of 57%. In addition, they found that relapses were successfully treated by further courses of ILIT. In this study, four (4) dogs (28%) had only partial remissions in which lesions improved without complete clearance and two (2) dogs (14%) did not respond.

Sigel and colleagues (Sigel et al., 1985) later reported in Methods in Enzymology that intra-lymphatic inoculation was a viable method to produce significant quantities of targeted IgG antibodies in rabbits. Their objective was not clinical treatment of disease in these animals, but rather a method for antibody production. They demonstrated the viability of intra-lymph nodal inoculation to produce large quantities of targeted IgG antibodies for research purposes.

More recently, Dr. Kündig and colleagues at the Experimental Immunology group at University Hospital, Zurich (Maloy et al., 2001), compared the immunogenicity of direct intra-muscular, intra-dermal, intra-

splenic, and intranodal injections of escalating doses of a naked immunogenic DNA in mice. They found that in mice, immunization with naked DNA by direct injection into a peripheral lymph node enhances lymphocyte responses relative to the other tested injection sites by more than three orders of magnitude.

In 2005, the same group (Johansen et al., 2005) compared the efficiency of different routes of immunization with MHC class I-binding peptide vaccines in a transgenic mouse model. They reported that peptide vaccine injections directly into the inguinal lymph node of mice enhanced cytotoxic CD8+ T cell responses by six orders of magnitude compared to the conventional intradermal and subcutaneous routes of administration and resulted in efficacy in both viral and tumor challenge models.

Building on this work, these authors examined allergen-specific antibody and T-cell responses using ELISA and flow cytometry in response to intralymphatic immunotherapy directed against Hymenoptera (bee) venom and cat fur allergens (Fel d 1 protein) in mice (Martinez-Gomez et al., 2009). In this study, they sought to assess the therapeutic potential of ILIT in separate groups of mice previously sensitized to the allergen of study. The sensitization protocol, using repeated intraperitoneal injections of the offending allergen resulting in positive control in an anaphylaxis model. They first performed biodistribution studies in these mice, which confirmed that injection directly into the lymph node delivered antigen more efficiently to subcutaneous lymph nodes than subcutaneous injection. Following this, they found that direct injection (ILIT) of the bee venom allergen, PLA2, or the cat fur allergen, Fel d 1, directly into inguinal lymph nodes of mice enhanced allergen-specific IgG and T-cell responses by a factor of 100 relative to subcutaneous injection using a 10-fold higher dose. In these studies, ILIT, but not SCIT, stimulated the production of the Th1-dependent subclass IgG2a antibodies (in addition to IgG1), which is associated with improved protection against allergen-induced anaphylaxis. This protective effect of ILIT-induced immune response was confirmed in their mouse-model of allergen-induced anaphylaxis.

In 2010, Dr. Ugur Sahin and colleagues at the Center for Translational Oncology and Immunology in Mainz, Germany reported positive findings using intra-lymphatic injection to improve the immunogenicity of RNA vaccines for anti-cancer immunotherapy (Kreiter et al., 2010). This study demonstrated rapid and profound production of antigen-specific, fully-differentiated CD8+ and CD4+ T cells in response to intra-lymph nodal inoculation. This result was orders of magnitude greater than subcutaneous or traditional intradermal inoculation. They extended this result to a 260-day survival benefit for mice that received intranodal inoculation and followed by a challenge with A20-HA lymphoma cells, versus mice that received subcutaneous or intradermal inoculations prior to the tumor challenge.

In 2016, Kim and colleagues (Kim et al., 2016) reported that intralymphatic injection of an allergenic extract to bacterial flagellin was superior to intranasal and sublingual administration in mice sensitized to flagellin protein in a mouse model of allergic rhinitis. Comparted to other AIT modalities, ILIT reduced symptom scores, reduced eosinophil infiltration in the nasal mucosa, reduced allergen-specific IgE levels, led to greater reductions in circulating cytokines, and a resulted in a relative reduction in local cytokines and chemokines within the nasal mucosa. Further, they found a two-dose ILIT protocol was more effective than a single injection for reduction in symptoms and chemical mediators in a murine model of allergic rhinitis.

Fischer and colleagues sought to examine the efficacy of ILIT as an alternative to SCIT, which is an accepted treatment for canine atopic dermatitis (Fischer et al., 2016). In this study, twenty (20) dogs of various breeds presenting to the dermatology clinic at the University of Zurich Veterinary School, were tested for IgE-mediated atopic dermatitis against common allergens using a standard protocol and commercially available allergenic extracts. Dogs were treated with ILIT using commercially-available, alum-precipitated allergens administered every four (4) weeks for at least three (3) and up to seven (7)

total ultrasound-guided injections directly into the popliteal lymph node. These authors assessed pets for Hill pruritus score, canine atopic dermatitis severity index (CADSI) score, use of rescue medications and adverse reactions over a follow-up duration of twenty-four (24) weeks. Twelve (12) of the canine patients (60%) were classified as responders based on composite clinical results including symptom scores and reduction in rescue medication use. Among responders, some dogs responded within four (4) weeks of treatment initiation (i.e., after one ILIT injection), while all responders showed a clinical response following three (3) injections. Treatments were continued in non-responders up to seven (7) total injections, (median 5.6 injections) without effect. Note that this data supports the three (3) injection ILIT series that has been used in numerous human clinical studies, as summarized below. All dogs were reported to have tolerated the ILIT procedure well and experienced no adverse effects during or after treatment. This study showed efficacy in canines for ILIT across a range of common allergens, including mites, grass pollens, fungi, weeds, and three species of tree pollen.

In addition to reports in mice, rabbits, and dogs, clinical studies of intranodal administration of anti-viral vaccines were reported as safe and effective for equine patients (Landolt et al. 2010) and non-human primates (Klavinskis et al., 1996). Landolt and colleagues note that in equine patients, typical vaccinations are given intramuscularly, but that sometimes this route fails to elicit an immune response, particularly for treatment of allergic diseases (immunotherapy). Furthermore, large amounts of antigenic substance may be required to vaccinate ponies due to their size and that this may not be commercially or logistically feasible. Therefore, they sought to compare a commercial vaccine given intramuscular with a DNA plasmid-based vaccine against a well-characterized strain of equine influenza virus (EIV). They found that ILIT provided similar antibody and T cell responses as both the commercial vaccination given intramuscularly and to a positive EIV exposed control group. In addition, ILIT provided comparable protection to the commercial vaccine in an EIV challenge model.

More recently, there have been veterinary studies comparing ILIT to SCIT for the treatment of equine insect bite hypersensitivity (IBH) caused by biting midges, a type of allergic dermatitis (Jonsdottir et al., 2015, 2016). This study showed safety and tolerability, with elicitation of IgG response and no subsequent reports of IBH after the horses were exported in Europe, where the biting midges are endemic. On balance, the animal data strongly supports ILIT as a safe and effective means to induce IgG and T cell responses with fewer treatments and at lower doses than sub-cutaneous injection across a range of small and large animal species. Several reports of animal studies show corresponding clinical efficacy in both vaccine challenge models and against atopic diseases across a range of allergens.

Summary of Previous ILIT Human Clinical Trials

Intralymphatic administration of immunogenic substances has been used previously, with several human clinical trials of cell-based cancer vaccines delivered via intralymphatic administration conducted in the 1970s. For example, in 1978, Julliard and colleagues at UCLA (Julliard et al., 1978) reported the results of their phase 1 cellular anti-cancer vaccine trial, in which they observed that, “intralymphatic injections of immunogenic materials are relatively safe and free from deleterious or painful side effects.” While they began their study in an in-patient observational setting, they quickly moved to out-patient intranodal injections. This modality remains common in the anti-cancer immunotherapy literature with many reported and ongoing clinical trials. In fact, the oncology literature includes dozens of clinical trials utilizing intranodal injections to deliver antigenic material. For example, in a review and meta-analysis for 200 cancer vaccine studies for renal cell carcinoma and prostate cancer, which included more than a dozen intranodal studies of dendritic cell vaccines, Draube and colleagues concluded that only mild side effects were reported and most adverse effects were local reactions at the injection site (Draube et al. 2011). This literature strongly supports the intranodal route as optimal for induction of immunologic response for cancer vaccines.

Since their initial proposal of the "geographical concept" of AIT response for allergy, the Experimental Immunology group at University Hospital, Zurich and others have conducted more than eight (8) controlled clinical trials of ILIT for atopic disease using commercially-available allergenic extracts. These studies, summarized below, treated more than 225 patients, including more than 175 patients treated with an active allergenic extract by direct injection into an inguinal lymph node.

The first study, led by Dr. Kündig (NCT00470457; Senti et al., 2008), was an open-label trial of 165 patients with grass pollen-induced rhinoconjunctivitis, who were randomized 3:2 to receive either fifty-four (54) subcutaneous injections with pollen extract over three (3) years, a standard SCIT regimen, or three (3) intralymphatic injections over two (2) months. In total, 183 patients with hay fever due to grass pollen allergy were recruited in Switzerland from June to August 2001. Of those, eighteen (18) did not meet the inclusion and exclusion criteria, sixty-six (66) were randomized to ILIT 99 patients were randomized to the conventional SCIT. Fifty-eight (58) ILIT patients started the treatment and received three (3) 0.1-ml injections with 1,000 standardized quality units (SQ-U) of aluminum hydroxide-adsorbed grass pollen extract (Alutard SQ; ALK-Abelló) and fifty-four (54) conventional SCIT patients started the treatment. In the intralymphatic group, 19 patients were lost to follow-up, and 1 patient withdrew consent. In the subcutaneous group, 17 patients were lost to follow-up, 4 patients withdrew consent, and 1 patient became pregnant.

SCIT patients received a cumulate dose of 4,031,540 standardized quality units (SQ-U) versus ILIT patients who received a cumulative allergen dose 3,000 SQ-U. Patients were evaluated after four (4) months, at one (1) year, and at three (3) years post-treatment by nasal provocation, skin prick testing, specific serum IgE measurements, and symptom scores. Three (3) low-dose intralymphatic allergen administrations increased tolerance to nasal provocation with pollen within four (4) months ($p < 0.001$). Tolerance was long lasting and equivalent to that achieved with three (3) years of standard SCIT ($p = 0.291$). ILIT ameliorated hay fever symptoms ($p < 0.001$), reduced skin prick test reactivity ($p < 0.001$), decreased specific serum IgE ($p < 0.001$), caused fewer adverse events than SCIT ($p = 0.001$), enhanced compliance ($p < 0.001$). The authors concluded that, "intralymphatic allergen administration enhanced safety and efficacy of immunotherapy and reduced treatment time from 3 years to 8 weeks."

The authors reported that the intralymphatic injections were not technically not difficult and on patient-assessment measures, reported as less painful than venipuncture ($p = 0.018$) and no more painful than subcutaneous injections. This is to be expected, as there is little sensory innervation of lymph nodes. Furthermore, they reported that the subcutaneous inguinal lymph nodes can be found easily by ultrasound as the nodes are approximately 1.5 cm in length, hypoechoic compared to surrounding tissue, and only a few millimeters under the skin surface, even in obese patients.

Following this strongly encouraging result, additional studies were conducted by this group and others. In 2012, a double-blind, placebo-controlled study of ILIT in twenty-five (25) patients with cat allergies using a modular antigen transport (MAT) vaccine generated from a recombinant major cat dander allergen (Fel d1) fused to a translocation sequence and part of the human invariant chain was reported (Senti et al. 2012). In this study, treatment with a sequence of three (3), low-but-escalating doses of the recombinant MAT protein vaccine as 1, 3, and 10mg of MAT–Fel d 1 or placebo in 0.1 mL. The ILIT procedure was reported as virtually painless and led to few adverse events, with more AEs reported in the placebo group, although the difference was not statistically significant. In addition to a structured report for local and systemic allergic reactions, the authors conducted pulmonary function testing (PFT), ECG monitoring, as well as blood chemistry, hematology, and urinalysis. None of those safety assessments revealed any abnormalities in either group. Following three (3) intranodal injections over two months, nasal tolerance to a cat dander challenge increased by a factor of twenty-five (25) relative to placebo treatment ($p < 0.001$). In addition, this study showed that ILIT with the MAT-Fel d1 fusion protein stimulated regulatory T-cell responses ($p = .026$ versus placebo) and increased cat dander–specific IgG4

antibody levels by 5.66-fold ($p = 0.003$). Their IgG4 result also positively correlated with IL-10 production ($p < 0.001$). In a follow-up report (Freiberger et al., 2016), they found that ILIT with MAT-Fel d1 protein elicited higher levels of total IgG, with a strong increase in cat-specific IgG4, a modest increase in IgG2, and no change to cat-specific IgE through 12 months.

In 2013, Cardell and colleagues (Hylander et al. 2013) reported the results of an open-label pilot study and subsequent, double-blind, placebo-controlled trial of ILIT for patients with aeroallergen-induced AR. In this study, they randomized thirty-six (36) patients with pollen-induced rhinoconjunctivitis to treatment with either active allergen 1,000 SQ-U of grass pollen extract or placebo control via three (3) ILIT injections of 0.1 mL volume. Using a structured assessment tool for local and systemic reactions, they reported that the active ILIT group in this study had more local adverse events than the placebo ILIT control group, primarily in the form of lymph node swelling, redness, and itch, but all reported events were mild and resolved spontaneously. One patient in the active group dropped out following a mild, local reaction (urticaria) at the first injection. They reported a significant improvement in patient-reported symptoms ($p = 0.047$). They did not find a change in patient use of rescue medications but did find that the active ILIT group consisted of two distinct populations of "responders" and "non-responders". The responder group reported significant improvements in symptoms (VAS improvement between 5 and 10 cm), while the non-responder group, reported no improvements (VAS improvement between 0 and 1 cm). There were no patients in the active ILIT in between. Responders reported improvements in nasal symptoms score that was rapid and sustained through the next allergy season. Responders were found to have increased circulating, allergen-specific IgG4, while non-responders and control patients did not.

At that time, Witten et al reported the results of their double-blind, placebo-controlled study of ILIT for grass-pollen induced rhinoconjunctivitis. Briefly, this trial recruited eighty (80) patients, of whom forty-five (45) with grass pollen allergy confirmed by SPT and serum IgE testing were randomized to one of three groups, a six injection ILIT regimen with 10,000 SQ-U/mL standardized grass pollen extract given as a three injection ILIT regimen with 1,000 SQ-U in 0.1 mL, and a three intra-lymphatic injection of placebo in 0.1 mL. The groups were balanced and the prior season's allergy QOL score was used as a control. This study controlled for the week-by-week symptom scores in both seasons relative to the pollen count each week. This was a methodologically strong study with careful conduct and control, however they deviated from previous work in a one key regard.

ILIT dosing was performed every other week, rather than every four weeks. The authors found no significant differences in symptoms or QOL score. This result was discussed extensively in the literature (Hylander et al. 2013; Kündig et al. 2014; Malling et al, 2014) and speculated to be due to the frequency of injections. Kündig and colleagues noted that no FDA–approved vaccines are labeled with less than a 4-week interval between injections and that the Centers for Disease Control and Prevention (CDC) guidelines consider a vaccination ''invalid'' if the time interval is shortened to less than the recommended minimum. In fact, all other studies of ILIT in humans have chosen a 4-week time interval between injections in accordance with the general believe in vaccine immunology that the timing between inoculations is essential to allow phases in which little antigen is present in lymph follicles so that competition for the antigen can positively select high-affinity B cells memory B-cell formation and affinity maturation. Witten and colleagues did however observe significant improvement in chemical mediators, including an increase in IgG4 levels after treatment (for the x6 group: $p = 0.0099$, for the x3 group: $p = 0.0128$). In addition, they found a 2.8-fold increase ($p = 0.0005$) was seen in the x6 group and a 2.9-fold increase was seen in the x3 group for IgG4 levels, and levels of T cell cytokines were significantly lower IFN-gamma level in the x3 group relative to placebo with a tendency toward higher IL-10 levels indicating some modulation of the immune system in the treated patients.

In terms of safety and tolerability, the authors reported that the procedure was generally found tolerable, and only 1 patient withdrew because of discomfort during the examination. All adverse events during the study were mild with no moderate, severe, or life-threatening AEs reported, but most (83%) patients in the active treatment groups experienced some local, injection site discomfort or lymph node swelling. These authors emphasize that additional double-blind, placebo-controlled trials of ILIT are warranted and suggest that in accordance with FDA's Guidance for Industry and the World Allergy Organizations taskforce on AR that a composite symptom and medication score be the basis for efficacy assessments in future studies.

In a small mechanistic study, ("Effect of Intralymphatic Immunotherapy on Basophil Response and Plasma Cell Kinetics in Allergic Patients"; EudraCT 2012-005227-33), Schmid and colleagues (Schmid et al., 2016) reported on the results from a series of three (3) monthly injections of 0.1 ml Alutard Phleum pratense, 10,000 SQ-U/mL (ALK-Abelló) ILIT to patients with rhinoconjunctivitis due to confirmed grass pollen allergy. This study enrolled seven (7) subjects and utilized prior year symptoms scores as the baseline. Despite the small sample size, symptoms scores were reduced after ILIT ($p = 0.0625$). and tolerance to a grass pollen nasal challenge and titrated SPT both increased significantly ($p = 0.031$ and $p < 0.006$). Further, they found the concentration of circulating, allergen specific, non-IgE plasmablasts increased after ILIT, more than doubling in all patients after the first injection, increasing five-fold after the second injection, and doubling again after the third injection. This change in concentration was statistically significant compared with the baseline visit ($p = 0.016$). There was not a corresponding, statistically-significant increase in allergen-specific IgE+ plasmablasts. The authors did not find basophil sensitivity or reactivity changed in response to ILIT. In this study, three patients experienced mild adverse effects. One (1) participant experienced local swelling at the injection site, another had local itching at the injection site, and a third patient had a decrease by 19% in PEF, that quickly normalized after inhalation of beta2-agonist. All side effects were considered mild and occurred after the first ILIT injection.

More recently, Patterson and colleagues (Patterson et al., 2016) reported the first ILIT study in US patients. This study was a randomized, double-blind, placebo-controlled, parallel-group study in American young adults and adolescents aged 15 to 24 years (NCT01982474). This was the first reported study of ILIT in adolescents, although there is now an ongoing pediatric study in Europe (EudraCT 2017-001861-25) using an auto-antigen for treatment of type 1 diabetes mellitus. Fifteen (15) patients were randomized 1:1 to active ILIT or placebo (diluent) in a double-blind manner. In total, seven (7) subjects received active ILIT and eight (8) received placebo. All patients received three (3) escalating doses of 0.1 mL, 0.2 mL, and 0.5 mL, separated by four weeks, of 20,000 PNU / mL aluminum hydroxide adsorbed grass pollen extract or diluent as placebo control. In this study, a total safety score (TSS) based on a structured assessment questionnaire for local and systemic adverse effects was utilized as the primary safety endpoint. Scoring ranged from 0 for no reaction to 4 for a serious systemic reaction. The sum of each injection score was the overall total safety score (TSS) for each participant. All 15 patients completed all three injections. Safety scores between groups were comparable with the mean TSS for active ILIT at 0.6 versus the mean for placebo at 0.4 ($p = 0.80$). Reactions were localized to the injection site and consisted of itching ($n = 2$), wheal ($n = 2$), and tenderness ($n = 5$). All reactions resolved spontaneously without treatment.

Importantly, these researchers concluded that a key clinical finding of their pediatric study was that ILIT is remarkably safe. The highest recorded total safety score was 2 (out of a possible score of 9) and occurred in a patient receiving placebo, underscoring that safety for ILIT is well within acceptable clinical standards for AIT.

Efficacy was a secondary endpoint and was assessed using daily allergy and asthma symptoms to create a daily symptom score (DSS) plus medication use to create a daily medication score (DMS), both of which

were recorded in an electronic diary during the grass pollen season. The total symptom score (TCS) was the primary efficacy endpoint and was a composite of the DSS and DMS for the entire season, following the method of Blaiss and colleagues (Blaiss et al., 2010). This approach is consistent with EAAAI (Pfaar et al., 2014), FDA (Guideline Allergic Rhinitis: Developing Drug Products for Treatment Guidance for Industry, Draft Guidance Feb 2016), and WAO recommendations for use of an integrated efficacy assessment of both symptoms and medication usage (. Participants in the active treatment group reported lower mean TCSs during grass pollen season were lower on 69 of 78 days and during the 2-week peak pollen period, the mean TCS was 16 (in the placebo group and 8 in the active ILIT group ($p = 0.08$, effect size = 0.97). findings suggest that an escalating dose, ILIT regiment with three (3) preseason injections conducted at least four weeks apart may be clinically effective for decreasing symptoms and medication use during grass pollen season.

In 2017, Dr. San Min Lee and colleagues at Gachon University Gil Medical Center reported the results of their clinical trial of ILIT in Korean AR patients. They sought to address a perceived gap in the human clinical trial literature examining ILIT for common indoor aeroallergens from dust mites (*Dermatophagoides farina*, Df, and *Dermatophagoides pteronyssinus*, Dp), and dog allergens. This study included patients with allergic symptoms and positive skin prick and serum IgE tests for any of the four qualifying allergies, both dust mites, dogs, and cats. This was an open-label study in which eligible patients were treated with a series of three ILIT treatments spaced four (4) weeks apart with aqueous extract to the allergen identified with their symptoms and confirmed by positive serum IgE and skin prick testing. They recruited twenty-four AR patients and enrolled a total of thirteen subjects, two of who dropped out after the first ILIT treatment. Eleven (11) subjects completed the treatment and one-year follow-up.

While this was a small, pilot study without a blinded control arm, it nevertheless provides important information. This study included patients with PAR due to sensitivity to multiple indoor aeroallergens, for which they were treated in the same series of ILIT injections and used a formalized dose-escalation/dose de-escalation protocol for standardized extracts based on a starting dose at a 1,000-fold dilution of the maximal SCIT dose for each allergen in a total volume of 0.1 mL. Second, this study included many patients with food allergies and asthma as comorbidities, suggesting a population with more serious atopic disease compared to some other ILIT trials, which focused on patients with SAR to outdoor aeroallergens. Third, this is the first study of ILIT to report cases of anaphylaxis.

In this study, PAR symptoms assessed with the SNOT-20 were alleviated ($p=0.012$) and quality-of-life assessed with the RQLQ was improved ($p = 0.007$) four (4) months after ILIT. These treatment responses were durable at one (1) year after ILIT ($p = 0.047$ and $p = 0.009$, respectively). Patients utilized fewer rescue medications at four (4) months and one (1) year post-treatment ($p = 0.04$). At one (1) year post-treatment, patients' Nasal symptoms to a nasal challenge were reduced. ($p < 0.05$). While these authors assessed IgE and IgG, the results did not show a clear trend for dog and cat allergenic extract treatment due to the small number of patients involved. However, it was noted that both IgE and IgG were somewhat increased in the group treated with Dp allergenic extract. This is consistent with observations that AIT leads to a transient increase in allergen-specific IgE followed by blunting of seasonal increases in IgE and a marked increase in IgG, particularly IgG4 (Wachholz et al., 2003). This study showed strong and sustained efficacy, with only one patient not demonstrating a clinical response on both assessments at month four and one year.

After the first injection, the allergen concentration was escalated 3-fold for the second injection, and 10-fold on the day of the third injection for patients that experienced no or only mild local or systemic hyper-sensitivity reactions. The allergen concentration did not change on the second or third injection if there was a moderate local or systemic reaction. The allergen concentration was decreased by 3- to 1,000-fold

from the previous concentration if there was a severe local or systemic reaction. When 2 or more allergens were injected into the inguinal lymph node, the allergen mixture was produced in a volume of 0.1 mL to preserve the target concentration of each allergen.

This is the first clinical trial of ILIT for AR that reported more than mild adverse events. The authors observed three serious reactions. Two (2) cases of anaphylaxis, one (1) case of a moderate-to-severe local reaction (urticaria) at the injection site following ILIT. These reactions were all in multi-allergen patients treated for dust mite allergies. Subject 5, a 22-year old female with a 20-year history of AR and food allergies, experienced anaphylaxis at the initial dose of 30 units each of the Dp and Df extract. Her second dose of ILIT was at a 1,000-fold dilution, while the third treatment was escalated 3-fold. This patient completed treatment and follow-up without subsequent AEs and with significant symptomatic improvement at the month four and one-year follow-up visits. Subject 6, a 50-year old female with a two-year history of AR and asthma, experienced anaphylaxis at the second ILIT treatment which was an escalation from 30 units of Df and Dp to 100 units of each allergen. At the third ILIT treatment, this subject's dose was reduced to 1 unit of each allergen, which did not elicit any adverse reaction. Subject 6 completed the study without additional adverse events and with symptomatic improvement on both clinical assessments. Subject 11 was a 46-year old female, with a 2-year history of PAR due to dust mites and dog dander. She had a history of urticaria, food allergies, and atopic dermatitis. At her third ILIT treatment, in which she received 300 units each of Df and Dp allergen with a 1:1 dilution of dog allergen, representing the maximal dose a patient in this study was eligible to receive based on prior dose-escalations without event, she experienced urticaria, which was graded as moderate-to-severe. This patient also completed the study without subsequent AEs and experienced symptomatic relief on both clinical assessments, which was sustained at the one-year time point.

In subjects who showed moderate-to-severe systemic reactions (subjects 5, 6, and 11), ILIT using Df and Dp at concentrations that, in SPT, led to allergen to histamine ratios in wheals of more than 1 caused systemic reaction in some but not all patients. These authors suggest that the allergen concentration for ILIT be reduced in hypersensitized patients, consistent with the recommendations by the manufacturer (HollisterStier, New Orleans, USA) of the allergenic extracts that they used. Specifically, they propose that SPTs be performed with serial dilutions of allergens to determine an initial dose of allergen in ILIT not exceed the maximal concentration of allergen that leads to an allergen to histamine ratio in wheals of less than one.

In a separate article, these authors (Kim et al., 2017) propose a mechanism by which this reaction might occur. Specifically, they suggest that some amount of allergen may leak from the internal cortex of the lymph node into the medullary sinus, which includes the vessel-like spaces that the lymph flows through and nodules within the sinuses containing arterioles, which connect to the lymphatic hilum. The hilum of the lymph node is the portion from which the efferent lymphatic vessels exit the concave side of the node. It is also the point at which the vasculature connects to the lymph node. While there is a theoretical case to suggest that allergenic extract delivered via intranodal injection could leak into the bloodstream, Dr. Lee and colleagues used injections for each escalating dose of only 0.1 mL. In contrast, Patterson and colleagues, and others have used larger volumes, including in younger patients, without similar systemic reactions.

Use of Glycerinated versus Aluminum-Hydroxide Precipitated Allergenic Extracts

Among previous ILIT studies, some studies used extracts precipitated with aluminum hydroxide, while others used glycerin. Aluminum hydroxide precipitates must be stored and diluted using phenol-saline with human serum albumin as a stabilizer. The aluminum absorption process reduces the binding of allergens to glass packaging and slows the degradation over time. Other allergenic extracts use glycerin

as stabilizer. At higher concentrations, glycerin is also bacteriostatic. Phenol is used in both types of extract to maintain sterility. Mountain cedar allergens used in this study are available as a glycerinated extract. The following section provides additional background and information about the use of glycerin in allergenic extracts and the appropriateness of the use of glycerinated extracts for intranodal administration.

The normal clinical concern with glycerin in immunotherapy is injection site pain. According to the ACAAI's Allergen Immunotherapy Extract Preparation Physician Instruction Guide, revision January 2017, care is advised when administering a volume greater than 0.2 mL of an extract in 50% glycerin because of the potential discomfort and pain. The rate and mechanism of local reactions across various concentrations of allergenic extract and glycerin were thoroughly investigated in the GILL study (Calabria C, et al., 2008). Although this was for subcutaneous immunotherapy, among the 9,678 subjects receiving immunotherapy injections for treatment of aeroallergens, small local reaction rates increased significantly with increasing allergen concentrations, from 7.3% (1:1000 v/v) to 23.0% (1:1 v/v; $P < .001$), but with not higher glycerin concentrations, up to 50% (v/v). Large local reactions were infrequent and did not significantly increase with allergen or glycerin concentration.

The dose-response relationship between glycerin and injection site pain or discomfort was also studied by Van Metre and colleagues (1996). They concluded that 1) injected glycerin produces pain that is proportional to total injected dose of glycerin and 2) absolute total doses of glycerin of less than 0.05 mL rarely produce clinically important pain, while reactions to higher doses of glycerin were of "trivial clinical importance." Notably, the volume used in the TASC-MC-ILIT-2018 study is 0.1 mL of 5% glycerin, or ten-fold lower than the threshold mentioned by Van Metre and colleagues. Prior intralymphatic immunotherapy studies have used both alum-precipitated and glycerinated extracts, although the final glycerin concentrations were not reported.

As a component of cell membrane phosopholipids and a product of the hydrolysis of lipids, glycerol (also known as glycerin, glycerine, propane-1,2,3-triol, CAS Number 56-81-5) is naturally present at detectable levels in most mammalian tissues. Due to the role of the lymphatic portal system in the absorption and metabolism of dietary fats, glycerin is naturally present in lymph at concentrations higher than many other tissues. For example, in healthy human volunteers, the concentration of glycerin in lymph is more than double the concentration in blood plasma (Nanjee, et al., 2000). Anatomically, lymph nodes themselves are always found within and tightly bound to adipose tissues which is likely due to the important physiological role of fat metabolism by lymphoid tissues. The metabolism of fats by lymphoid tissues provides a direct energy source in the form of both glycerol and free fatty acids, is important for the maturation and proliferation of lymphocytes, and required for the generation of immune signaling molecules. Lymphoid tissues utilize fatty acids through direct hydrolysis of triacylglycerols into non-esterified fatty acids and free glycerol. (Pond & Mattacks, 1995). In culture, a high proportion (60-80%) of glycerol from hydrolysis of fats is taken up by directly lymphocytes (Calder, et al.,1994).

Within the circulatory system itself, glycerin has been used clinically for many years to manage intracranial and intraocular pressure at doses much higher than that used in the TASC-ILIT-MC-2018 study. This use was reviewed by Frank and colleagues (Frank et al., 1981). More recent clinical trials have examined the use of glycerol to control intracranial pressure. For example, Berger and colleagues infused a 15 g dose of glycerin delivered in a 10% (v/v) concentration via a central venous line into patients with large middle cerebral artery infarction (Berger C, Sakowitz O, Kiening K, Schwab S. Neurochemical Monitoring of Glycerol Therapy in Patients with Ischemic Brain Edema. Stroke 2005;36:e4-e6.). This resulted in an increase in glycerol concentration in the CSF from .080 mmol/L—which is also the normal concentration in lymphatic fluid reported by Nanjee and colleagues— up to a peak of .280 mmol/L, without adverse effect. Notably, the concentration in plasma, normally about .040

mmol/L increased to a peak of approximately 1.4 mmol/L during infusion. Because of the rapid metabolism of glycerol in the body, the concentration of glycerol and the reduction in intracranial pressure returned to pre-treatment levels seventy (70) minutes after infusion.

The inguinal lymph nodes selected for injection in this study are about 1.0 cm in diameter, which equates to a volume of about 0.5 mL. The injections in the TASC-ILIT-MC-2018 study are 0.1 mL of 5% (v/v) glycerin, which is visibly retained in the node for a short time immediately after injection. This is a dose of 6.6 mcg and would equate to a theoretical peak concentration of glycerol in the node of about .187 mmol/L or only about 2.3 times the normal concentration in the node. Diffusion into the lymphatic system and metabolism in the node should lead to a rapid return to normal levels of free glycerol. Therefore, the use of glycerinated extracts for intranodal administration is appropriate for this study.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

This study will use commercially-available, FDA-approved allergenic extract for diagnostic testing and commercially-available, FDA-approved allergenic extract for therapeutic use (Mountain Cedar Pollen, ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) with sterile saline with 0.4% phenol as a diluent or placebo control. The risks described in the approved product labels are described in the following section. The package inserts are attached in Appendix 1 and Appendix 2.

Immediate and Short-term Risks

Administration of allergenic extracts for diagnostic testing and AIT has the potential to cause IgE-mediated hypersensitivity reactions in sensitized individuals due to the presence of allergen-specific IgE-bearing mast cells in tissues that are exposed to the environment, such as the dermis and mucosa, where they perform a surveillance function. Physiologic data support the belief that mature, allergen-specific IgE-bearing mast cells are not normally found in the lymph nodes (Liu et al. 2013). Thus, the purpose of this research study is to assess whether intra-lymphatic administration of a commercially-available, FDA-approved allergenic extract for Mountain Cedar allergy is safe and effective. Previous data has shown few and mostly mild reactions following intralymphatic injection of many commercially-available allergenic extracts, with comparable efficacy at much lower doses, but no data for ILIT with allergenic extract of Mountain Cedar pollen has been reported. However, there are significant risks inherent in the administration of any allergenic extract skin testing or immunotherapy.

AIT must only be performed by licensed medical personnel experienced in administering allergenic extracts and trained to provide immediate emergency treatment in the event of a life-threatening reaction. Allergenic extracts may potentially elicit a severe life-threatening systemic reaction, which can rarely result in death (Slater et al., 2012). Patients with unstable asthma or steroid dependent asthmatics and patients with underlying cardiovascular disease are at greater risk to a fatal outcome from a systemic allergic reaction, and thus the treating physician must carefully assess the individual risk and potential benefits for allergen testing and immunotherapy for those patients. This study will exclude patients that, in the opinion of the investigator meet the criteria for increased risk of fatal outcomes outlined in the label information for allergenic extracts. Therefore, emergency measures and personnel trained in their use must be available immediately in the event of such a reaction. The label information indicates that patients should be instructed to recognize adverse reaction symptoms and be observed in the office for at least 30 minutes after skin testing or AIT treatment. This study will include a sixty-minute observation period following ILIT to ensure that delayed hypersensitivity reactions, should they occur, are likely to be

observed in the clinic. Patients will be instructed to contact the physician's office if delayed-onset hypersensitivity symptoms occur.

Invasive medical procedures, including injections may cause some patients to experience pre-procedural anxiety. This may be characterized by feelings of anxiety and related physiologic responses, including increased heart rate, respiration, and/or blood pressure. In most cases, pre-procedural anxiety and the associated symptoms do not require any treatment and resolve spontaneously in less than one hour.

Allergenic extract should be temporarily withheld from patients or the dose adjusted downward if any of the following conditions exist: (1) severe symptoms of rhinitis and/or asthma; (2) infection or flu accompanied by fever; or (3) exposure to excessive amounts of clinically relevant allergen prior to a scheduled injection. Furthermore, immunotherapy should not be initiated during a period of symptoms due to exposure. Since the individual components of the extract are those to which the patient is allergic, and to which he or she will be exposed, typical allergic symptoms may follow shortly after the injection, particularly when the antigen load from exposure plus the injected antigen exceeds the patient's antigen tolerance.

Local Reactions

With diagnostic allergy testing, a positive skin reaction is to be expected in sensitive patients as part of the confirmatory diagnostic process. The positive reaction typically defined as an urticarial wheal with surrounding erythema (resembling somewhat a mosquito bite reaction) larger than the control site. The smallest reaction considered positive is erythema with a central papule at least 5 mm in diameter. In some cases, with no reaction at the control site, erythema can be considered an indication of sensitivity. In general, the size of wheal and erythema response correlates directly with the patient's sensitivity to the allergen. This study will employ histamine as a positive control. Thus, participants in this study can expect to experience at least one urticarial wheal associated with the allergy diagnosis.

For patients receiving AIT injection, some erythema, swelling or pruritus at the site of injection are common, the extent varying with the patient. Such reactions should not be considered significant unless they persist for at least 24 hours. Injections with allergenic extracts in 50% glycerin are known to cause discomfort at the site of the injection.

Local reactions (erythema or swelling) which exceed 4-5 cm in diameter are not only uncomfortable, but also indicate the possibility of a systemic reaction, especially if the dosage is increased. Large, persistent local reactions may be treated by local cold, wet dressings and/or the use of oral antihistamines. They should be considered a warning of possible severe systemic reactions and an indication of the need for temporarily reduced dosages. A mild burning immediately after the injection is to be expected. This usually subsides in 10 to 20 seconds.

Systemic Reactions

With careful attention to dosage and administration, systemic reactions to immunotherapy are infrequent, but it cannot be overemphasized that in sensitive individuals, any injection could result in anaphylactic shock. Therefore, physicians administering allergenic extracts understand and must always be prepared for the treatment of severe reactions. Most severe systemic reactions will begin within a 30-minute period, with few cases having been reported after sixty minutes, but systemic reactions may occur at any time after skin tests or immunotherapy. Symptoms can range from mild to life-threatening (due to anaphylaxis). Other possible systemic reactions which may occur in varying degrees of severity are laryngeal edema, fainting, pallor, bradycardia, hypotension, angioedema, cough, wheezing, conjunctivitis, rhinitis, and urticaria. Adverse reaction frequency data for allergenic extract administration for testing and

treatment show that risk is low. Based on decades of clinical experience with mountain cedar allergenic extract for diagnosis and immunotherapy, anaphylactic reactions to this allergen are comparatively rare, but there were three reported occurrences (including one in the placebo group) in a clinical trial conducted by Fling and colleagues (Fling et al., 1989). That publication provides limited methodologic information and used an aggressive dose-escalation schedule. Other immunotherapy clinical trials with mountain cedar have not reported anaphylactic reactions.

However, if a systemic or anaphylactic reaction does occur, patients will be treated according to the instructions on the label information and the 2015 Joint Practice Parameter Updated for Treatment of Anaphylaxis (Lieberman et al. 2015), as described below. Specifically, adults require an injection of 0.3 mL to 0.5 mL of 1:1,000 epinephrine-hydrochloride intramuscularly or subcutaneously into the lateral thigh (*vastus lateralus* muscle). This should be repeated after five to ten minutes, if necessary. Doses of epinephrine may be repeated as frequently as every 20 minutes thereafter, depending on the severity of the condition and the response of the patient. After administration of epinephrine, profound shock or vasomotor collapse should be treated with volume expanders and vasopressor agents to reverse hypotension. Airway patency should be insured. In case of respiratory obstruction, oxygen should be given by mask and intubation may be necessary. Inhalation bronchodilators and parenteral aminophylline may be required to reverse bronchospasm. Intravenous antihistamine, theophylline and/or adrenal corticosteroids may also be used if necessary after adequate epinephrine and circulatory support has been given. Emergency resuscitation measures and personnel trained in their use must be available immediately in the event of a serious systemic or anaphylactic reaction not responsive to the above measures. Therefore, patients may require cardiopulmonary resuscitation. Rarely are all these measures necessary as epinephrine is usually sufficient to produce a prompt response. However, the physician administering immunotherapy must be prepared in advance for all contingencies. Promptness in the initiation of emergency treatment measures is of utmost importance. Patients experiencing severe reactions may be transferred to a hospital for further treatment. There may be costs to the patient for treatment received in that setting. Severe systemic reactions mandate a decrease of at least 50% in the next dose, followed by cautious increases thereafter. Repeated systemic reactions, even of a mild nature, are sufficient reason for the cessation of further attempts to increase the reaction-causing dose allergenic extracts. Rates of systemic reactions reported in clinical trials of SCIT, SLIT, and ILIT appear similar.

Long-term Risks

Clinical studies of AIT have not demonstrated long-term risks. Long-term risks associated with ILIT are unknown, but no risks have been reported in clinical studies of ILIT with follow-up durations out to three-years (Hylander et al. 2016) while sustained immunologic response has been documented to persist over that time. AIT has been shown in several studies to have long-term benefit in terms of sustained reduction in symptoms and rescue medication for at least three years, and longer-term developmental studies have shown a reduction in the development of allergic asthma over a follow-up period of ten years (Canonica and Durham. 2016).

Treatment Alternatives

AIT is the only proven treatment that can limit the natural course of atopic disease. The alternative to ILIT is treatment with SCIT or treatment with drugs to mitigate allergy symptoms. Previous studies have shown that the SCIT regimen deters many patients that would benefit from AIT. Whether or not a patient participates in this study, they will receive prescriptions for drug treatments to alleviate allergy symptoms.

2.3.2 KNOWN POTENTIAL BENEFITS

Allergenic extracts are indicated for use in diagnosis and immunotherapy of patients presenting symptoms of allergy (hay fever, rhinitis, etc.) to specific environmental allergens. Participation in this study may enable diagnosis of the patient's allergy using SPT.

Multiple studies have demonstrated the effectiveness of AIT for allergic disease, including rhinitis, conjunctivitis, asthma, atopic dermatitis, and stinging insect envenomation for both children and adults. The degree of effectiveness varies for individual patients, but clinical improvements typically occur within or soon after the first year of SCIT treatment, and this benefit may improve with continued treatment. There is no consensus on how long to continue or when to discontinue AIT for patients experiencing clinical benefit but benefits for some individuals are sustained for years after discontinuation of therapy, and indefinitely in others. While SCIT has been demonstrated to induce tolerance, these benefits typically require 30 or more injections over three (3) or more years. SLIT has been shown effective for three products to date, demonstrating benefit following a daily regimen of approximately one year in length. To date, eight (8) clinical trials of ILIT have demonstrated clinical efficacy (symptom scores, medication usage, and/or quality-of-like) or favorable immune changes (increase in allergen-specific IgG, reduced reactivity to skin or respiratory challenge testing) following one to three injections, typically spaced four weeks apart. Several studies have shown sustained efficacy or favorable immune changes for one or more years.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

AIT has been an accepted treatment for allergies for more than 100 years and is the only proven disease-modifying therapy. Diagnosis and treatment of allergies using conventional AIT is rarely associated with serious systemic reactions. Allergists conducting diagnosis or immunotherapy treatment must be highly trained in the use of allergenic extracts and the treatment of potentially life-threatening reactions as part of normal clinical practice (Cox et al., 2011).

Confirmation of allergy to mountain cedar pollen will follow the procedures described in the Prescribing Information and the immunotherapy dosing will be customized based on individual patient sensitivity during confirmatory testing. This is in accordance with the normal, FDA-approved clinical use of mountain cedar allergenic extract for AIT.

This study will involve minimal deviations from the normal conditions of use for AIT. AIT requires that allergists must modify and adjust the dose-titration of allergenic extracts using clinical judgment and patient response on the MQT, in accordance with guidance from the approved label (Appendix 1) and the clinical practice guidelines (Cox et al., 2011), as part of their routine care and management of patients in clinical practice. Adjustments to dosing and related safety precautions, as described above for the management of local and systemic reactions, including the management of potential anaphylactic reactions, will not vary significantly from the procedures described in the approved label for the allergenic extract (Appendix 1) and the current, best practice standard of care as described by the AAAAI Practice Parameter for Anaphylaxis diagnosis and treatment (Lieberman et al., 2015).

The major difference between SCIT and ILIT is the site of injection and the total number of injections. In this study of ILIT, the injection site will be into an inguinal lymph node guided by ultrasonography rather into the outer aspect of the upper arm. Patients will receive up to three (3) injections spaced four weeks apart consistent with vaccine best practices. This contrasts with the 30-70 weekly or semi-weekly injections typically used for SCIT. Thus, this study will involve one-tenth or fewer allergen exposures than traditional immunotherapy and at lower doses. It should also be noted that the cumulative dose of allergenic extract used for ILIT is approximately 1/1,000th of the cumulative dose used for SCIT, but the injections will be reconstituted using the same diluent and injected using the same gauge needle and same

size syringe according to the same clinical and pharmacy SOPs used for SCIT and consistent with the AAAAI Practice Management Resource Guide, 2014 edition.

Based on the eight previous ILIT studies, changing the injection site and reducing the dosage level does not significantly increase the risks or reduce the acceptability of risks associated with allergen immunotherapy. There is sufficient data for other allergenic extracts to believe that exposure to allergens via ILIT has a similar chance of treatment success as SCIT, but given that allergen-specific mast cells are not normally in lymph nodes (as compared to the dermis) and the allergenic extract doses involved are much lower for ILIT than conventional SCIT, the overall risk-to-benefit ratio of this study is likely to be similar to or more favorable than that for allergic patients receiving standard-of-care SCIT treatment. In addition, this trial may support an expedient dosing regimen for mountain cedar AIT that would enable more patients to undertake AIT and experience the clinical benefits of AIT sooner.

Therefore, participation in this study may reduce the patient's allergy symptoms during the 2018-2019 mountain cedar allergy season, and possibly in future allergy seasons. Reduction of symptoms and associated allergy symptom medication including a reduction in out-of-pocket medication cost is a potential benefit to study patients. Participation may also help provide information that will benefit other allergy patients, including those suffering from Mountain Cedar allergies, and possibly others, by improving the understanding of how different treatment regimens with approved allergenic extracts can reduce allergy symptoms. Thus, the overall risk-benefit assessment for this study is favorable.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINT(S)	JUSTIFICATION FOR ENDPOINT
Primary		
To evaluate the safety of ILIT for Mountain Cedar pollinosis relative to placebo control based on the proportion of subjects receiving allergenic extract versus the proportion of subjects receiving placebo that: 1. experience anaphylaxis, or 2. are treated with epinephrine, or 3. experience any other treatment-emergent, serious adverse event (SAE) within 60 minutes of the ILIT procedure.	1. Relative proportion of subjects experiencing a serious, treatment-emergent AE up to 60 minutes after the ILIT procedure.	Risk of anaphylactic reactions is inherent in AIT and dose adjustments are required to continue therapy. This study will evaluate the frequency of these and related serious, treatment-emergent reactions to understand whether the individualized starting dose yields a safety profile for ILIT treatment of mountain cedar allergy that is consistent with other immunotherapies. Rates of anaphylactic reactions following in studies of SCIT and SLIT have been reported at about 1% of patients, which is similar to the frequency across all previous studies of ILIT.
Secondary		
1. To evaluate the efficacy of ILIT relative to placebo during the 2018-2019 Texas Mountain Cedar allergy season using total combined score (TCS), a composite of the daily symptoms score (DSS) and daily medication score (DMS). .	1. Difference in reported average daily total combined score (TCS) between ILIT and placebo groups during the 2018-2019 Mountain Cedar pollen season. Relative number of days during the allergy season that TCS is lower in the ILIT group relative to the placebo group.	The European Academy of Allergy and Clinical Immunology (EAACI; Pfaar et al., 2014) recommends the TCS, a composite of daily symptom score (DSS) and daily medication score (DMS) as the standard primary endpoint for randomized controlled trials in AIT for allergic rhinoconjunctivitis. This endpoint has been used in previous pivotal trials of AIT (for an example see Blaiss et al. 2010).
To evaluate the safety profile of ILIT versus placebo using a standardized scoring system, the total safety score (TSS) for systemic and local AEs of interest	1. Composite safety score of subjects receiving ILIT relative to placebo based on the severity of local and systemic reactions as assessed by	The TSS rates each local and systemic AEI on a scale of 0-4 (0 for no reactions up to 4 for a serious systemic reaction). The AEIs include: lymph node swelling, localized itch, itching

OBJECTIVES	ENDPOINT(S)	JUSTIFICATION FOR ENDPOINT
(AEIs) up to 60 minutes post-procedure	the total safety score (TSS)	at any other location, localized redness, nasal symptoms, pulmonary symptoms (chest congestion or wheezing), urticaria/hives and angioedema, abdominal symptoms (cramping), light-headedness, or swelling of the tongue or throat (see Appendix 3)
To establish the tolerability profile of ILIT for Mountain Cedar pollinosis patients at immediately and 30 minutes post-procedure using patient-reported outcomes	2. Patient reported pain immediately after and 30 minutes post ILIT procedure using the NRS-11 rating scale	The NRS-11 is a widely used and studied instrument for patient-reported pain measures and ranges from 0 (no pain) to 10 (worst imaginable pain), with a mid-point anchor at 5 (moderate pain; see Appendix 3).
To assess the patients' self-reported satisfaction with treatment at the end-of-study.	Patient-reported treatment satisfaction at the end of the study using four Likert-style question, PGI-S and two selected questions modified from the Patient Experience Questionnaire (PEQ), "Are you satisfied with the treatment" and "Would you recommend the treatment to your friends or family?"	Previous studies of ILIT have not reported on the subjective patient experience related to treatment satisfaction. Patient reported outcomes provide valuable information about the patient's perspective on the treatment, symptoms, or other aspects that cannot be readily observed.
To assess the induction of tolerance to the causative allergen by ILIT treatment in an antigen-specific serum IgE test at the end-of-study.	Average relative reduction in allergen-specific IgE baseline to the end-of-study visit between active ILIT and placebo.	An immunological endpoint provides correlative evidence to support that a reduction in symptoms is mediated by a reduction in allergen sensitivity in an IgE-mediated test.
Tertiary/Exploratory		
To assess the effect of ILIT on asthma rescue medication use.	Relative use of rescue inhalers by patients with comorbid asthma between active and placebo groups during the 2018-2019 Mountain Cedar pollen season.	Allergic asthma is a common comorbidity. There is some evidence that AIT may reduce frequency or severity of asthma. As an exploratory assessment, the use of rescue inhalers during allergy season will be evaluated.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This investigator-initiated study will be conducted to evaluate the safety, tolerability and efficacy of an alternative injection site and associated dose and injection regimen for allergen immunotherapy. Specifically, this study will examine a three-dose treatment series of ultrasonography-guided, intra-lymphatic immunotherapy (ILIT) injections over a two-month period prior to the start of the 2018-2019 Mountain Cedar allergy season of a commercially-available, FDA-approved allergenic extract (ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) for the treatment of Mountain Cedar pollinosis versus placebo (diluent phenol-saline, ALK-Abelló, Inc., Port Washington, NY). The hypothesis of this research study is that treatment with three intralymphatic injections of allergenic extract of mountain cedar allergenic extract will be safe and well tolerated relative to placebo treatment and provide superior reduction in allergic rhinoconjunctivitis symptoms and associated medication usage during the 2018-2019 Texas Mountain Cedar pollen season. To minimize bias, the study will use a randomized, double-blind, parallel-group, placebo-controlled design.

Patients will be recruited from any of sixteen (16) clinics associated with the Texan Allergy & Sinus Center in the Central Texas cities of Austin, Dallas, and San Antonio and the surrounding metro areas. Enrolled patients will be treated at three (3) TASC clinics (Austin, TX; Dallas, TX, or San Antonio, TX).

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

AIT is well established for the treatment of atopic disease for a very wide range of allergens through dozens of randomized, double-blind, placebo-controlled clinical trials. To date, most trials have involved subcutaneous or sublingual administration of AIT, although intralymphatic use has been studies as described in section 2.2. These routes of administration involve some established risks due to the presence of allergen-specific mast cells in the dermis and mucosa, although the risk-benefit ratio for SCIT and SLIT is clearly established. This trial aims to establish the safety, tolerability and efficacy of an alternative injection site with associated modifications to the treatment dose and regimen for AIT relative to placebo. Specifically, this trial will assess the intranodal administration of allergen immunotherapy (ILIT) versus placebo injections at the same location. The control group will receive diluent administered intranodally, following the same procedures and in a double-blind manner. Patients in both groups will receive prescriptions for rescue medications or instructions for over-the-counter medications for their AR symptoms, as needed, including oral antihistamines, eye drops, and inhaled short-acting beta agonists. Use of rescue medications for AR and asthma symptoms will be incorporated into the composite efficacy endpoint, the TCS, as described in section 3.0.

4.3 JUSTIFICATION FOR DOSE

As noted in section 2.2, previous clinical trials in humans and animals, as well as mechanistic studies demonstrate that for proteins and peptides, approximately 1/100th or less of the antigen administered subcutaneously enters the distal lymph nodes. Thus, previous studies have typically initiated ILIT at a 1/100th of the indicated or target dose following escalation under a standard SCIT protocol. Previous dosing data has largely been for standardized extracts. For non-standardized allergenic extracts, such as Mountain Cedar, the dose determination procedure used clinically for SCIT is based on individual patient sensitivity testing.

Starting dose for immunotherapy is related directly to a patient's sensitivity as determined by carefully executed skin testing. Degree of sensitivity can be established by determination of D50.11. A general rule is to begin at 1/10 of the dose that produces sum of erythema of 50 mm (approximately a 2+ positive skin test reaction). For example, if a patient exhibits a 2+ intradermal reaction to 1 AU/mL, the first dose should be no higher than 0.05 mL of 0.1 AU/mL. Dosage may be increased by 0.05 mL each time until 0.5 mL is reached, at which time the next 10-fold more concentrated dilution can be used, beginning with 0.05 mL, if no untoward reaction is observed.

4.4 END OF STUDY DEFINITION

Following the end of the 2018-2019 Mountain Cedar pollen season, anticipated to be in March 2019, study subjects will be contacted and asked to return for an End-of-Study assessment visit. A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Events (SoE), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Both male and female adult patients with a history of SAR with bothersome symptoms due to Mountain Cedar pollinosis confirmed by skin prick testing (defined as a wheal greater than or equal to 3 millimeters larger than the diluent control)
2. Patients must be willing to provide written, informed consent
3. Patients must be willing and able to comply with study procedures
4. Women of childbearing potential must agree to use an acceptable form of contraception during the trial, defined as barrier or hormonal methods.

There are no contraindications for patients over age 60 for the diagnostic or therapeutic use of allergenic extracts, but those patients may have reduced responsiveness to SPT and intradermal testing. Since assessment of IgE-response as a mediator of treatment effect is an objective of this study, patients over age 60 will be included only if they have a qualifying diagnostic result from the MQT.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Patients less than 18 years of age
2. Clinically-significant chronic sinusitis, as determined by the investigator
3. History of systemic allergic reaction, e.g. anaphylaxis with cardiorespiratory symptoms, generalized urticaria, or severe facial angioedema.
4. Participation in another clinical trial or use of an experimental medication within 30 days of enrollment
5. Medically significant co-morbidities that, in the opinion of the investigator, place the subject at increased risk during the study or may confound the interpretation of results, including but not limited to:
    a. Autoimmune diseases, other than AR, allergic asthma, eczema and food sensitivities

   b. Pulmonary or respiratory diseases other than stable asthma
   c. Cancer other than basal cell carcinoma
   d. Coronary artery disease or hypertension treated with beta-blockers
   e. Clinically significant impairment of renal or hepatic function
   f. Clinically-significant lab abnormalities
6. Use of concomitant medications that may reduce the effectiveness of rescue treatments for anaphylaxis (e.g. beta-blockers) or alter the immune response to AIT (e.g., immunosuppressants, systemic corticosteroids)
7. Previously completed SCIT or SLIT for Mountain Cedar pollinosis
8. Inability to access suitable lymph nodes for ILIT injections
9. Plans to leave the area for a significant period of the upcoming Mountain Cedar pollen season
10. Pregnant women, women who plan to become pregnant during the study and nursing mothers

Patients that display hypersensitivity, defined in Section 5.2 as a ratio of wheal size greater than 1.0 for the allergen relative to histamine control during the MQT, will be allowed to enroll as these patients are highly likely to benefit from AIT. The starting dose for hypersensitive patients is reduced following the procedure outlined in Section 6.2. Only patients that experience or have a prior history of anaphylaxis to with cardiorespiratory symptoms, generalized urticaria, or severe facial angioedema will be excluded. This study is intended to be equitable in the opportunity for potential patients. Exclusion criteria are determined based on ensuring subject safety and avoiding confounding elements, such as previous experience with AIT with Mountain Cedar allergenic extracts. Because published data on the dosing and use of ILIT for pediatric patients is limited to one study, patients under the age of 18 will be excluded from this study. Since AIT is indicated for pediatric populations, future studies may examine ILIT in pediatric groups, but Mountain Cedar pollinosis generally develops later and the incidence peaks in adulthood, supporting the initial testing of ILIT with Mountain Cedar allergenic extract in an adult population. According to the approved label, controlled studies of hypo-sensitization with moderate to high doses of allergenic extracts during conception and all trimesters of pregnancy have failed to demonstrate any risk to the fetus or to the mother. However, based on histamine's known ability to contract uterine muscle, the release of significant amounts of histamine from allergen exposure or hypo-sensitization overdose should be avoided on theoretical grounds. Therefore, pregnant patients and those that become or intend to become pregnant during the study will be excluded.

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to potential queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not initially meet the criteria for participation in this trial (screen failure) may be rescreened, if the investigator reasonably believes that the criteria that was not met could change during the enrollment period. For clarity in record-keeping, rescreened participants will be assigned the same participant number as for the initial screening.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Up to sixteen (16) centers in the United States that are affiliated with TASC will recruit patients. Interested and eligible patients will be enrolled and treated at one of three (3) TASC-affiliated clinics in

San Antonio, Dallas, and Austin, Texas. Because the target sample size is thirty-two (32), each treatment site is anticipated to enroll approximately 11 subjects.

TASC uses standard procedures to recruit allergy patients using a variety of tools, including social media, on-line advertising using Google AdWords, television and radio advertisements, and physician referrals. Because allergy patients often do not know which allergen causes their symptoms, advertisements and outreach efforts are not targeted to Mountain Cedar or other allergens specifically. At presentation, patients with suspected allergies undergo a series of diagnostic procedures, including sensitivity testing that covers up to 58 common seasonal and environmental allergens in accordance with standard-of-care medical practices. For this research study, subjects will be recruited from among allergy patients presenting at TASC clinics that test positive for an allergy to Mountain Cedar pollen. Study-specific flyers or tri-fold handouts may be provided, but any study-specific recruitment tools or advertising will be subject to IRB review and approval prior to use.

Subject retention for this study will be managed using TASC's standard appointment reminder system, currently a software platform called Solution Reach. Dairy notifications will be sent through the HIPAA-compliant patient dairy feature within the EDC system.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The intervention in this study will be a series of three (3) injections (0.1 mL) of a commercially-available, FDA-approved allergenic extract (Mountain Cedar Pollen, ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) or diluent as placebo control (sterile saline solution containing 0.4% phenol as a preservative; ALK-Abelló, Inc., Port Washington, NY) given every four weeks prior to the start of the 2018-2019 mountain cedar allergy season into a superficial inguinal lymph node through guidance via ultrasonography using a 1-mL hypodermic syringe with a 25-gauge or smaller needle. The approved product label is included in Appendix 1.

6.1.2 DOSING AND ADMINISTRATION

According to the approved label, subcutaneous and deep subcutaneous routes of administration for allergenic extracts have been proven to be safe. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Study participants will have the injection site prepared for aseptic injection, of study treatment into a superficial inguinal lymph node on the right side using ultrasonography. Injections may be made into the left-side at the discretion of the patient or investigator, and if so, should be noted in the chart and CRF. The selected lymph node will be identified and marked on a sonogram to be retained in the subject's record for reference at subsequent injections. Subsequent injections will be performed into the same lymph node, if possible. If for any reason, the same node is not injected, that information will be recorded in the chart and CRF.

Allergenic extracts should not be injected intravenously, therefore aspiration of the syringe will be performed to avoid inadvertent intravascular administration. Before each injection, after inserting the needle into the selected lymph node, but before injecting the dose, the investigator will pull the plunger of

the syringe slightly. If blood returns in the syringe, the syringe and its contents will be discarded. A new lymph node will be selected, and a new syringe prepared. The injection procedure described above will be followed. After each injection, subjects will be closely monitored for one hour with vital signs checked at fifteen (15) minute intervals, and adverse events, if any, recorded using the TSS worksheet (Appendix 3). Patients that experience a treatment-emergent adverse reaction to the extract will be monitored in the office until they are stable.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Commercially available, FDA-approved allergenic extract and diluent will be procured from the manufacturer according in accordance with TASC's Standard Operating Procedures (SOPs). Treatment sets are provided in three and four vial sets in serial dilutions prepared for therapy. Maintenance vials are in five (5) mL and ten (10) mL vials, while concentrates are provided in multiple dose vials in 10 mL and 50 mL vials with potency expressed in PNU/mL (up to and including 100,000 PNU/mL) or W/V (up to and including 1:10 W/V), aqueous or in 50% glycerin, to be diluted prior to use at a ratio of 1:10 weight to volume (W/V). Sterile Diluent for Allergenic Extracts (Phenol Saline) is supplied in vials of 4.5 mL, 9.0 mL, 30 mL and 100 mL. Standard aseptic precautions will be used when making dilutions.

Dilution to 1/100th the maximal SCIT dose will be performed in pharmacy by an unblinded pharmacist who will prepare three vials, one for each clinic location, to be distributed to and stored on-site at the clinic pharmacies at the Texan Allergy & Sinus Center locations in Austin, Dallas, and San Antonio, Texas. Extracts for this study will be prepared at a 1/100th concentration of active allergenic extract with diluent and another three vials of diluent with a matched concentration of glycerin. After visually confirming that the active group and placebo group are visually identical, the lots of investigational product will be labeled and securely stored under the control of the unblinded pharmacist only. Once patients are enrolled, the unblinded pharmacist will request the treatment assignment via the EDC system and prepare a patient-specific vial with the correct active or control solution. The vial will be labeled for the individual patient's use according to the clinic and pharmacy SOPs for preparation and storage of allergenic extracts. Patient-assigned vials will be retained in the pharmacy in secure storage, separate from the unblinded solutions for the duration of the study according to the TASC's SOPs for storage of AIT vials and consistent with the AAAAI Practice Management Resource Guide, 2014 edition, Allergen Immunotherapy Extract Preparation Manual. Records of dilution, mixing and administration of extracts will be maintained in accordance with the clinic and pharmacy SOPs. Subsequent dilutions prepared according to the patient-specific dosing regimen in section 6.2 will be made by the investigator (blind to the actual contents of the vial) immediate before performing the injection procedure.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

This study will use commercially-available, FDA-approved allergenic extract of Mountain Cedar pollen manufactured and purchased directly from ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256. Sterile therapeutic extracts are supplied in either Phenol Saline Diluent or in Diluent containing Glycerin 50% (v/v) for subcutaneous injection. Inactive ingredients may include: Sodium Chloride for isotonicity, Glycerin, and Sodium Bicarbonate as buffering agents. These products are compounded and diluted on a w/v or PNU basis. Pollens are individually extracted from pure pollen extracted in a phenol-preserved sodium bicarbonate solution. Pollen extracts are filtered aseptically and, after final packaging, they are tested for sterility and safety.

The bulk sterile extracts used for this study are allergenic extract of Mountain Cedar (ALK-Abelló, Inc., Port Washington, NY 11050; US Government License No. 1256) Lot #0003252197, expiration date 10/10/2021 containing 0.4% phenol and 50% glycerin supplied at a 1:20 w/v ratio. The phenol saline diluent is also manufactured by ALK-Abelló, Inc., and contains 0.9% sodium chloride, 0.4% phenol (preservative) and water for injection. The lot number is LOT # L 2090617, with an expiration date of 09/2020. The glycerin stock used was 50% (v/v) also manufactured by ALK, Lot # L 2112217, expiration date of 11/2023. Accordingly, the expiration date of mixtures prepared with these stocks should be no later than the earliest expiration of the components used, namely 09/2020, however, as noted in the following section, individually-labelled patient vials of diluted extracts have a shorter stability period.

6.2.3 PRODUCT STORAGE AND STABILITY

Once a vial is assigned to a patient, it is labeled specifically for the use of that patient and stored in accordance with TASC's SOPs and the AAAAI Practice Management Resource Guide, 2014 edition, Allergen Immunotherapy Extract Preparation Manual. To maintain stability of allergenic extracts, proper storage conditions are essential. Bulk concentrates and diluted extracts are to be stored at 2° to 8° C even during use. Bulk or diluted extracts are not to be frozen. Potency of stored extracts declines over time and extracts are not to be used after the expiration date shown on the vial label. However, due to the short duration of treatment in this study, treatment with prepared investigational materials (allergenic extract or matched placebo) will occur before the lots used will expire.

As noted above in section 6.2.2, the stability of diluted extracts is less than that for the concentrated products and based on guidance from the manufacturer, the expiration of extracts prepared for patients in this study is considered to be March 28th, 2019. Photographs of the labels and complete records are maintained on site in the clinic pharmacy.

This preparation procedure below (Section 6.2.4) yields a final concentration of 5% (w/v) glycerin and 0.35% phenol. The stability of diluted extracts prepared for individually-labeled patient vials of immunotherapy has been studied in published literature provided by the manufacturer, ALK-Abelló, Inc. The guidance from the manufacturer is that 1:100 diluted allergenic extract of mountain cedar prepared with glycerinated phenol-saline is stable for up to six months at 4° C, which would give an expiration date of March 28th, 2019 for the vials used in this study. ALK has provided the following published studies for reference: Plunkett, 2008; Nida et al., 2016; and, Plunkett, 2016. Note that this stability guidance is also consistent with the ACAAI's Allergen Immunotherapy Extract Preparation Physician Instruction Guide, revision January 2017.

6.2.4 PREPARATION

Preparation of syringes and, if necessary patient-specific extract dilutions will follow TASC's SOPs and the AAAAI Practice Management Resource Guide, 2014 edition, Allergen Immunotherapy Extract Preparation Manual. Specifically, bulk extracts will be diluted at the investigational drug pharmacy at a standard 1:100th dilution using Sterile Diluent for Allergenic Extracts. Dilutions will be made with sterile disposable syringes using aseptic technique using two sequential, 10-fold dilution processes to achieve the desired concentration for initiation and continuation of immunotherapy. For example, transferring 0.5 mL of a 10,000 PNU/mL extract into 4.5 mL of diluent will yield 5 mL of extract at 1,000 PNU/mL. For weight volume products, a 1:100 w/v dilution may be prepared from a 1:10 w/v by transferring 0.5 mL of the 1:10 w/v to 4.5 mL of diluent. Prepare as many additional serial dilutions as necessary to reach the appropriate concentration.

For patients that display a hypersensitivity reaction to the extract, as defined in section 5.2 (a wheal for allergenic extract greater than that for the positive control), the initial dose will be at an additional 1:10 dilution. For patients that experience a systemic reaction to any ILIT injection, as defined in section 2.3.2, subsequent ILIT injections will be made at a 1:10 dilution from the preceding treatment. Thus, the dosing will be as follows:

Baseline	Not Hypersensitive to Allergenic Extract on MQT				IS HYPERSENSITIVE to Allergenic Extract on MQT			
Injection 1	Inject 0.1 mL of undiluted IP				Inject 0.1 mL of **1:10** diluted IP			
Injection 2	No Systemic Reaction at Injection 1 Inject 0.1 mL of undiluted IP		**SYSTEMIC REACTION** at Injection 1 Inject 0.1 mL of **1:10** diluted IP		No Systemic Reaction at Injection 1 Inject 0.1 mL of **1:10** diluted IP		**SYSTEMIC REACTION** at Injection 1 Inject 0.1 mL of **1:100** diluted IP	
Injection 3	No Systemic Reaction at Injection 2 Inject 0.1 mL of undiluted IP	**SYSTEMIC REACTION** at Injection 2 Inject 0.1 mL of 1:10 diluted IP	No Systemic Reaction at Injection 2 Inject 0.1 mL of 1:10 diluted IP	**SYSTEMIC REACTION** at Injection 2 Inject 0.1 mL of **1:100** diluted IP	No Systemic Reaction at Injection 2 Inject 0.1 mL of **1:10** diluted IP	**SYSTEMIC REACTION** at Injection 2 Inject 0.1 mL of **1:10** diluted IP	No Systemic Reaction at Injection 2 Inject 0.1 mL of **1:100** diluted IP	**SYSTEMIC REACTION** at Injection 2 Inject 0.1 mL of **1:1,000** diluted IP

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Once enrollment, subjects in this study will be randomized in a 1:1 ratio to receive either ILIT with 1:100 diluted Mountain Cedar pollen allergenic extract or diluent as placebo control. Preparation of the diluted allergenic extract or placebo will be performed by the pharmacy according to the procedures defined in Section 6.2. Neither the patient nor the investigator will know the treatment assignment. Randomization information will be stored in the EDC system. Emergency unblinding is unlikely to be required. Patients receiving AIT are at risk of systemic hypersensitivity reactions and such reactions will be treated according to the procedures outlined in Section 2.3.1 regardless of treatment assignment. Blinding may be imperfect in cases where strong systemic reactions occur, but in previous trials of AIT, injection site reactions and systemic reactions, including anaphylaxis have been reported to occur in both groups, but at a lower rate in the placebo group. Thus, this risk of unblinding due to obvious treatment-related side effects is possible, but not definitive. Dose adjustments will be made by serial dilution following the procedures outlined in Section 6.2 and do not require knowledge of the treatment assignment. The end-of-study assessment will include a question for subjects regarding their belief about which treatment they received. This will be used to assess the maintenance of the study blind.

6.4 STUDY INTERVENTION COMPLIANCE

Administration of active immunotherapy or placebo by intranodal injections will be performed by an investigator and the procedural details will be entered into the subject's electronic Case Report Form (CRF). Randomized subjects that do not receive their first intranodal injection will be categorized as

screen failures in accordance with exclusion criterion nine (9). Subjects will receive symptom and medication diary questions via text message or email directly from the electronic data capture system. Subject will be instructed to complete these questions. Patients that repeatedly fail to complete dairy questions will receive a phone call from the study site to assess the subject's status and encourage completion of the diary questions. Patient that receive intralymphatic injections will not be withdrawn for non-compliance with diary procedures, but patient non-compliance will be analyzed and reported as the percentage of days during the allergy season that they responded to at least one diary question divided by the number of days during the 2018-2019 Mountain Cedar pollen season that the patient received an electronic diary question, notification. Adjustments to the study analysis population datasets for non-compliance and the handling of missing data are described in section 9.3, Analysis Populations, and 9.4, Statistical Analysis.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications and over-the-counter medications other than the prescribed rescue medications noted below, which will be captured in the subject diary on the daily total medication score (DMS) questionnaire. Supplements will not be considered concomitant therapy for the purposes of this study.

6.5.1 RESCUE MEDICINE

At enrollment, patients will be provided rescue medications to treat their AR symptoms, including an oral antihistamine (Zyrtec), nasal glucocorticosteroid spray (Flonase), and a prescription for eye drops (olopatadine). Study subjects will be instructed to administer oral antihistamines, eye drops, and a nasal glucocorticosteroid spray as needed, according to the recommendation of the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines and record the use of these medications in their daily total medication score diary. Use of rescue medications for treatment of AR symptoms will be a component of the primary efficacy outcome, the daily TCS.

As noted in section 2.3.2, subjects in this study may receive concomitant treatments as rescue medications for systemic or anaphylactic reactions. In the event any or all of these rescue medications are required, they will be provided by the investigator. These treatments include, but are not limited to epinephrine-hydrochloride, volume expanders, vasopressor, oxygen, inhalation bronchodilators, aminophylline, oral or intramuscular antihistamines, theophylline, and adrenal corticosteroids. Use of rescue medications for treatment of systemic reactions will be recorded in the CRF and the use of epinephrine will be a component of the primary safety outcome.

In the event that study subjects require transport to an Emergency Room facility for ongoing treatment emergencies, such transportation, and therapy and treatments received outside the premises of TASC will not be provided by TASC and the payment for such treatments will be the responsibility of the patient.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Participants may choose to discontinue ILIT treatment at any time without prejudice. Discontinuing participation in the study will not preclude patients from continuing alternate allergy treatments. Patients may choose to continue as a patient of TASC or seek treatment elsewhere at their own expense. Discontinuation of ILIT treatment does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol to ensure patient follow-up through the end of the allergy season. Reasonable efforts will be made to contact the patient to encourage orderly completion of study discontinuation procedures. If a clinically significant finding is identified (including, but not limited to new medical conditions, adverse effects, or worsening of symptoms) after enrollment, the investigator or qualified designee will determine if any change in clinical patient management is needed. New clinically relevant finding will be reported as an adverse event (AE). For patients that discontinue the study prior to the scheduled end-of-study visit, the reason for discontinuation will be collected.

The data to be collected at the time of discontinuation will include the following:

- Adverse events, concomitant medications, reasons for discontinuation, vital signs

ILIT treatment of subjects may be discontinued at any time by the investigator any at any time the investigator determine that it is clinically a clinical determination that it is in the interest of the patient to discontinue treatment.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. The investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study non-compliance either an inability or unwillingness to complete the study treatments or patient diary
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant will be leaving the central Texas area for an extended period during the allergy season
- If the sponsor of this research, TASC, chooses to discontinue the study for any reason.

The reason for participant discontinuation or withdrawal from the study will be recorded on in the CRF. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for more than one scheduled visit and is unable to be contacted by the TASC personnel using reasonable efforts.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within one week and counsel the participant on the importance of maintaining the assigned visit schedule during the treatment period and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three (3) telephone calls and, if necessary, a certified letter to the participant's last known mailing address.
- These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The following procedures will be used to evaluate the efficacy of ILIT:

- **Demographics and Medical History** will be collected to assess sex, age, concomitant asthma, or other co-variates that may moderate treatment effect
- **Special assays** include the MQT, to confirm sensitivity to Mountain Cedar pollen, assess the relative sensitivity to determine whether or not a dose-reduction is required, and serum IgE testing for immunologic response at the end of study as a mechanistic confirmation of treatment response. The MQT requires skin prick testing and intradermal testing and will be performed in accordance with the manufacturer's instructions (Appendix 2) and routine clinical practice.
- **Administration of questionnaires or other instruments** for patient-reported outcomes will include an e-diary for assessment of daily symptoms score (DSS) and daily medication score (DMS) use during the allergy season to calculate a daily total combined score (TCS). These assessments are recommended by the EAACI as the optimal integrated measure of for efficacy measurement of immunotherapy for rhinoconjunctivitis (Pfaar et al. 2013).

Certain diagnostic information for this study and other baseline information, including medical history and concomitant medications and MQT required for subject screening may come from the patient's existing medical chart or the results of diagnostic tests performed as part of an individual's regular medical care. Such information, when collected following the patient's enrollment into this clinical trial will be done in accordance with existing TASC policies and procedures for compliance with the Health Insurance Portability and Accountability Act (HIPAA) rules, Texas state laws, as applicable.

8.1.1 Visit Schedule and Assessments

The Trial Flowchart, Schema, and Schedule of Events table are found in Section 1. All study procedures will be performed by the investigator or a suitably qualified designee.

8.1.1.1 Visit 1 (Screening)

The following procedures will be performed:

- Review the study and procedures, provide the patient with the opportunity to review the consent form ask questions, and have their questions answered.
- Obtain written informed consent and document in the subject's chart
- Collect patient demographics, medical history including concomitant medications

  - For women of childbearing potential, confirm adequacy of birth control methods and document in the subject's medical record
- Perform standard physical exam
- Perform MQT
- Order allergen specific serum IgE testing
- Collect vital signs, including heart rate, blood pressure, and respiration
- Confirm patient eligibility by assessing whether the patient satisfies the study inclusion and exclusion criteria
- Prescribe symptomatic treatment / rescue medications including an intranasal corticosteroid and oral antihistamine
- Randomize patient using the on-line EDC system and label the assigned vial for individual patient use
- Complete entry of patient information into the EDC system (eCRF)

8.1.1.2 Visit 2 (Treatment Visit 1)

The following procedures will be performed:

- For women of childbearing potential, perform a urine pregnancy test to confirm continued eligibility for the study
- Collect vital signs
- Review ILIT procedure, allow patient to ask and have questions answered
- Perform ILIT procedure following the procedures outlined in detail in section 6.1.2:
  - Identify and select a right-sided superficial inguinal lymph node using ultrasonography
  - Prepare the area for aseptic injection
  - Verify assignment of the subject's assigned vial of allergenic extract or matched placebo
  - Inspect vial for clarity or discoloration
  - For hypersensitive patients, prepare a 1:10 dilution using aseptic procedures from the assigned vial and standard Allergenic Extract diluent (phenol-saline), as described in Section 6.2
  - Draw 0.1 mL of allergenic extract from the vial or the 1:10 dilution, as appropriate using a 1 mL syringe.
  - Tighten the patient's skin around the lymph node using thumb, index finger, and middle finger to pull skin taunt and allow for controlled placement of the injection volume.
  - Using a sterile 25-gauge or smaller needle, approach the lymph node with the needle at a 90° angle to the skin
  - Insert the needle into the selected lymph node and aspirate the syringe by pulling the plunger slightly to avoid accidental intravenous administration
    - If blood is drawn up, discard and replace the syringe and extract, prepare a new syringe and repeat
  - Slowly inject into the entire volume into the lymph node and during injection, observe the lymph node volume through the skin to confirm injection by visible enlargement.
  - Administer a sterile dressing, if necessary
  - Record injection information in the patient's medical chart

- Collect vital signs every fifteen minutes for one hour (60 minutes) post-injection
- Collect patient-reported pain immediately after and 30 minutes post-injection using the NRS-11
- Record any local or systemic reactions on the TSS worksheet
    - Patients that are stable and do not have any evidence of a systemic reaction will be monitored in the office for sixty (60) minutes post-injection
    - Patients that experience a systemic reaction or other serious, treatment-emergent adverse event will remain in the office for observation until stable
- Administer rescue medications, if necessary, according to the procedures in section 2.3.1
- Schedule the next visit
- Complete entry of patient information into the EDC system (eCRF)

8.1.1.3 Visit 3 (Treatment Visit 2)

The following procedures will be performed:

- For women of childbearing potential, perform a urine pregnancy test to confirm continued eligibility for the study
- Collect vital signs
- Assess patient for any changes to medical history, including adverse events since the previous visit, resolution of ongoing adverse events from Visit 1, or changes in concomitant medication use
- Perform ILIT procedure following the procedures outlined in detail in section 6.1.2:
    - Identify and select a right-sided superficial inguinal lymph node using ultrasonography
    - Prepare the area for aseptic injection
    - Verify assignment of the subject's assigned vial of allergenic extract or matched placebo
    - Inspect vial for clarity or discoloration
    - For patients that experienced a reaction at a prior injection, prepare a 1:10 or 1:100 dilution using aseptic procedures from the assigned vial and standard Allergenic Extract diluent (phenol-saline), as described in Section 6.2
    - Draw 0.1 mL of allergenic extract from the vial or the 1:10 dilution, as appropriate using a 1 mL syringe.
    - Tighten the patient's skin around the lymph node using thumb, index finger, and middle finger to pull skin taunt and allow for controlled placement of the injection volume.
    - Using a sterile 25-gauge or smaller needle, approach the lymph node with the needle at a 90° angle to the skin
    - Insert the needle into the selected lymph node and aspirate the syringe by pulling the plunger slightly to avoid accidental intravenous administration
        - If blood is drawn up, discard and replace the syringe and extract, prepare a new syringe and repeat
    - Slowly inject into the entire volume into the lymph node and during injection, observe the lymph node volume through the skin to confirm injection by visible enlargement.
    - Administer a sterile dressing, if necessary
    - Record injection information in the patient's medical chart
- Collect vital signs every fifteen minutes for one hour (60 minutes) post-injection

- Collect patient-reported pain immediate after and 30 minutes post-injection using the NRS-11
- Record any local or systemic reactions on the TSS worksheet
    - Patients that are stable and do not have any evidence of a systemic reaction will be monitored in the office for sixty (60) minutes post-injection
    - Patients that experience a systemic reaction or other serious, treatment-emergent adverse event will remain in the office for observation until stable
- Administer rescue medications, if necessary, according to the procedures in section 2.3.1
- Schedule the next visit
- Complete entry of patient information into the EDC system (eCRF)

8.1.1.4 Visit 4 (Treatment Visit 3)

The following procedures will be performed:

- For women of childbearing potential, perform a urine pregnancy test to confirm continued eligibility for the study
- Collect vital signs
- Assess patient for any changes to medical history, including adverse events since the previous visit, resolution of ongoing adverse events from Visit 2, or changes in concomitant medication use
- Perform ILIT procedure following the procedures outlined in detail in section 6.1.2:
    - Identify and select a right-sided superficial inguinal lymph node using ultrasonography
    - Prepare the area for aseptic injection
    - Verify assignment of the subject's assigned vial of allergenic extract or matched placebo
    - Inspect vial for clarity or discoloration
    - For patients that experienced a reaction at a prior injection, prepare a 1:10 or 1:100 dilution using aseptic procedures from the assigned vial and standard Allergenic Extract diluent (phenol-saline), as described in Section 6.2
    - Draw 0.1 mL of allergenic extract from the vial or the 1:10 dilution, as appropriate using a 1 mL syringe.
    - Tighten the patient's skin around the lymph node using thumb, index finger, and middle finger to pull skin taunt and allow for controlled placement of the injection volume.
    - Using a sterile 25-gauge or smaller needle, approach the lymph node with the needle at a 90° angle to the skin
    - Insert the needle into the selected lymph node and aspirate the syringe by pulling the plunger slightly to avoid accidental intravenous administration
        - If blood is drawn up, discard and replace the syringe and extract, prepare a new syringe and repeat
    - Slowly inject into the entire volume into the lymph node and during injection, observe the lymph node volume through the skin to confirm injection by visible enlargement.
    - Administer a sterile dressing, if necessary
    - Record injection information in the patient's medical chart
- Collect vital signs every fifteen minutes for one hour (60 minutes) post-injection
- Collect patient-reported pain immediate after and 30 minutes post-injection using the NRS-11

- Record any local or systemic reactions on the TSS worksheet
    - Patients that are stable and do not have any evidence of a systemic reaction will be monitored in the office for sixty (60) minutes post-injection
    - Patients that experience a systemic reaction or other serious, treatment-emergent adverse event will remain in the office for observation until stable
- Administer rescue medications, if necessary, according to the procedures in section 2.3.1
- Instruct patient on completion of daily symptom and medication diary, reconfirm contact information for e-diary distribution
- Complete entry of patient information into the EDC system (eCRF)

8.1.1.5 Patient Diary

During the allergy season, patients will receive a daily diary with a series of numeric questions to be answered electronically for measurement of the daily symptom and daily medication score, including use of a rescue inhaler for patients with asthma. Patients will be considered compliance if they complete at least 50% of expected daily responses. Patients will receive reminders from the EDC system via text message or email, as well as general outreach from TASC regarding their AIT journey.

8.1.1.6 End-of-Study Visit

At the conclusion of the 2018-2019 Mountain Cedar pollen season, TASC will contact study subjects to schedule and end-of-study visit. At this visit, the following procedures will be performed:

- Assess patient for any changes to medical history, including adverse events since the previous visit, resolution of ongoing adverse events from Visit 3, or changes in concomitant medication use
- Physical exam
- Order allergen specific serum IgE testing
- Collect vital signs
- Assess patient for any changes to medical history, adverse events since the previous visit, or changes in concomitant medication use
- Administer patient-reported outcomes, including treatment satisfaction
- Complete entry of patient information into the EDC system (eCRF)

8.2 SAFETY AND OTHER ASSESSMENTS

The following procedures will be performed as part of the safety and other assessment of the patient during this study:

- **Physical examination** including height and weight, body systems review
- **Vital signs** including temperature, pulse, respirations, and blood pressure
- **Special assays or procedures** include a Urine Pregnancy Test (UPT) for women of childbearing potential. As noted in Section 2, the risk of AIT during pregnancy is low, but immune sensitivity reactions and use of histamine in diagnostic allergy testing can be associated with pre-term delivery.

- **Administration of questionnaires** for patient-reported outcomes, including the NRS-11 for injection site pain, and treatment satisfaction.
- **Assessment of adverse events.** AEs/SAEs will be collected spontaneously and using the Total Safety Score (TSS) a structured worksheet for collecting and evaluating expected adverse events of interest (AEIs) associated with AIT.

In the event that a subject experiences either:

1. A treatment-emergent, serious adverse event that is related to study treatment, or
2. A grade 3 systemic allergic reaction according to WAO criteria, as assessed on the TSS worksheet that is possibly or definitely related to study treatment, or
3. Any other treatment-emergent adverse event resulting in death

The study will be placed on hold to further evaluate the risks and benefits of the study before a decision is made on whether or not to continue or terminate the study.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Any untoward medical occurrence in a clinical investigation subject administered immunotherapy or matched placebo which does not necessarily have a causal relationship with the treatment and is not otherwise related to their underlying medical condition or a comorbid condition present at the time of enrollment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the immunotherapy or other medicinal product, whether or not related to the medicinal product, that has an onset after enrollment in the study and the initiation of treatment, defined as the injection of study treatment and the seven day period following. Events reported via the patient diary on the daily symptom score are by definition symptoms of the underlying disease and are therefore not adverse events. Symptoms may fluctuate and thus changes in symptoms are not considered adverse events but are rather measures of treatment efficacy for the underlying condition. Pre-planned procedures are not considered adverse events. Pre-existing conditions, unless worsened are not considered adverse events. Whenever possible, adverse events will be consolidated into the underlying diagnosis rather than a collection of separately reported symptoms, as determined by the investigator.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) is considered "serious" if, in the view of the sponsor-investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of an existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon 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.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All AEs will be assessed by the investigator using a protocol defined grading system. AEIs captured on the Total Safety Score worksheet will not be reported separately as adverse events but will be graded according to the scoring system associated with that tool, as appropriate for a study endpoint. For adverse events (AEs) not included in the protocol defined grading system or AEI list, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events that interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the approved product label attached in Appendix 1 or is not otherwise consistent with the risk information described in Section 2.3 of the study protocol.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews, or other contacts with a study participant during the treatment and follow-up period.

All AEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product as assessed by the investigator, and date of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately, according to the definitions in Section 8, regardless of

relationship. All AEs will be followed to adequate resolution or the end-of-the study. Local and systemic reactions during the 60-minute observation period will be captured on the Total Safety Score report form which includes specific criteria for assessing and grading adverse reactions to AIT. For patients experiencing a reaction, the observation period will be extended until the patient is stable. In accordance with Section 2.1, after each treatment visit, patients are counseled on how to recognize the signs of delayed-onset reactions, including worsening pain, tenderness, redness, swelling at the injection site and to assess the injection site for these symptoms for seven (7) following treatment. If they recognize these or other symptoms of a delayed-onset reaction, they are instructed to report these symptoms by phone.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's pre-existing condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator or designee will record all events, defined as serious, unexpected, and related adverse drug reactions with start dates occurring any time after the initiation of treatment until seven (7) days after the third treatment will be reported in accordance with the requirements of 21 CFR 312, if applicable, to the manufacturer using their post-marketing safety report system, and to the IRB as an Unanticipated Problem in accordance with Section 8.4. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Unresolved adverse events will be followed for outcome information until the end of the study.

8.3.5 ADVERSE EVENT REPORTING

Adverse events will be analyzed and reported in a peer-reviewed publication following the completion of this study and tabulated and reported in Clinicaltrials.gov if 42 CFR 11 is applicable and this study is conducted under an IND.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The investigator will report to the IRB any serious adverse event (SAE) that meets the IRB's definition of an Unanticipated Problem (UP) in accordance with the IRB's reporting procedures for Unanticipated Problems, as described in Section 8.4. Serious, unexpected, and related adverse drug reactions will be reported under the requirements of 21 CFR 312, as applicable.

All SAEs will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event will be made available to the IRB and FDA upon request.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

In the event that the IRB determines that the risk-to-benefit ratio of the study has changed or additional information, as determined by the sponsor-investigator or IRB, has emerged during the course of the study, including information developed during the study as part of this research, or other information in published literature reports of ILIT, the ICF will be revised and an updated ICF will be provided to study participants for review and discussion to determine whether they wish to continue their participation in

the study. Should they agree to continue participation, subjects will sign the revised informed consent and their agreement will be noted in their medical record.

8.3.8 EVENTS OF SPECIAL INTEREST

Adverse Events of Interest (AEIs) include symptoms associated with local and systemic allergic reactions to AIT, up to and including anaphylaxis. Such events will be evaluated on the TSS worksheet rather than reported on separate AE or SAE forms. Specific AEIs associated with AIT include: Confusion, hypotension/collapse, unconsciousness, bradycardia, pallor, and urinary or fecal incontinence, which will be used to define severe reactions on the TSS worksheet. Diaphoresis, vomiting, presyncope, dyspnea, stridor, wheeze, chest/throat tightness or laryngeal edema, nausea, vomiting, and abdominal pain will define moderate reactions and reactions limited to the skin, including urticaria, erythema, injection site pruritis and angioedema will be defined as mild.

8.3.9 REPORTING OF PREGNANCY

Pregnancy is not considered an adverse event, but subjects that become pregnant prior to completion of study ILIT treatment will be discontinue from treatment. Those patients may continue follow-up procedures through the end-of-study. Pregnancy is required to be reported to the IRB as an UP, in accordance with the procedures in section 8.4.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given:
  a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and
  b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems to the reviewing IRB using the IRB's UP report portal and will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

To be reported Immediately:

- Changes in research that were initiated without IRB review and approval to eliminate apparent immediate hazards to the human subjects to ensure the continued safety and welfare of subjects
- Any significant new findings developed during the course of the research and after study completion which may relate to the subject's willingness to continue participation will be provided to the subjects.
- Modifications to previously approved documents
- Receipt of Investigator/Site 483, Determination letter or Warning letter
- If the Principal Investigator's medical license is suspended, revoked, placed on probation or restricted in any state or country
- Safety information that may help to provide additional protections for subject's safety and well-being, throughout the course of the study and after study completion.
- Communication of results from a research study to subjects when those results directly affect their safety or medical care
- Reports of pregnancy

To be reported within 10 (calendar) days of discovery:

- Unanticipated problems reports – Unanticipated problems should be reported regardless of whether they occur during the study, after the study completion, or after participant withdrawal or completion. Unanticipated problems involving risks to human subjects or others that are (1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; (2) related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures in the research); and (3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. Examples of problems or events that may meet the definition of unanticipated problems involving risk to subjects or others may include, but are not limited to the following:
    - Imminent threat of a reportable event that has not yet occurred
    - Information indicating a change to the risk/benefit ratio of the research
    - Death
    - Breach of confidentiality, including lost or stolen study documents/data
- Non–compliance issues – such as failure by the sponsor-investigator or a sub- to follow the IRB's requirements, applicable regulations or to protect human research subjects, including but not limited to the principles of the Belmont Report
- Serious non–compliance issues – non-compliance as defined as above and as determined to be serious in a way that adversely affects the rights and welfare of human subjects following the investigation and review by the IRB

- Continuing non–compliance issues – a pattern of repeated non-compliance or serious non-compliance as determined by the IRB
- Significant deviations – those that deviate from the approved protocol, informed consent process and affect or can potentially affect the safety of subjects.
- Revisions to the report of prior investigations, as applicable

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Reporting of UP to study participants will occur as required in accordance with the IRB's procedures and at the IRB's direction in accordance with the IRB's written procedures.

9 STATISTICAL CONSIDERATIONS

Statistical analysis will be performed by the sponsor-investigator or qualified designee using appropriate statistical analysis software. Analyses requiring significance testing will be two-sided at a 5% significance level, unless otherwise specified. All confidence intervals will be two-sided 95% confidence intervals.

Before unblinding, the programming and testing of the analysis programs and datasets will be completed in accordance with the specifications within the protocol. Assessment tools, including the TSS, TCS, DMS, DSS, NRS-11, and other PROs are attached in Appendix 3.

9.1 STATISTICAL HYPOTHESES

The null hypothesis for the primary and secondary endpoints is the equality of ILIT treatment with allergenic extract and intranodal injection of placebo (diluent) control. The null hypothesis for the safety endpoints is that ILIT treatment with allergenic extract is non-inferior to placebo. To control for multiplicity, efficacy tests will be performed stepwise in pre-specified order of hypotheses to be tested. Post-hoc and exploratory analyses will be noted as such in the publication of trial results.

Safety Endpoints:

1. Proportion of subjects experiencing a serious, treatment-emergent AE up to 60 minutes after the ILIT procedure.
2. Proportion of subjects experiencing systemic reactions to ILIT relative to placebo and severity of reactions relative to placebo as assessed by the Total Safety Score (TSS)
3. Proportion of subjects reporting local injection site reactions relative to placebo and severity of local reactions relative to placebo, as assessed by the TSS

Primary Efficacy Endpoint:

Secondary Efficacy Endpoints:

1. Average daily TCS during the 2018-2019 Mountain Cedar pollen season.
2. Proportion of days during pollen season for which active patients experience a lower TCS than placebo patients
3. Patient reported pain or discomfort immediately after and 30 minutes post ILIT procedure
4. Patient-reported treatment satisfaction at the end of the study
5. Reduction in allergen-specific serum IgE between the baseline and end-of-study visit

Exploratory Endpoints:

1. Use of rescue inhalers by patients with asthma

9.2 SAMPLE SIZE DETERMINATION

To determine sample size, the following were assumed:

- Tests will be two-sided and performed at the $\alpha = 0.05$ significance threshold, as noted in Section 9
- Study participants will complete the diary assessments (DSS, DMS) to yield a daily TCS on at least 50% of the possible days during the allergy season
- The difference between the average daily TCS for the placebo group and the ILIT group will 8.0 with an assumption that the standard deviation of the average daily TCS is 9.0 across both groups
- The randomization will be 1:1
- Using a priori samples size for multiple linear regression with an effect size 0.50 (f^2) for the active treatment group
- The screen failure rate is estimated to be approximately 12%

- A total of 26 screened patients will be required to enroll a total of 23 subjects into this two-treatment parallel-design study (up to 16 per group), the probability is 80% that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is a 0.50 effect size under multiple linear regression with two predictors (Soper, D.S. (2018). A-priori Sample Size Calculator for Multiple Regression [Software]. Available from http://www.danielsoper.com/statcalc).

9.3 POPULATIONS FOR ANALYSES

- Intent-to-Treat (ITT) Analysis Dataset: will include all randomized participants and be used for subject listings and demographics tables.
- Modified Intention-to-Treat (MITT) Analysis Dataset: will include all participants who received at least one (1) study intervention and completed at least one (1) complete daily e-diary assessment and be used for production of efficacy tables, subject listings, and demographics tables.
- Safety Analysis Dataset: will include all participants who took received at least one (1) dose of study intervention regardless of whether they provided any diary data and will be used for safety tables and tolerability tables.
- Per-Protocol (PP) Analysis Dataset: will include the subset of participants in the MITT data set who received three study treatment injections and completed at least 50% of e-diary responses during the allergy season (follow-up period), and has no other major protocol violations, and will be used for production of supplementary efficacy tables and analyses.
- Additional datasets for sub-population will include the adjusted ITT, MITT, Safety Dataset, and PP dataset for the subset of patients with asthma at baseline.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

This study is committed to the use of valid and reliable statistical methods in accordance with ICH E9, Statistical Principles for Clinical Trials for exploratory clinical trials. Thus, while this trial employs pre-specified objectives and tests of pre-defined hypotheses, this trial may require a more flexible approach to analysis in response to accumulating results. Statistical methods using appropriate transformations and non-parametric testing will be applied. Tests of underlying assumptions of specified tests will be performed. If required assumptions are not met, alternative analytic tests will be applied, as appropriate, and the reasons for selection will be reported in the publication.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The efficacy analysis will be secondary to the relative assessment of safety between groups. For efficacy, the primary analysis will be based on the difference of average daily total combined score (TCS), which is the sum of the daily symptom score (DSS) and daily medication score (DMS) between groups analyzed using a general linear model with daily pollen count at the study site and treatment as fixed effects, adjusting for different error variation for each treatment group. An exploratory adjustment using allergic asthma at baseline as a co-variate will also be performed. Additional nonparametric analysis with the Wilcoxon rank sum test may be conducted on the DMS if the data are not normally distributed and heavily weighted by zero values. There will be no imputation for missing data.

9.4.3 ANALYSIS OF ADDITIONAL SECONDARY EFFICACY ENDPOINT(S)

Secondary efficacy criteria will be compared between treatment groups using appropriate methods according to their nominal or quantitative nature. Specifically, the percentage of days for which the TCS is lower will be assessed using the $\chi 2$ test, while the NRS-11 and immunologic response will be analyzed using the Mann-Whitney test. Treatment satisfaction may be presented as a dichotomized variable, in which case it will be analyzed using a paired samples t-test. Co-variates will be assessed using a regression model for ordinal data. Data will be presented in the form of descriptive statistics and appropriate tables and graphs with a conclusion on whether or not the significance level allowed the null hypothesis for each test to be rejected.

9.4.4 SAFETY ANALYSES

Safety analyses will compare treatment groups using appropriate methods according to their nominal or quantitative nature. The total safety score (TSS) and proportion of subjects with any treatment-emergent SAE, as defined in the composite safety endpoint, will be assessed using the Mann-Whitney test. Data will be presented in the form of descriptive statistics and appropriate tables and graphs with a conclusion on whether the significance level allowed the null hypothesis for each test to be rejected.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Subject disposition information for the total analysis dataset will be generated. Descriptive statistics will be generated in summary tables comparing the active treatment and placebo groups across demography, baseline MQT results, comorbid allergic asthma, duration of Mountain Cedar pollen allergy, number of baseline concomitant medications for the ITT, MITT, PP, and Safety datasets. Nominal variables will be described by number and percentages of patients in each category. Quantitative variables will be described using means, standard deviations and percentiles, as appropriate. Number and percentage of missing values will be tabulated and reported, as appropriate.

9.4.6 PLANNED INTERIM ANALYSES

The will be no planned interim analysis for this study.

9.4.7 SUB-GROUP ANALYSES

Subgroup analyses for efficacy and safety will be conducted for dichotomized sub-groups based on sex (M/F) and age (18-65 years versus / >65 years). Sub-group analysis will be exploratory. Identification of additional co-variates and use of additional co-variates in regression models will be considered exploratory.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Tables of individual, numerically coded participant data will be generated for serious adverse events of interest only with dose, demographics, and medical history information.

9.4.9 EXPLORATORY ANALYSES

Efficacy analysis will be repeated on the subset of the PP dataset that was positive for allergic asthma at baseline. In addition, this subset will be assessed for differences in frequency of rescue inhaler use. Safety assessments may be repeated as exploratory using allergic asthma as a baseline co-variate. The use of immunologic response on end-of-study skin prick testing may be used for exploratory correlative analysis of primary and secondary efficacy outcomes measured by the TCS. Additional predictors in the efficacy analysis multiple regression model will be assessed, including baseline allergen-specific serum IgE levels.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to administering the study intervention. An informed consent form will be submitted to the IRB with this protocol. Any study-specific recruitment materials will be provided in advance to the IRB for review and approval, prior to implementation.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise during that discussion. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the medical record (including the date), and the form will be signed before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, IRB, and posted on Clinicaltrials.gov. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will be responsible for promptly informing study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- PI or sponsor-investigator decision
- Determination of unexpected, significant, or unacceptable risk to participants

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

The study may be resumed once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or regulatory authorities, as applicable.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the sponsor-investigator, sub-investigators, their staff, and their contractors, consultants, vendors and associates. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor-investigator, unless required by a lawful court order.

All research activities will be conducted in as private a setting as possible.

The study monitor, designated by the sponsor-investigator, and other authorized representatives of the sponsor-investigator, representatives of the Institutional Review Board (IRB), regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records and pharmacy records for the participants in this study. The clinical study site will permit access to such records. This disclosure is to protect the rights, safety, and welfare of subjects and ensure the integrity of the study.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location or secure electronic format for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored only to those authorized to receive it. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number in the analysis dataset exports. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at in a secure, HIPAA-compliance data collection system until completion of the study. After the study is completed, the de-identified, archived data will be retained on a secure cloud-storage system hosted by the data collection vendor, until completion of the analysis and publication, at which time it will be securely archived in electronic format at the TASC and removed from the data collection vendor's servers.

Biological samples **will not** be collected or stored for future use.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Sponsor Investigator	**Sub-Investigator**
Christopher Thompson, MD	Stacy Silvers, MD
Texan Allergy and Sinus Center	Texan Allergy and Sinus Center
5929 Balcones Dr, Ste 200 | Austin, TX 78731	5929 Balcones Dr, Ste 200 | Austin, TX 78731
(512) 799-4110	(512) 969-6210
drthompson@texanallergy.com	drsilvers@texanallergy.com

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the sponsor-investigator and qualified sub-investigator. Dr. Silvers is board certified in allergy and immunology by the American Board of Allergy and Immunology. Dr. Thompson is a board-certified Otolaryngologist, with a subspecialty in head and neck surgery. They will be responsible for medical and safety oversight of the study as well as medical management and treatment of study participants.

10.1.7 CLINICAL MONITORING

Clinical monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Monitoring for this study will be performed by qualified personnel designated by the sponsor-investigator. Monitoring for this investigator-initiated study will be risk-based with an emphasis on ensuring documentation of compliance with 21 CFR 50 and 56 (informed consent and IRB review and notifications) as well as remote review of data in the EDC system to identify and address missing data, identify protocol deviations, and if necessary, perform targeted review of key study data, including but not limited to IP storage and accountability but without 100% source data verification. Independent audits are not planned for this study.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The sponsor-investigator will ensure an adequate quality system to ensure that this study is conducted in accordance with the protocol, ICH-GCP and other applicable regulations. Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be investigated for clarification and/or resolution. Written, study-specific Standard Operating Procedures (SOPs), will be established to ensure that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP). The sponsor-investigator will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the IRB and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the investigator and qualified designees. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into the RedCap EDC system, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study-related documents will be maintained, and no records will be destroyed until this research has been terminated or completed, and all required reports have been made to the public trial registry and IRB. Records may be retained indefinitely beyond that period in a secure archival format at the discretion of the sponsor-investigator.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or other procedures implemented to ensure the conduct of this study. Noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the sponsor-investigator to use continuous vigilance to identify and report deviations to the IRB in accordance with the IRB's reporting guidelines. All deviations should be documented and addressed in study source documents, and incorporated into the analysis datasets in accordance with the specified definitions in Section 9.3. A listing of deviations, should they occur, will be produced and provided to the IRB in accordance with their procedures.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the ICMJE publication and data sharing policies. The sponsor-investigator is responsible for submitting the manuscript for publication within one-year of study completion. Every attempt will be made to publish results in a suitable, peer-reviewed journal of the sponsor-investigator's choosing.

This trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov either directly or through a reference to the peer-reviewed manuscript after publication.

10.1.12 CONFLICT OF INTEREST POLICY

This investigator-initiated study is being conducted independently from outside influence. The allergenic extract manufacturers have not been consulted on the design and will not participate in the data analysis. Contractors and consultants may be hired by the sponsor-investigator to support specific aspects of the study conduct and should any of those personnel be involved in the design, conduct, analysis, publication, or any aspect of this trial and have an actual conflict of interest, such conflict of interest will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The purpose of this study is publication and the reporting of results, including conflict of interest disclosures will comply with ICMJE requirements.

10.2 ADDITIONAL CONSIDERATIONS

None.

10.3 ABBREVIATIONS

AE	Adverse Event
AEI	Adverse Event of Interest
AIT	Allergen Immunotherapy
AU	Allergen Unit
AR	Allergic Rhinitis
AAAAI	AMERICAN ACADEMY OF ALLERGY, ASTHMA & IMMUNOLOGY
ACT	American Thoracic Society Asthma Control Test
APC	Antigen Presenting Cell
CRF	Case Report Form
CDC	Center for Disease Control
cm	centimeter
CMP	Clinical Monitoring Plan
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
DMS	Daily Medication Score
DSS	Daily Symptom Score
DSMB	Data Safety Monitoring Board
DC	Dendritic Cell
DNA	deoxyribonucleic acid
DHHS	Department of Health and Human Services
Df	Dermatophagoides farina
Dp	Dermatophagoides pteronyssinus
ECG	electrocardiogram
eCRF	Electronic Case Report Forms
ELISA	Enzyme-linked immunosorbent assay
EIV	Equine Influenzas Virus
EAACI	European Academy of Allergy and Clinical Immunology
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ISM	Independent Safety Monitor
IBH	Insect Bite Hypersensitivity
IRB	Institutional Review Board
ITT	Intent-To-Treat
ICMJE	International Committee of Medical Journal Editors
ICH	International Conference on Harmonisation

ISO	International Organization for Standardization
ILIT	Intralymphatic Immunotherapy
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IB	Investigator's Brochure
MHC	Major Histocompatibility Complex
MC	Mast Cell
MedDRA	Medical Dictionary for Regulatory Activities
MD	Medical Doctor
mL	milliliter
mm	millimeter
MITT	Modified Intent-to-Treat
MQT	Modified Quantitative Test
NCT	National Clinical Trial
NIH	National Institutes of Health
N/A	Not Applicable
NRS	Numeric Rating Scale
OHRP	Office for Human Research Protections
OIT	Oral Immunotherapy
PEQ	Patient Experience Questionnaire
PGI-I	Patient Global Impression-Improvement
PP	Per Protocol
PAR	Perennial Allergic Rhinitis
PLA2	Phospholipase A2
PI	Principal Investigator
PNU	Protein Nitrogen Units
QA	Quality Assurance
QC	Quality Control
RQLQ	Rhinosinusitis Quality of Life Questionnaire
SoE	Schedule of Events
SAR	Seasonal Allergic Rhinitis
SAE	Serious Adverse Event
SPT	Skin Prick Testing
SOP	Standard Operating Procedure
SQ-U	Standard Quality Unit
SAP	Statistical Analysis Plan
SLIT	Sublingual Immunotherapy
TASC	Texan Allergy & Sinus Center
TCS	Total Combined Score
TSS	Total Safety Score
UP	Unanticipated Problem
US	United States
UPT	Urine Pregnancy Test

W/V	Weight to Volume ratio
WAO	World Allergy Organization

10.4 PROTOCOL AMENDMENT HISTORY

IRB-approved Version	Date	Description of Change	Brief Rationale
2.0	19 Dec 2018	Safety moved ahead of efficacy in objectives and aims	

11 REFERENCES

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12 APPENDIX 1 – PACKAGE INSERTS & LABEL FOR ALLERGENIC EXTRACTS

JUNIPERUS ASHEI POLLEN

mountain cedar injection, solution

PRODUCT INFORMATION			
Product Type	NON-STANDARDIZED ALLERGENIC	**Item Code (Source)**	NDC:0268-6614
Route of Administration	PERCUTANEOUS		

ACTIVE INGREDIENT/ACTIVE MOIETY		
Ingredient Name	**Basis of Strength**	**Strength**
JUNIPERUS ASHEI POLLEN (UNII: 544F8MEY0Y) (JUNIPERUS ASHEI POLLEN - UNII:544F8MEY0Y)	JUNIPERUS ASHEI POLLEN	0.05 g in 1 mL

INACTIVE INGREDIENTS	
Ingredient Name	**Strength**
GLYCERIN (UNII: PDC6A3C0OX)	0.5 mL in 1 mL
PHENOL (UNII: 339NCG44TV)	0.004 mL in 1 mL
SODIUM CHLORIDE (UNII: 451W47IQ8X)	0.009 g in 1 mL
SODIUM BICARBONATE (UNII: 8MDF5V39QO)	0.00275 g in 1 mL
HYDROCHLORIC ACID (UNII: QTT17582CB)	
SODIUM HYDROXIDE (UNII: 55X04QC32I)	

PACKAGING				
#	**Item Code**	**Package Description**	**Marketing Start Date**	**Marketing End Date**
1	NDC:0268-6614-06	5 mL in 1 VIAL, MULTI-DOSE		

MARKETING INFORMATION			
Marketing Category	**Application Number or Monograph Citation**	**Marketing Start Date**	**Marketing End Date**
BLA	BLA103753	01/01/1965	

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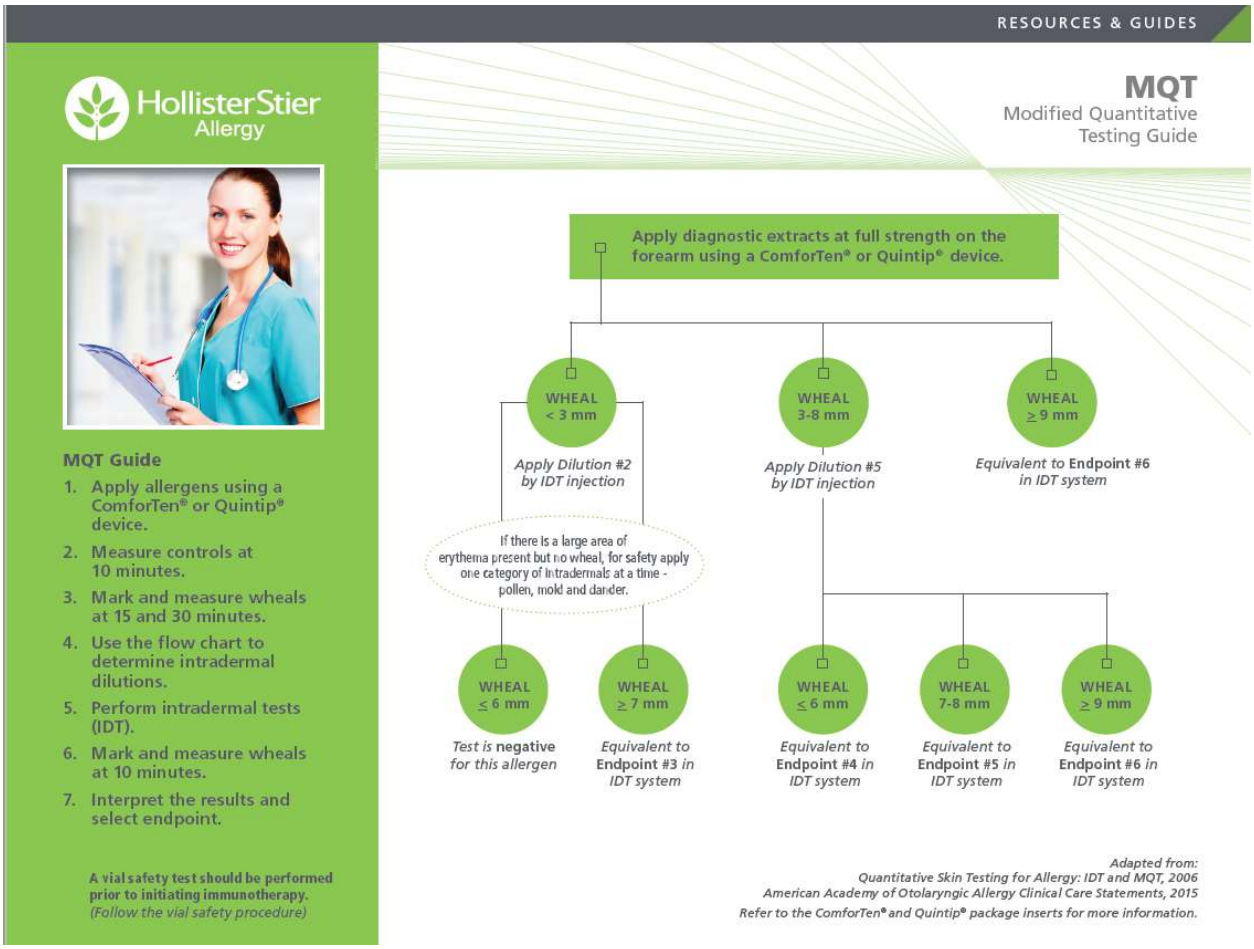
Prescribing Information Attached Separately

13 APPENDIX 2 PRODUCT INFORMATION FOR MODIFIED QUANTITATIVE TEST



14 APPENDIX 3 ASSESSMENT TOOLS AND RATING SCALES

Total Safety Score (TSS) Worksheet

Subject ID:
Injection # (write in 1, 2, or 3):

RECORDING INSTRUCTIONS: Mark/code the box(es) indicating reactions experienced by this subject following injection (D = definitely related to study, P = probably related to study, N = not related to study)

ILIT protocol defined grading scale for severity# of adverse events			
	Mild	Moderate	Severe
LOCAL REACTIONS			
Lymph node swelling			
Itch			
Redness			
Other*			
SYSTEMIC REACTIONS			
Rhinorrhea			
Sneezing			
Dyspnea			
Cough			
Wheeze			
Pruritus			
Rash			
Angioedema			
Nausea			
Vomiting			
Diarrhea			
Headache			
Other*			

SCORING INSTUCTIONS: Give one score for most severe reaction (see scoring key on back) - min score 0, max score 4

SCORE for this injection	

Total Safety Score Scoring Guide

Safety Scoring Key	
Reaction	Point value
None	0
Local - if any marks in white boxes	1
Systemic mild (WAO Grade 1/2) - if any marks in green boxes	2
Systemic moderate (WAO Grade 3) - if any marks in yellow boxes	3
Systemic severe (WAO Grade 4) - if any marks in red boxes	4

Severity will be determined by study staff. Local reactions will be divided into: 1) mild: events require minimal or no treatment and do not interfere with the patient's daily activities; 2) moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning; 3) severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating. Systemic reaction severity will be based on the World Allergy Organization grading for systemic allergic reactions (mild = grades 1/2, moderate = grade 3, severe = grade 4/5).

*Will be specified if unexpected, unlisted reaction occurs.

Note: if epinephrine is used, the dose(s) will be recorded in the chart and CRF.

Total Combined Score

Total Combined Score (TCS) is the sum of the daily symptom scores (DSS) and daily medication scores (DMS) for rhinoconjunctivitis.

Daily Symptom Score (DSS)

	no symptoms = 0	mild symptoms = 1	moderate symptoms = 2	severe symptoms = 3
1. runny nose				
2. stuffy nose				
3. sneezing				
4. itchy nose				
5. gritty/itchy eyes				
6. watery eyes				

Total DSS =_____

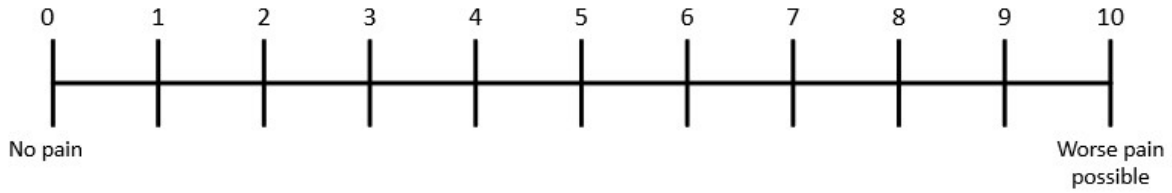
Daily Medication Score (DMS)

	No	Yes	
Did you use an oral antihistamine (Zyrtec) today?			If yes, score = 6
Dis you use antihistamine eye drops (olopatadine) today?			If yes, score = 6
Did you use a nasal corticosteroid (Flonase) today?			If yes, score = 8

Total DMS = _____

TCS = DSS + DMS = _____

Numeric Rating Scale (NRS) – 11



Instructions for use: The numerical rating scale (NRS) is perhaps the most common pain assessment tool used. It is important to recognize this is an 11-point scale (0-10 not 1-10). Ask the patient whether or not they are experiencing discomfort right now. If so, ask them to describe the discomfort.

To ascertain their understanding of the scale, ask if they would recognize:

- zero is a state of no pain (“0”) (or specific discomfort they describe)
- and the worst pain (or specific discomfort) imaginable (“10”).

Have the patient rate the intensity of their pain/discomfort “right now” verbally with a number or by pointing to the number that represents their pain intensity • This process can be repeated with similar questions about emotional distress

Patient Global Impression of Improvement (PGI-I)

Check the box next to the number that best describes how your allergy is now, compared with how it was before you had treatment:	
Very much better	1 □
Much better	2 □
A little better	3 □
No change	4 □
A little worse	5 □
Much worse	6 □
Very much worse	7 □

Selected questions modified from the Patient Experience Questionnaire (PEQ),

Are you satisfied with the treatment that you received?	( ) Yes	( ) No
Would you recommend the treatment to your friends or family?”	( ) Yes	( ) No