EVALUATING THE CURRENT STATUS OF ALLERGEN IMMUNOTHERAPY AND ESTABLISHING A BASOPHIL ACTIVATION TESTING PROTOCOL FOCUSING ON SHRIMP ALLERGY TO IMPROVE ORAL IMMUNOTHERAPY

A Thesis

by

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Abstract

Allergy immunotherapy continues to be an essential treatment in the armamentarium of allergy management not only because it is the only known therapy that could modify underlying pathophysiology, but also it can lead to significant symptom and medication reduction. With recent studies of food allergy and a novel oral immunotherapy for peanut approved by the FDA in 2020, patients with food allergy have new options and allergen immunotherapy has dramatically changed their quality of life. However, questions and challenges remain, including the indication and consideration of allergen immunotherapy, identification and selection of appropriate patients for allergen immunotherapy, and lack of a comprehensive review to scrutinize the details regarding allergen immunotherapy.

In this thesis, a stepwise approach is proposed, and the results of this project are to provide (1) a comprehensive systematic review appraising allergen immunotherapy and identify important aspects and considerations and (2) a basophil activation testing protocol to enhance the accuracy and safety for allergy diagnosis and improve oral immunotherapy outcome in shrimp allergy.

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CHAPTER-1

APPRAISE AND SUMMARIZE THE CURRENT STATUS OF ALLERGEN IMMUNOTHERAPY AND IDENTIFY FACTORS AND CONSIDERATIONS THAT MAY AFFECT ALLERGEN IMMUNOTHERAPY

Introduction

Ever since hay fever, aka allergic rhinitis, was first described by John Bostock in 1819, allergen sensitization has become recognized as a major role in rhinoconjunctivitis, asthma, atopic dermatitis, and other allergies including drug and food. (1-2) These allergic diseases represent a substantial health burden in both developed and developing countries and have increased in prevalence over the past decades. For each of the two most common allergies, respiratory allergies, including allergic rhinitis and asthma, and food allergy, has affected approximate 8-10% of both pediatric and adult populations. (3-5) Allergic rhinitis, asthma, and food allergy are frequent clinical diagnoses, but they may not be well controlled by standard management.

Allergen immunotherapy (AIT), among all other available treatments for allergic diseases, is a unique remedy. AIT is composed of a series of allergen administration either through injectable, sublingual, or oral route to an allergic individual over a defined period which results in decreased sensitization or even induced tolerance to the disease-causing allergens. Although the sites for allergen uptake in AIT may be different, such as the skin and

gastrointestinal tract for subcutaneous and oral AIT, respectively, the immunological effects for both AIT are similar. At present, it is the only therapy known to not only modify the underlying allergic immune cascades, but can also lead to symptom mitigation, quality of life improvement, and overall medication dose reduction. Because of the significance of AIT in the Allergy & Immunology field, the term allergen "extract" has been replaced by allergen "vaccine" by the World Health Organization (WHO) to reflect that AIT is an immune modifier with proved long term benefits. (6)

Another perfect example to demonstrate the importance of AIT is oral allergen immunotherapy (OIT). Until January 2020 when a first-in-class OIT for peanut was approved by the United States Food and Drug Administration (7), patients with food allergy could only perform strict avoidance and carry epinephrine auto-injectors (EAI) in case of an emergency. The advent of OIT has significantly changed their quality of life.

Despite the recent groundbreaking advance in OIT research and clinical trials, there are challenges, including appropriately diagnosing and selecting the "true" food allergic patients who are suitable for immunotherapy. Basophil activation testing (BAT), as a novel and non-invasive investigational assay for precisely diagnosing food allergy, may inform targeted OIT and elucidate the important role of immune dysregulation in patients with food allergy.

Methods

Both AIT and food allergy remain an active area of research, and the overlap between the two, i.e. OIT, is on the horizon. A comprehensive and systematic summary and

understanding of AIT can often reveal the important aspects that may inform novel laboratory tests and pharmaco-immunotherapies to improve disease outcome, especially in food allergy.

Shellfish, one of the top eight food allergen categories and the most common adult-onset food allergy, is a well-known culprit to cause allergic reactions and emergency visits and may lead to life-threatening anaphylaxis. (8) The most prevalent allergen among shellfish allergies is shrimp. From previous adult seafood allergy epidemiological studies in the United States, shrimp accounted for about 70% of shellfish allergies. (9) However, due to the high risk of developing severe allergic reactions, shrimp OIT is not well-studied compared to other food allergens. By studying the BAT assays, we should be able to implement better diagnosis and treatment of shrimp allergy.

This project evaluated the current status of AIT and proposed a BAT protocol focusing on shrimp allergy to improve outcomes in patients with shrimp allergy.

1. Conduct a systematic review regarding the current status of AIT and identify factors and considerations that may affect AIT. Appraise and summarize the pharmaco-immunotherapies in treating patients with immunoglobulin E mediated hypersensitivity.
2. Establish a BAT protocol to enhance the accuracy and safety for diagnosing shrimp allergy. Utilize the accumulated knowledge in food allergy to assess BAT as a clinical measure of food allergy and OIT for future multicenter shrimp OIT trial.

Results

For the first objective, a comprehensive systematic review is conducted to gauge the current status of AIT. Because of the relatively new and limited evidence regarding OIT, the

most evidence-based and experienced subcutaneous immunotherapy for allergic rhinoconjunctivitis and asthma is selected to identify important clinical factors and considerations that may influence the AIT outcome. Table 1 shows the index of the full review followed by each section described in detail.

A. Allergen, Atopy, and AIT B. Indications C. Pathophysiology D. Efficacy D.1. Mono- Versus Multi-Allergen D.2. Disease Prevention E. Beginning of Immunotherapy F. Precautions F.1. AIT during Pregnancy F.2. AIT in Children	G. Follow-Up and Duration of Immunotherapy H. Unresponsiveness from Immunotherapy I. Safety and Adverse Events I.1. Local Reactions I.2. Systemic Reactions I.3. Pre-injection Assessment J. Treatment of Adverse Events J.1. Local Reactions J.2. Systemic Reactions K. Summary

Table 1: Index of the allergen immunotherapy systematic review project

A. Allergen, Atopy, and AIT

Previously, the term “allergen” was used to define an antigenic substance that induces the production of specific immunoglobulin E (IgE) antibodies. (10) However, there may be two misunderstandings with such definition. First, not all individuals having specific IgE antibodies develop clinical symptoms. Second, an allergen response may not be limited only to IgE as it can be cell or other antibodies such as IgG-mediated. The more tailored definition of “allergen” is a type of antigen that cause an immunological hypersensitivity in which the immune system reacts to a harmless substance. Such immunological hypersensitivity is named as “allergy”. While historically almost all the allergens are known to be proteins, there is a new allergen,

galactose-alpha-1,3-galactose (often abbreviated as “alpha-gal” in the medical literature), that comes from a mammalian carbohydrate which can cause a delayed IgE-mediated allergy. (11) This new finding suggests that medical terms in the Allergy & Immunology field may need to be updated periodically to keep up with the times.

Another common term used in the allergy field is atopy. The word “atopy” was coined first to describe a specific type of sensitization state. Over the years, atopy has been redefined as a personal or familial tendency to allergy according to the revised nomenclature of allergy by World Allergy Organization (WAO) on the previous European Academy of Allergology and Clinical Immunology position statement. (12) Furthermore, atopy should be reserved to describe the genetic predisposition to common IgE-mediated allergic diseases, e.g. allergic rhinoconjunctivitis, asthma, and atopic dermatitis, and not to be used until an IgE sensitization has been demonstrated by specific IgE antibodies in vitro or by positive skin testing in vivo. Less common allergens such as drug or Hymenoptera, are not considered to be “atopy” although genetic susceptibility may exist in the spectrum. (13)

Vaccine is defined as a product that stimulates a person’s immune system to produce immunity to a specific disease, protecting the person from that disease. Similarly, AIT has the immune modification effect for each individual extract corresponding to the specific allergen and is recognized as a vaccine by WHO in 1998. (6) Nonetheless, there are differences among conventional versus allergen vaccines. The primary goal for conventional vaccines is to stimulate and/or boost immune response to a pathogen as in antibody production and immunological memory, whereas allergen vaccines aim to transform and/or suppress immune

reaction to an allergen by modulating specific IgE antibodies and allergen-specific T cells. Both allergen and traditional vaccines are antigen specific.

B. Indications

AIT should be considered for any patient who has demonstrated allergic symptoms along with specific IgE to clinically relevant allergens. (14) It has been used in the treatment of allergic rhinoconjunctivitis, asthma, atopic dermatitis, Hymenoptera allergy, and most recently, food allergy. For Hymenoptera allergy, venom immunotherapy is the treatment of choice other than insect prevention and as-needed EAI to dramatically decrease the risk of future systemic allergic reaction (SAR). Candidates for venom immunotherapy include patients with moderate to severe SAR to Hymenoptera stings. (15) On the other hand, an emerging success on food OIT trials has shed light on future management as there are very few treatment choices, same with Hymenoptera allergy, such as triggering food avoidance and EAI prescription. (16) Compared to Hymenoptera and food allergy in which the medical managements are limited, there is no absolute indication for AIT in respiratory allergies. Allergen avoidance, patient counseling, pharmacotherapy, and device technique for sprays and inhalers all constitute the basic managements for allergic rhinoconjunctivitis and asthma. AIT, when appropriate, should be utilized adjunctively with environmental control and medical therapy.

Numerous randomized, prospective, single- or double-blinded, placebo-controlled clinical trials have demonstrated the efficacy of AIT for respiratory allergies. However, due to active allergen ingredients in the AIT, there is small but existing risk during AIT to develop an immunotherapy-related SAR (IR-SAR). AIT, therefore, has to be balanced on a risk-benefit ratio.

The relative indication in prescribing AIT for respiratory allergies depends on the degree of symptom control that can be achieved by allergen avoidance, pharmacologic treatments, education, and adherence to medications. The decision of initiating AIT may also be influenced by patient's preference, adverse events to pervious medications, and socioeconomic status. (14)

In contrast, there is no absolute contraindication for AIT in respiratory allergies either. The proposed relative contraindications before starting AIT include: 1. uncontrolled or severe asthma, 2. past severe IR-SAR, 3. poor adherence to AIT, 4. significant cardiovascular or pulmonary diseases, 5. pregnancy, 6. status of mentally or physically unable to communicate clearly with the physician. (14) These medical conditions may add more risk and reduce patient's ability to survive in the setting of a severe or life-threatening IR-SAR. Precautions to some of the special groups are discussed in subsequent sections.

Herein lies the important step-by-step considerations for AIT:

1. Allergic versus non-allergic condition

The concept of AIT is to administer a certain amount of allergen content to desensitize a patient who is known to have allergic disease caused from the responsible allergen. It is a precise and individualized medicine designed for allergic diseases. For allergic rhinoconjunctivitis and asthma, physicians must first determine whether patient's disease is an allergic versus non-allergic type or, in a real-world situation where there is often an overlap in between, a predominant allergic versus non-allergic nature before implementing AIT. To maximize the treatment effect of AIT, it is pivotal for physicians to determine the proportion of

allergy component in each allergic disease and select the right patient with an allergy dominance. The stratification may not be easy, but most allergy-prone patients could be carefully identified based on a good history and physical examination supported by appropriate procedures, laboratory, and radiology findings.

2. Relevant versus irrelevant sensitization

Once a patient's disease was considered to have an underlying allergy in nature, physicians need to clarify what are the causative allergens for AIT to be prescribed. This evaluation is usually conducted by an allergist/immunologist who perform a series of skin and/or serum testing to find out the culprit allergen(s). Patient's age, influence by concomitant medications, and improper techniques or materials can all cause significant false positive or negative results. (17) An allergist/immunologist is a physician specially trained to diagnose, manage and treat allergies, asthma, and immunologic disorders including primary immunodeficiencies. Thus, an allergist/immunologist is experienced in performing the testing, interpreting the results, and treating any adverse events that may happen during the testing. As noted previously, AIT should only be considered for a patient who has done either skin or serum testing with demonstrated specific IgE antibodies to a clinically relevant allergen.

The hallmark of allergic diseases is the production of a specific IgE antibody, which is dependent on the allergen exposure and a collaboration between innate and adaptive immune system. The typical sequence of events in allergy consists of exposure to a low dose of allergen, activation of T and B cells specific for the allergen, production of specific IgE antibodies, binding of the IgE antibodies to the mast cells followed by repeated allergen exposure to trigger the

activation of the mast cells. The stimulation of mast cells with various mediator release can lead to immediate and delayed pathological hypersensitivity.

Percutaneous or intracutaneous skin testing can elicit an in vivo allergic response by applying a small amount of allergen on a picked or injected skin where mast cells are abundant in the dermis, respectively. Serum testing is an indirect measurement of total and specific IgE antibodies. There is a general agreement about 85 – 95% between skin and serum testing. Skin testing is more sensitive but less specific than in vitro serum testing. (18)

Despite a positive result in either a skin or serum testing confirms the presence of specific IgE antibodies, i.e., allergen sensitization, it does not always guarantee the presence of allergic symptoms or diseases. Allergen sensitization without correlative allergic symptoms or diseases is quite common and found to be in 8 – 30% of the population when performing a skin testing for aeroallergen. (19) Whether a positive result for an asymptomatic individual is a false alarm or a herald sign of future onset of allergy is a continuing debate. However, with appropriate history correlation, one study has shown that skin or serum testing can increase the predictive value to 97 – 99% compared with 82 – 85% of history alone for respiratory allergies. (20) In contrast, a negative skin or serum testing with a negative history suggest a non-allergic condition. Physicians should always correlate the testing results with the pertinent clinical history which is the best way to verify a relevant or irrelevant allergen sensitization.

3. Responsive versus unresponsive to traditional therapy

Many patients with allergic diseases receive treatments with pharmacological and/or non-pharmacological therapies, including but not limited to, antihistamines,

glucocorticosteroids, leukotriene modifiers, anticholinergics, bronchodilators, novel biologics, environmental control, and nasal irrigation. These therapies have all been shown to be effective in treating allergic diseases. Because of the easy access to over-the-counter medications, physicians often have to manage patients who do not respond to conventional allergy therapies and feel strongly towards to commencing AIT. Nonetheless, AIT is not considered as first line therapy and listed as add-on therapies in allergic rhinoconjunctivitis and asthma treatment guidelines in step 2, 3 or above, meaning in the moderate or severe disease category, due to its potential severe adverse effects. (21-22) Before AIT is initiated, a failure of control of symptoms with medical therapies should be documented unless primary allergy prevention is the goal. (23) A physician should diligently assess a patient to make sure all other treatments are optimized.

For allergic rhinitis and asthma, considerations to optimize treatment response are listed as following:

a. Have allergen avoidance and environmental control been evaluated and improved? Have skin and/or serum testing been done to identify possible trigger(s)?
b. What are the characteristics (phenotype/endotype) of the patients' disease? Do current managements cover the whole disease category especially if the patient has overlap pattern such as mixed rhinitis or asthma-COPD overlap syndrome?
c. Is there any comorbidity of the disease that makes it refractory to management?
d. Have the patient education and counseling been optimized including medication adherence, device technique, and allergen and/or irritant avoidance?

e. Without creating a medical or economic burden for the patient, have all other alternative therapies been tried? For each patient having a different pharmacokinetic profile, is the medication dosage sufficient for the specific patient?

4. Adherence, cost, and preference

Other important aspects to be incorporated into AIT consideration include adherence, cost, and preference. AIT is a series of allergen vaccine administration which at least have to be 2 years and at best to be extended to a total duration of 3 - 5 years in order to have long term benefits, and it has been proven cost-effective in an analysis of USA Medicaid population. (24) While AIT is economically advantageous, adherence to AIT could be a problem because of the treatment duration. (25) Therefore, patient preference should also be taken into account.

A summary for abovementioned AIT considerations is shown in Table 2. The advantages of AIT include reduction of medication burden, more flexible schedule than traditional therapy, and long-term vaccination benefit, while the disadvantages of AIT are potential adverse reactions, prolonged treatment time, and increased time and resources for the health facility. These once again highlight the importance of involving both patients and doctors in the decision of AIT.

Principle Questions for Allergen Immunotherapy Initiation	
1. Is the disease allergic or allergy dominance? 2. Is the sensitization relevant to the disease? 3. Are conventional therapies optimized? 4. Does patient prefer AIT after a discussion including benefits/risks, adherence, and cost?	
Relative Indication	Relative Contraindication
(1). A diagnosis of an allergic disease that may benefit from AIT (2). Specific IgE antibodies to clinically relevant allergens demonstrated by skin and/or serum allergen testing	(1). uncontrolled or severe asthma (2). past severe immunotherapy-related systemic allergic reaction (3). poor adherence to AIT (4). significant cardiovascular or pulmonary diseases (5). Pregnancy (6). status of mentally or physically unable to communicate clearly with the physician.

Table 2: A summary of AIT considerations

C. Pathophysiology

For respiratory allergies, airway mucosa is exposed to allergens through inhalation. Upon contacting and infiltrating through the mucosa, allergens bound to allergen specific IgE antibodies which cross-link sensitized mast cells and basophils. Once mast cells and basophils are activated, they release various preformed and newly synthesized mediators and cytokines that provoke symptoms and trigger further allergic immune cascades. The features for allergy symptom are pruritus, vasodilation, increased vascular permeability, mucus secretion, and for lower airway prominently, smooth muscle contraction. These responses, especially immediate

reactions, may be considered an original self-defense to protect individual from potential exposure to hazardous substance, therefore, the body threshold for stimulation is set at a relative low antigen level. However, this safety net may turn into pathologically allergic hypersensitivity when having exaggerated responses to a harmless molecule. In some patients, the early response could be followed by a late phase response characterized by multiple cell infiltrations including eosinophils, neutrophils, activated T cells, and macrophages. The recruitment and content release from these cells are responsible for a prolonged inflammation and tissue damage.

Dosage of AIT, in contrast, is approximately 100 times of the estimated maximal annual exposure to a natural allergen. (26) This quantitative difference will elicit intense immune effect through immune deviation and tolerance. An important observation is that the decrease of mast cell and basophil sensitivity and tendency for degranulation could take place in early AIT stage and lead to the inhibition of both the immediate and delayed responses in the conjunctiva, skin, nose, and lungs. In other words, a reduction in mediators and cytokines release from mast cells and basophils can prevent further inflammation and cell recruitment. Following initial desensitization of end organs with AIT administration, changes in the cellular and humoral responses ensue.

Allergic patients have increased numbers of allergen-specific CD4+ type 2 T helper cells in the serum, but normal levels of antigen-specific type 1 T helper cells and CD4+ CD25+ regulatory T cells. (27) Commonly recognized major alternations for both humoral and cellular immunity following a successful AIT are listed as below:

Cellular Immunity

1. An increase of regulatory T cell numbers and their inhibitory cytokines
2. A reduction of type 2 T helper cell responsiveness to specific allergen and an immune deviation toward to type 1 T helper cell subset.

Humoral Immunity

1. An elevation of allergen-specific IgA and IgG levels, particularly IgG4 isotype
2. An initial rise of allergen specific IgE level followed by gradual decline

There are several points to be noted. First, the abovementioned immunologic changes do not happen in sequence but rather overlap and interact with each other simultaneously. Second, the immune modification is complex and therefore, the exact mechanism is difficult to be put together and fully depicted as a whole. However, the succinct concepts are immune deviation and tolerance. (14) Immune deviation is a term indicating a modification of immune response to antigen exposure, whereas immune tolerance is a state of unresponsiveness of the immune system to previous reaction-eliciting antigen. In both situations, regulatory T cells appear to play the pivotal role. AIT has been shown to induce regulatory T cell releasing key cytokines including interleukin (IL)-10 and transforming growth factor-β. (28) The presence of such regulatory cytokines has been described to decrease B cell antigen specific IgE while increasing in antigen specific IgA and IgG4 production, induce type 1 T helper cell response (producing interferon-γ) while suppressing type 2 T helper cell cytokine release (producing IL-4, IL-5 and IL-13), and prevent long term inflammation and inflammatory cell recruitment such as eosinophils. (29) A simplified cell-to-cell interaction during AIT is represented in Figure 1.

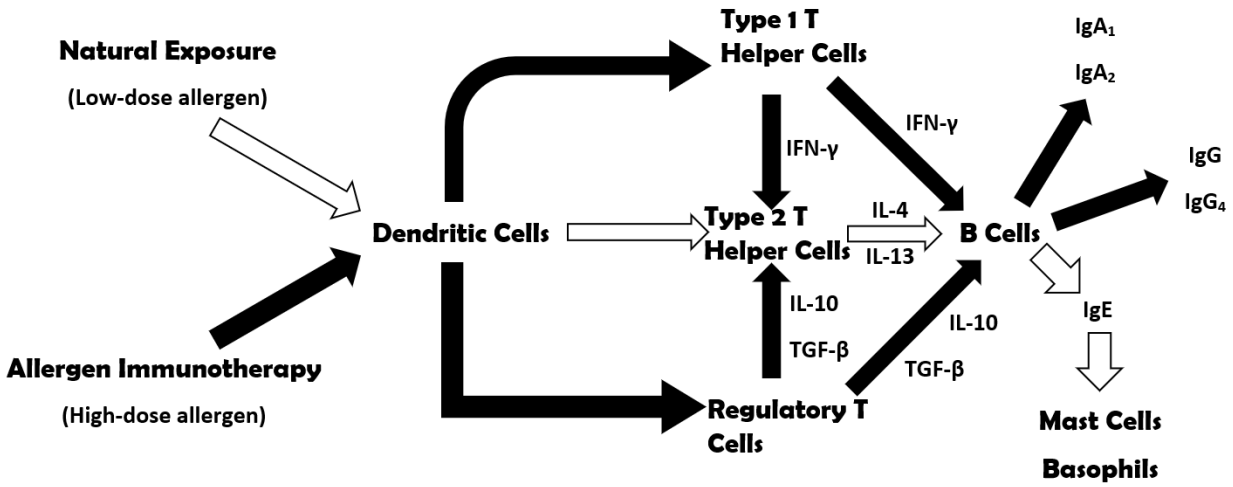

Figure 1: A pathophysiologic comparison in allergy versus allergen immunotherapy. White and black arrows denote allergy- and immunotherapy-related immune cascades, respectively. Abbreviations: IL, interleukin; IFN-γ, interferon gamma; TGF-β, transforming growth factor-β

Even with observed correlation between post-AIT immune alterations and clinical improvement, no distinctive immunological biomarkers have been proven useful for prediction of responsiveness, risk of adverse events, and periodic monitoring. Likewise, the immune deviation and tolerance induced by AIT should not be considered as a complete immunological transformation, nor a total elimination of allergies either symptomatically or histologically. Besides the risks for having IR-SAR from direct allergen injection, AIT seems to be safe in terms of their immunological modification. To date, there is no definite cause-and-effect relationship established between AIT and its theoretical probability of precipitating autoimmune diseases from circulating IgG4 immune complex, immunosuppression from regulatory T cells, and helminth infections from type 2 T helper cell deviation. If indeed there is a case-and-effect

relationship, as noted in anecdotally reported cases, the occurrence of such immune complications caused by AIT administration is extremely rare. (14, 30-34)

D. Efficacy

Many well-designed studies, systematic reviews, meta-analyses, and written guidelines have attested AIT as an effective treatment for allergic diseases. In this section, the efficacy of AIT in two major allergic airway diseases, allergic rhinoconjuctivitis and asthma, is discussed.

AIT can achieve multiple clinical improvements in allergic rhinoconjuctivitis such as in reducing nasal and ocular symptoms, decreasing total medications, enhancing quality of life, delaying disease progression, and even preventing new sensitizations. (35-37) However, not all categories in AIT, e.g. fungi, could provide sufficient data to support their efficacy, and the degree of improvement should not be considered as universal to all treated patients. (38) Depending on different research population, method design, and primary outcome, there may be substantial differences among study results. Different AIT regime may also lead to various clinical outcomes due to quality and quantity of each allergen vaccine. Standardized allergen vaccine quality is more consistent when compared to non-standardized one.

Compared to allergic rhinoconjuctivitis, data supporting AIT in asthmatics are less robust. According to the Global Initiative for Asthma report, the efficacy of AIT in asthmatics is demonstrated but limited. (22) The reasons are that the efficacy data are extrapolated from many studies including primarily allergic rhinoconjuctivitis, but not directly for asthma, and only mild asthmatics. There are scant studies comparing AIT with pharmacotherapies or using standard outcomes such as asthma exacerbations. It is concluded that the potential benefit of

AIT in asthmatics who have prominent allergy and allergen sensitization(s) must be weighed against the risks of adverse events, adherence, and cost to the patient and health system. (22) The other systematic review and meta-analysis report has demonstrated that AIT can reduce shot-term symptoms and medication scores, improve quality of life, and diminish allergen-specific airway hyperreactivity with modest increased risk of systemic and local adverse events in asthmatics. (39) The recent report from Agency for Healthcare Research and Quality also endorses that AIT may reduce quick-relief and long-term control medications, improve lung function and quality of life, and have glucocorticosteroid-sparing effect. Local and systemic allergic reactions are frequent but infrequently required a change in treatment with rarely reported life-threatening adverse events including anaphylaxis. (40)

D.1. Mono- versus Multi-allergen

From an immunology point of view, it is possible to give multiple traditional vaccines simultaneously and achieve each disease protection and, theoretically, the similar effect should apply to AIT as well. Nonetheless, efficacy for multi-allergen AIT is controversial. Most of the double-blind, placebo-controlled studies that have demonstrated the efficacy of AIT in allergic rhinoconjuctivitis and asthma are conducted with single allergen vaccine while few studies investigate multi-allergen AIT. Among those few studies, both the heterogeneity of the trial designs and the negative outcomes in some studies have made it difficult to convincingly document the advantage or disadvantage to use multi-allergen AIT. (14) The deep discussion with the potential methodological pitfalls in the positive and negative studies is beyond the scope of this section, but there are several important factors to be considered. First, comparing to traditional vaccination that each vaccine is given at different site or time, the characteristic

of multi-allergen AIT is to mix several allergen vaccines into a single vial which to be administered as a single injection at each time, and therefore, there may be a diluting effect by mixing the extracts and lowering the dose of each allergen below the optimal threshold. Second, some allergen vaccines with enzymatic activities, especially insects and fungi, should be separated from other allergen vaccines because of potential degradation. Due to the controversy between mono- versus multi-allergen immunotherapy, there is nationwide practice variation in the usage of AIT. The typical AIT prescription in the United States is multi-allergen based on The Allergen Immunotherapy: A Practice Parameter Third Update, published by a joint task force for the American Academy of Allergy, Asthma & Immunology, American College of Allergy, Asthma & Immunology, and Joint Council of Allergy, Asthma and Immunology. (14) Whereas in Europe, mono- or oligo-allergen AIT is a more common practice, from both the Guideline on Allergen Products: Production and Quality Issues from European Medicine Agency and the Allergen Immunotherapy Guideline from European Academy of Allergy and Clinical Immunology. The European guidelines have recommended only homologous allergens that are taxonomically related, for example a mixture of grass allergen vaccines, can be mixed. (41-42)

D.2. Disease Prevention

Although allergic rhinoconjuctivitis and asthma control can be achieved in most patients, there is no known cure. Primary prevention of any disease, including allergic rhinoconjuctivitis and asthma, is ideal. AIT has demonstrated and remained to be the only successful therapies in allergic disease modification. As a result, AIT has been studied for potential prevention of new sensitization and asthma development in allergic rhinoconjuctivitis patients. From a recent systematic review and meta-analysis, there are total of 6 and 2

randomized controlled trials with inconsistent results for short- and long-term prevention of new identified sensitization, respectively. (43) Among these studies, there are three low (44-46), one moderate (47), and two high (48-49) risk of bias clinical trials for short-term sensitization prevention in contrast to one moderate (50) and one high (51) risk of bias trials for long-term sensitization prevention. Even though the meta-analysis demonstrated benefit in short-term risk reduction of new sensitization, the overall risk reduction becomes negative excluding the two high risk of bias studies. (43) However, data in preventing the development of asthma in allergic rhinoconjuctivitis patients have shown good outcomes. Within the total of 6 randomized controlled trials studying asthma prevention effect up to two years post AIT, the systematic review and meta-analysis have demonstrated a significant asthma prevention effect in allergic rhinoconjuctivitis patients. Additionally, a subgroup analysis of utilizing AIT favors more in pediatric versus adult population. (43) Long-term asthma preventive effect could not be seen but this may be due to strict diagnostic criteria for primary outcomes. (52-53) In summary, there is no good evidence to conclude the usage of AIT for both short- and long-term new sensitization prevention as immune deviation and tolerance might be more allergen-specific. However, some positive data, even though in high risk of bias, suggests that a small group may attain benefit and the consideration should be a case-by-case scenario. There may be good evidence of implementing AIT in pediatric allergic rhinoconjuctivitis group for future asthma prevention but multiple facets, including risks, adherence, and cost, need to be evaluated to reach an agreement between patients and physicians.

E. Beginning of Immunotherapy:

In view of the complexity of decision-making as who will be beneficial from AIT (previously discussed in the indication section), along with the depth of appropriate dosage range, preparing, and mixing for each relevant allergen vaccines, it is clear that the prescription of AIT should be made under physicians with special training in Allergy & Immunology. The Allergen Immunotherapy: A Practice Parameter states that the physician prescribing AIT should be trained and experienced in prescribing and administrating AIT which is derived from patient's clinical and allergen exposure history and the results of either in vitro or vivo testing for specific IgE antibodies. (14) Instead of going deeply through writing AIT prescription, mixing proper extracts, and making a tailored schedule, for the purpose of this section, the aim is to convey important issues of what should be concerned before and during AIT.

1. What is the indication?

As mentioned earlier, it is noteworthy to emphasis again the necessity of a clear indication to initiate AIT. The risk of having a SAR or even potential life-threatening anaphylaxis exists across all AIT although it can be minimized to a certain degree. A detailed consultation between both patients and physicians and an informed consent should be conducted and obtained, respectively. All the other aspects of AIT such as preference, adherence, and cost should be co-evaluated and achieved mutually at best.

2. What is the schedule?

There are two phases in AIT: the initial build-up and maintenance phase. During the build-up phase, patients get incremental dosage of the allergen vaccines at each injection. Once

patients reach the effective dosage target, they are switched to the maintenance phase which is mostly one injection per month and stay on the same dosage over a period of time. In general, patients need to be on the maintenance therapy for at least 3 to 5 years in order to have a long-term protection benefit. (14) In terms of the build-up phase, there are three type of injection schedules, including conventional, cluster, and rush immunotherapies. The conventional schedule contains an injection 1 to 3 times a week. This is consistent with the allergen vaccine package insert which indicates a weekly schedule. Patients usually reach their maintenance dose within 3 to 6 months depending on the initial starting dose and adverse events during the build-up phase that may need schedule adjustment. Alternatively, the cluster and rush schedule can be used to accelerate the build-up phase. A cluster immunotherapy schedule begins with AIT administration 1 or 2 times a week with each time 2 or more injections are given at a 30-minute interval to achieve the maintenance dose as brief as 4 weeks. For a rush or even a faster ultra-rush immunotherapy schedule, patients are given AIT on an intense schedule to reach the therapeutic maintenance dose within hours to days. The advantages of fastened schedules are that these schedules permit patients to complete the build-up phase more rapidly than a conventional protocol, but either cluster or rush AIT has more risk of causing SAR. (14) Patients should be fully explained with the risks and benefits of accelerated schedules, premedication usage before injections, and monitored closely during the build-up phase. Anithistamines, leukotriene modifiers, and other drugs have been reported to be useful as premedications. (54-56) Management for adverse events during both build-up and maintenance phase is discussed in subsequent sections.

3. What allergen vaccine(s) is prescribed?

While allergists/immunologists are usually the physicians who select and prescribe AIT, it is also important for patients to know the rationale of how allergists/immunologists or other doctors who are trained and experienced in AIT choose the allergen extracts. First, the prescribing physician must obtain a detailed clinical history, confirm with the appropriate testing, and identify the correct patient to receive AIT. The corresponding allergens that contributing to seasonal or perennial allergies may vary substantially depending on regions of different climate, geography, and location. For instance, a patient who has typical seasonal allergies, his/her testing results should correlate with a particular season, such as Spring for tree, Summer for grass, and Fall for weed pollens. Similarly, inner-city subjects with perennial allergies should be evaluated for cockroach and/or rodent allergens. Second, when possible, standardized allergen vaccines should be utilized to prepare the AIT, which include several grass pollens, short ragweed, house dust mites, cat hair and pelt, and Hymenoptera venoms. The advantage of choosing standardized extracts is that their allergen content and activity are much more consistent, and therefore both retaining of therapeutic effect and reduction of adverse events could be accomplished. Third, cross-reactivity and enzyme activity must be considered when multiple allergen vaccines are mixed. Allergen cross-reactivity is the elicitation of same or similar patient's immunologic response to a single or multiple allergen(s) which share the overlapped or similar biochemical structure. It is not advisable nor necessary to include the allergen vaccines that share significant cross-reactivity due to undesirable dilution of other allergen extracts and unwanted risks of SAR from too much of the same or similar allergen constituents. Manufacturing companies may offer mix of the compatible pollen species that

belong to the same or different genera, and, ideally, prepare extracts based on cross-reactivity to further assist physicians in selecting the most appropriate allergen vaccines for treatment. Likewise, allergen vaccines such as cockroaches and fungi should be separated from others due to their proteolytic enzyme that can degrade other allergenic proteins. (57) Other studies have shown that pollens, house dust mites, and cat allergens could be mixed. (58) If high proteolytic allergen vaccines are required, it is necessary to prepare 2 or more vials to assure the therapeutic dose of each allergen and meanwhile avoid extract-to-extract interactions.

F. Precautions:

Since no single allergen vaccine is considered completely safe for an allergic individual, a general layer of precaution should be applied to every patient on AIT. AIT should be administered only by trained personnel who are sophisticated in administrating injections, adjusting dose, and managing adverse events appropriately. An established protocol established at the office or hospital clinic for managing different kinds of adverse event is prerequisite, especially in case of anaphylaxis, a life-threatening situation, which needs to be treated promptly with epinephrine. Early recognition and immediate response to an SAR is imperative to prevent further reactions. It is prudent to identify and recognize patients who are on AIT that is at higher risks for IR-SAR (14):

1. Uncontrolled and/or currently symptomatic asthma.
2. Significant seasonal or non-seasonal exacerbation of allergic symptoms, particularly asthma (for example, severe asthma symptoms during springtime or exposure to pet animals).

3. Other serious comorbidities or specific function decline, primarily with cardiac and pulmonary diseases and/or cardiopulmonary functional impairments.
4. Previously demonstrated a high degree of hypersensitivity on either skin or serum aeroallergen testing or even having allergic reaction from skin testing.
5. On certain medications that may interfere with the treatment of an adverse event from AIT. Examples would be β-blockers or angiotensin converting enzyme inhibitors.
6. An accelerated AIT schedule such as cluster, rush, or ultra-rush immunotherapy.
7. AIT administration from new vials, particularly to non-standardized allergen vaccines or their mix due to inconsistent allergen quality and quantity.
8. Special populations including children under 5 years of age, during pregnancy, and systemic mastocytosis

Notably, although there is no absolute contraindication in AIT, the aforementioned groups at risk may be considered as relatively contraindicated for AIT administration depending on the risk and benefit ratio and this is often a case-by-case scenario. The same precaution rule is also true for an elderly patient due to there is no absolute upper age limit for initiation of AIT. Elderly patients are not included in the special populations because the comorbidities may be present on younger subjects as well, albeit they occur more frequently in older subjects. (14) Other obstacles or illnesses that may complicate AIT including poor adherence or severe psychological disorders should be to be carefully reviewed as whether such patients are suitable for immunotherapy. A further detailed precaution regarding certain risky patients is discussed below.

F.1. AIT during Pregnancy

A physician prescribing AIT must know the risks and benefits of continuing immunotherapy among pregnant women. There are two concerning major risks that may occur for AIT during pregnancy: uterine smooth muscle contraction and fetal injury from rescue medication usage during an adverse allergic reaction. Because of the small but serious risk concern on the fetus, mother, or both, including spontaneous abortion, preterm labor, and fetal hypoxia, AIT is usually not initiated for pregnant patients unless a life-threatening potential exists, such as moderate to severe Hymenoptera hypersensitivity. (59) Discontinuation of AIT should be considered for any schedule during the build-up phase because of the non-therapeutic dosage and increased risk of having a reaction while updosing. For pregnant women who are on the maintenance phase of immunotherapy, AIT would be continued. As for concerns regarding the changes in fetal development and immune function, despite there is no single large prospective study investigating the safety of AIT during pregnancy, several retrospective studies have found that there is no greater risk of prematurity, toxemia, abortion, congenital malformation, neonatal death, or other adverse outcomes in women who receive AIT during pregnancy and there might be potential prevention effect of allergen sensitization in the newborns. (59-63) Whether the maternal AIT will truly benefit the offspring remains unanswered, and this is unlikely to be formally and prospectively studied owing to possible but clear risk of having SAR from AIT administration. There is no evidence to suggest an increased risk of commencing or continuing AIT for a breastfeeding mother and her breast-fed child.

F.2. AIT in Children

AIT in the pediatric population has been shown to be effective for both allergic rhinoconjunctivitis and asthma. The clinical indication of beginning AIT is similar for both adults and children except there may be more concentration on the prevention of new sensitization and/or asthma development, despite not all the preventive studies have strong evidence as discussed earlier. Experience suggests that AIT injections may be stressful in young children. (42) Aside from moderate to severe Hymenoptera hypersensitivity and food allergy, AIT is usually not considered for infants and toddlers in view of the fact that repeated AIT administration is traumatic to younger children and there is difficulty in communication if an allergic adverse event occurs. AIT is suggested to be avoided in children who are younger than 5 years of age, however, there are studies that have reported efficacy in this age group. (64-65) This is not an absolute contraindication to be restrained from receiving AIT. (66) Consequently, the Allergen Immunotherapy: A Practice Parameter clearly states that AIT can be considered as a disease modifying treatment for patients at all ages, and the risk and benefit assessment along with detailed clinical history and diagnostic testing results must be evaluated in every situation. (14)

G. Follow-up & Duration of Immunotherapy:

Since there is no good immunological biomarker that can correspond with clinical improvement and no prediction can be made for the timing when will a patient notice a clinical response, routine follow-up is critical. Studies have demonstrated that physiological and clinical response can often be observed when patients are close to or reach their maintenance dosage.

(67-68) It is appropriate to follow up with patients shortly after achieving their maintenance phase. Similar rule applies to cluster and rush SCIT schedules, yet a shorter follow-up is needed. Patients who are on active AIT should be evaluated at least every 6-12 months on a regular basis. (14) The purpose of a follow-up is not only assessing the clinical efficacy, but also to monitor adverse events, reinforce good adherence, and determine whether the dosage should be adjusted. Other aspects, such as severity of disease, level of clinical improvement and medication reduction, patient adherence, time, cost, and convenience should all be considered for the continuation or discontinuation of AIT.

Once the patient has allergic symptom amelioration from AIT, clinical trials and observations suggested that AIT should be continued at least for 3-5 years to achieve a long-term protection. (14) Vice versa, this also indicates that AIT can be stopped after 3-5 years of successful immunotherapy treatment. There are patients that have demonstrated prolonged symptom remission or disease relapse after AIT discontinuation. At present, no specific clinical and laboratory markers can distinguish between both groups and therefore, the continuation of AIT is an agreement between physicians and patients after a full explanation and discussion. Experience suggests that when symptom relapses after AIT is discontinued, a response to restarting such immunotherapy happens more rapidly than the original course of AIT. (69)

H. Unresponsiveness from Immunotherapy:

As a result of the great heterogeneity among patient status, allergen characteristics, and allergen vaccines, individual response to AIT is different. A general rate of successful AIT treatment among the trials and studies should not be extracted and implemented to a single

patient. However, this does not preclude a physician to investigate a patient who has no improvement from AIT administration and simply claim the patient as unresponsive to immunotherapy treatment. If there is no obvious clinical improvement after one year of maintenance immunotherapy, possible reason(s) explaining the AIT unresponsiveness should be pursued. (14) Such reason(s) of lack of efficacy might include, but not limit to, (a) failure to reduce significant allergenic exposure or continuous exposure to high levels of allergen (e.g., receiving cat AIT but there are cats in the house), (b) inappropriate treatment due to dominant non-allergy-mediated diseases (e.g., vasomotor rhinitis or neutrophilic asthma), (c) continued exposure to non-allergen triggers or irritants (e.g., tobacco smoke), (d) incomplete identification and treatment of clinically relevant allergens, (e) failure to treat with adequate doses of each allergen because of low-potency AVs or low-dosage immunotherapy prescription, or (f) a co-existing condition which accounts for patient's symptoms (e.g., chronic rhinosinusitis or nasal polyps). If none is found, discontinuation of AIT should be considered and discussed with patients and other alternatives may be evaluated.

I. Safety and adverse events:

I.1. Local reactions:

Adverse events associated with AIT can be either local or systemic. Local reactions, including one or more symptoms of pruritus, burning sensation, erythema, and injection-site swelling, are quite common with AIT. The frequency can range from 26 up to 82 % in all patients receiving AIT and 0.7 to 4% per injection. (70-72) Of one survey conducted in patients having AIT, over 80% of patients who have local reactions did not perceive local reactions to be

bothersome and 96% of the local reactors continue their treatment of AIT. (73) From a safety perspective, published studies have demonstrated that a single local reaction does not predict subsequent a local or systemic reaction (74-75), however, with more frequency of having local reactions, there may be more risk of having future systemic reactions. (76) Some of the local reactions, specifically pain or burning sensation, are attributed from the glycerin content in allergen vaccines. Higher concentration of the glycerin is associated with higher chance of pain at the injection site. (77) Other local reactions or the sizes of local reaction are not particularly associated with glycerin even when the glycerin concentration is elevated up to 50%. (78) The comparable local reaction rates between aeroallergen and Hymenoptera AIT, for which the Hymenoptera extracts are lack of glycerin component have indicated that allergen content in the allergen vaccines plays a bigger role in local reactions. (78)

I.2. Systemic reactions:

Severity of SAR related to SCIT can range from mild generalized pruritus and rhinitis symptoms to severe or even life-threatening anaphylaxis. There is a 5 graded classification system developed by WAO based on the severity of reactions and number of organs involved. (79) The prevalence of conventional schedule AIT-related SAR has been reported to be 0.1 to 0.2% per injections and 2 to 5% of all patients receiving AIT. (80) As for the rate of fatal and near-fatal reaction, for which a near-fatal reaction is defined as respiratory compromise, hypotension, or both, evaluated by survey studies from Allergy & Immunology society physician members, it is estimated to be once in every 2 to 2.5 million injections for fatal reactions versus 1 to 5.4 events in every 1 million injections for near-fatal reactions between the year from 1990 and 2001. (81-84) In the recent report from a national surveillance study from 2008-2013, there

has been a few AIT-related fatalities in which there are 2 out of 4 deaths occurred under the care of allergist. (85) The rate of SAR remained stable, including 1.9% of all AIT-treated patients and 0.08% and 0.02% for grade 3 and 4 SAR, respectively. Precaution of not giving AIT to uncontrolled asthma patients has significantly reduced the grade 3 and 4 systemic reactions. Appropriate pre-injection evaluation should be taken to minimize the risk of IR-SAR. Recently, the WAO AIT grading system has been reviewed and updated as shown in Figure 2. (86)

<table>
<tr><th colspan="5">Grading System for Systemic Allergic Reactions</th></tr>
<tr><th>Grade 1</th><th>Grade 2</th><th>Grade 3</th><th>Grade 4
(Anaphylaxis)</th><th>Grade 5
(Anaphylaxis)</th></tr>
<tr><td rowspan="2">Symptom(s)/sign(s) from 1 organ system present

• Cutaneous
Urticaria and/or erythema-warmth and/or pruritus, other than localized at the injection site
And/or
Tingling, or itching of the lips
Or
Angioedema (not laryngeal)
Or
• Upper respiratory
Nasal symptoms (eg, sneezing, rhinorrea, nasal pruritus, and/or nasal congestion)
And/or
Throat-clearing (itchy throat)
And/or
Cough not related to bronchospasm
Or
• Conjunctival
Erythema, pruritus, or tearing
Or
• Other
Nausea
Metallic taste</td><td>Symptom(s)/sign(s) from ≥ 2 organ symptoms listed in grade 1</td><td>• Lower airway:
Mild bronchospasm, (eg, cough, wheezing, shortness of breath) which responds to treatment
And/or
• Gastrointestinal
Abdominal cramps and/or vomiting/diarrhea

• Other
Uterine cramps

Any symptom(s)/sign(s) from grade 1 would be included</td><td>• Lower airway
Severe bronchospasm, (eg, not responding or worsening despite treatment)
And/or
• Upper airway
Laryngeal edema with stridor

Any symptom(s)/sign(s) from grades 1 or 3 would be included</td><td>• Lower or upper airway
Respiratory failure
And/or
• Cardiovascular
Collapse/hypotension
And/or
Loss of consciousness (vasovagal excluded)

Any symptom(s)/sign(s) from grades 1, 3, or 4 would be included</td></tr>
<tr><td colspan="4">Diagnostic Criteria for Anaphylaxis

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
AND AT LEAST ONE OF THE FOLLOWING
a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow, hypoxemia)
b. Reduced blood pressure or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
c. Reduced blood pressure or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced blood pressure after exposure to known allergen for that patient (minutes to several hours):
a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease in systolic blood pressure
b. Adults: systolic blood pressure of less than 90 mm Hg or greater than 30% decrease from that person's baseline</td></tr>
</table>

Figure 2: Represented updated grading system for AIT-associated systemic allergic reactions (upper portion) and anaphylaxis diagnostic criteria (bottom portion)

I.3. Pre-injection assessment:

Besides uncontrolled asthma, prior IR-SAR, and high sensitivity identified on skin testing patients, other risk factors for developing SAR from AIT have been identified. (6) These risk factors include erroneous AIT dosing, non-standardized allergen vaccine usage, concomitant β-blockers, cluster or rush schedules, and injections from new vials. Nevertheless, the risk of developing SAR and fatal anaphylaxis should be avoided or minimized whenever possible, and it may be achieved by pre-injection assessment. The pre-injection assessment consists of inquiries regarding asthma and/or rhinoconjunctivitis symptom control, change in health condition such as pregnancy, previous skin testing sensitivity and AIT-related systemic reactions, and concurrent medication use like β-blockers. Additional peak flow measurement may be included to concur that asthma is in a good control. Patients with any active systemic illness and/or prior adverse events from AIT should be evaluated by an allergist/immunologist before the next AIT injection.

J. Treatment of adverse reactions:

J.1. Local reactions:

There is no comprehensive study evaluating the treatment for local reactions during conventional build-up and maintenance phase although medications such as H1 & H2 antihistamines and leukotriene receptor antagonists are commonly used in clinical practice. The potential benefit of using these medications for local reactions is mostly extrapolated from rush venom immunotherapy studies for Hymenoptera allergy except one double-blind, placebo-controlled study showing benefit of loratadine premedication for cluster AIT. (87-91) Oral H1

antihistamines have been demonstrated to decrease local reactions while H2 antihistamines was not found to have any additional benefit if added to fexofenadine, an H1 antihistamine, premedication during rush venom immunotherapy. (88-90) In the other double-blind, placebo-controlled rush venom immunotherapy study, montelukast premedication was found to delay and decrease the size of local reaction when compared to placebo group, however, in the same study, there is no difference between the desloratadine premedication and placebo group. (91)

J.2. Systemic reactions:

The majority of IR-SAR, particularly most of the severe reactions, begin within 30 minutes after an AIT injection. (14) Any healthcare provider who administers AIT regardless of subspecialty should keep the patient under monitoring in the physician's office for at least 30 minutes following an injection. A longer time may be necessary for high-risk patients. In accordance, most of the extract manufacture's package inserts suggest a monitoring period of either 20-30 or 30 minutes after an AIT injection. It must be acknowledged that a delayed SAR may occur after the 30-minute monitoring period as these types of reactions have been reported up to 50% of all IR-SAR. (92-94) Furthermore, there may be biphasic reactions, defined as symptom recurrence after complete clinical symptom resolution of the initial reaction, reported up to 20% of all IR-SAR, that usually happen within 24 hours after initial injection. (95) There is no specific symptom of the initial reaction that can predict ensuing delayed and/or biphasic reactions, but fortunately, delayed and biphasic reactions are typically less severe than the original reactions. (14) Patient should be counseled on the chance of developing these reactions and an appropriate management plan with instructions especially on when to seek medical attention.

Importantly, physicians who prescribing and/or administering AIT must be aware of the potential risks of IR-SAR, promptly recognize the early signs and symptoms, and institute proper managements, if necessary. Assessing and maintaining of airway, breathing, circulation, and adequacy of mentation are the essential treatments. Epinephrine is the first-line therapy for anaphylaxis and there is no contraindication to give an epinephrine injection in an anaphylactic patient. It is paramount to administer epinephrine injection early in the management of anaphylaxis. Delayed epinephrine injection has been linked to fatalities resulting from severe respiratory and cardiovascular complications and biphasic reactions. (14) The preferable treatment recommendation for epinephrine injection is 0.2 to 0.5 ml intramuscular in the mid-outer thigh (1:1000 dilution) and should be repeated every 5 minutes, as necessary, to relieve and control adverse symptoms. If the clinical situation deems appropriate, the 5-minute interval may be shortened to permit more frequent injections. (14) Physicians should know the pharmacologic benefits and risks, as well as the potential lack of response to epinephrine injection especially when a patient is on β-blockers. In such case, glucagon, could be used to bypass the β-adrenergic receptor and reverse refractory bronchoconstriction and hypotension by directly activating adenyl cyclase during an anaphylaxis in a patient on β-blockers.

Indeed, the advocacy of epinephrine injection has brought more questions: how to define anaphylaxis and when to administer epinephrine if there is an IR-SAR? In 2006, the National Institute of Allergy and Infectious Diseases, Food Allergy and Anaphylaxis Network, and Food Allergy Research and Education assembled experts from different specialties and proposed the diagnostic criteria for anaphylaxis which is shown in Figure 2. (96) It should be noted that the proposed criteria are a balance between trying to include all patients with

anaphylaxis and avoiding unacceptably high number of mild to moderate SAR to be labelled as "anaphylaxis". Thus, the criteria suggest at least 2 system involvement or major organ compromise including pulmonary and/or cardiovascular system with a known allergen exposure. In the same report, a caveat was added: "there undoubtedly will be patients who present with symptoms not yet fulfilling the criteria of anaphylaxis yet in whom it would be appropriate to initiate therapy with epinephrine". This statement remains true, particularly with patients who are on AIT which contains known allergens. Likewise, the 2015 anaphylaxis Practice Parameter Update states that observational studies and analysis of near-fatal and fatal reactions have shown early treatment of any systemic reaction, even mild in severity, with epinephrine injection may prevent progression to more severe or life-threatening SAR. (97) In one study, the rapid administration of a single dose of epinephrine for mild SAR from AIT was able to cease further symptom development with no extra epinephrine injection needed. (95) Realizing this, physician and other health care professionals should not wait a SAR to evolve into anaphylaxis to justify an epinephrine injection given the fact that the benefit from such treatment outweigh the potential risk. Although there will be likely no consensus on determining which symptom(s) would be the perfect herald or threshold for ensuing anaphylaxis, any symptom listed in the WAO AIT grading system should be considered for potential indication of epinephrine injection to prevent deleterious anaphylaxis.

There are other second line therapies that have been implemented in the treatment of SAR consisting of oxygen administration, recumbent position placement with elevated lower extremities, intravenous fluid replacement, and intubation if clinically necessary for laryngeal edema. Ancillary medication such as nebulized β2 agonist for respiratory symptoms, H1 and H2

antihistamines, and glucocorticosteroid can be given as adjunctive therapy other than epinephrine injection. (97) The detailed discussion regarding efficacy of each management or medication is beyond the scope of this section but the concept is to provide options in addition to epinephrine administration. Clinicians who perform and administer AIT should have the appropriate medications and equipment available to treat any IR-SAR. Patient should also be instructed as when to seek for medical assistance if there is a delayed or biphasic reaction once they have been stabilized and discharged from the physician's office for the initial systemic reaction. If EAI is justified and prescribed, a patient must be instructed on the use of the portable epinephrine. The risks and benefits of continuing AIT in patients who have had a severe SAR should be carefully discussed and evaluated before next shot.

Summary and Conclusions

In summary, while allergen avoidance and pharmacotherapy are still valuable managements, AIT is considered to have the capacity to modify the natural course of disease by inducing long-term immunological deviation and tolerance. The risks of AIT can be minimized when immunotherapy is given to carefully selected patients in an appropriate setting. As exploring new technology and advancing knowledge of AIT, as in BAT and novel OIT, respectively, there will be even more ways to take advantage of that technology and knowledge and completely change AIT in the future.

CHAPTER-2

ESTABLISH A BASOPHIL ACTIVATION TESTING PROTOCOL TO ENHANCE THE ACCURACY AND SAFETY FOR DIAGNOSING SHRIMP ALLERGY

Introduction

Food allergy is defined by the Expert Panel of the National Institute of Allergy and Infectious Disease in 2010 as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food. (98) On the other hand, food intolerance is often confused with or mislabeled as food allergy because of the similarity of gastrointestinal symptoms and general improvement from food avoidance. The differences between these two diseases are crucial because OIT is designed for and may only treat food allergy, but not food intolerance. Compared with food intolerance in which the hypersensitivity reactions are localized to gastrointestinal organs, food allergy can affect not only local but the entire immune system, which may lead to fatal anaphylaxis. To mitigate the chance of developing severe reactions upon accidental exposure and potentially introduce food tolerance to the specific food allergens, OIT has been implemented with favorable outcomes.

However, the risks associated with OIT remain to be addressed. From a peanut OIT phase 3 trial data, around 12% of patients withdrew from treatment during OIT because of adverse reactions and high risks of conducting oral food challenges. (99) In another systematic review and meta-analysis evaluating the efficacy and safety in peanut OIT, the authors conclude

high-certainty evidence showing that peanut OIT significantly increases allergic and anaphylactic reactions over avoidance or placebo, despite peanut OIT reaching desensitization status in most patients with peanut allergy. (100) BAT, a flow cytometry functional assay measuring the degree of basophil degranulation and activation after allergen exposure, may provide an extra layer of safety in terms of mitigating risks during OIT and oral food challenges.

Methods

Human basophils, one leukocytes that makes up less than 1% of the circulating white blood cells, and like tissue-residing mast cells, express the high-affinity IgE receptor and can produce and secrete histamine, proteases, cytokines, chemokines, and lipid mediators to cause allergic reactions. Although activation of basophils can result from direct stimulation, the major way to activate basophils occurs upon cross-linking of the high-affinity IgE receptor with allergens or artificial agents. Because of their ability to cause type I hypersensitivity reactions, as seen with mast cells, isolated basophil tests may serve as a reliable in vitro functional assay due to their easier availability compared to mast cells, help to clarify equivocal cases, and avert detrimental provocation challenges.

Once the direct stimulants or allergens cross-link the IgE which is bound to the high-affinity IgE receptors, basophils express unique surface markers which is measured by BAT using flow cytometry. There are different markers that can be exploited to identify basophils and quantify their activation, including IgE, FcεRI, CD123, CCR3, CRTH2, CD63, CD69, and CD203c. (101) However, multiple labeling for surface marker is necessary for the identification of basophils because of overlaps with other blood cells. For example, IgE and FcεRI can also be

detected on dendritic cells, eosinophils, monocytes, macrophages, and B cells. CD123, the alpha subunit of the IL-3 receptor, can be expressed on plasmacytoid dendritic cells so additional staining with HLA-DR may be needed to confirm the HLA-DR negative basophil. In contrast, CD203c is a glycosylated type II transmembrane ectoenzyme that constitutively being expressed in low levels on the surface of peripheral basophils and therefore can be used as a single marker or in combination with other markers.

Following stimulation with allergens, up-regulation of surface markers could be quantified to determine basophils activation, namely CD63 and CD203, although most studies favor CD63 and occasionally use CD203c. While CD203c is constitutively expressed on the basophil surface and quickly up-regulated upon cell activation, CD63, also known as lysosomal-associated membrane protein-3, is contained within the intracellular secretory granules, and not expressed on the surface of resting basophils. During basophil activation and degranulation, these secretory granules begin to fuse with the plasma membrane and CD63 becomes expressed on the basophil surface. In addition, CD69 may also be useful as its expression is significantly increased when stimulated with IL-3; however, found to be weakly expressed upon IgE-mediated stimulation.

There are three steps in the BAT procedure: cell stimulation, staining, and flow cytometry. Blood specimens should be processed soon after collection because basophils would lose viability and reactivity considerably over time. 1-2 microliter of blood is required for BAT and allergen extracts can serve as stimulants. Whole blood collection for BAT should use either heparin or ethylenediaminetetraacetic acid. Different allergen concentrations should be used,

as the intrinsic sensitivity of the basophils and affinity of IgE to allergens varies among patients. (102)

Depending on using different markers such as CD63 and CD203c, the result interpretation of BAT may vary. BAT results for CD63 typically use the percentage of basophils expressing CD63 since resting basophils do not express CD63, whereas BAT results for CD203 usually use mean fluorescence intensity ratio comparing between the allergen stimulation and negative control. With different allergen concentrations, the basophil activation results in a bell-shaped dose-responsive curve. Various parameters are established and measured based on the dose-responsive curve to compare between subjects due to individual differences in terms of basophil activation. (103) CD-max is the concentration that corresponds to the maximum proportion of activated basophils at any allergen concentration. 50% effective concentration, i.e. EC50, is the effective dose at 50 % of the maximal basophil response. CD-max and EC50 are measures of basophil reactivity and sensitivity, respectively. The area under the dose-response curve is implemented more recently to assess basophil reactivity and sensitivity simultaneously.

Results

For the second objective, the aim is to utilize the accumulated knowledge in food allergy and AIT to establish a BAT protocol as a clinical measure to improve allergy diagnosis and OIT results.

Since no well-established BAT has been developed for shrimp allergy, a 14-step shrimp BAT protocol based on available evidence is proposed here in Table 3:

1. Blood specimen can be stored at 4°C for 24 hours. If the blood samples are stored, they must be put into a water bath at 37°C for 30 seconds right before implementing the BAT assay.
2. 100 µL of peripheral blood is mixed with anti-IgE (final concentration: 2 mg/mL) and commercially available shrimp extract diluted in the CMF-PBS at different concentrations (final concentrations: 1000, 100, 10, 1, and 0.1 ng/mL) dissolved in 100 µL of medium-only control in round-bottomed tubes with loose lids.
3. Incubate the sample at 37°C in a 5% CO2 incubator for 30 minutes.
4. Add 900 µL of cold 2.5 mM EDTA/CMF-PBS to stop basophil activation.
5. Centrifuge the samples at 4°C for 5 minutes. Remove the specimen supernatants.
6. Add multiple monoclonal antibodies at a total of 15 µL for surface marker staining and mix with pellets. The monoclonal antibody cocktail consists of 5 µL of FITC-conjugated anti-CD63 monoclonal antibody, 5 µL of PE-conjugated anti-HLA-DR mAb, and 5 µL of PerCP-conjugated anti-CD123 mAb.
7. Incubate the samples on ice for 20 minutes.
8. Add 3 ml of staining buffer (5% bovine serum albumin and 2 mM EDTA in CMF-PBS) and centrifuge the samples at 4°C for 5 minutes. Remove the specimen supernatants.
9. Add 1 ml Fix/Perm solution and mix with pellets.
10. Incubate the samples on ice for 30 minutes.
11. Add 2 ml of permeabilization buffer and again centrifuge the samples at 4°C for 5 minutes. Remove the specimen supernatants.
12. Add 150 µL of staining buffer.
13. Perform flow cytometry and analyze the data by gating basophils as CD123 positive and HLA-DR negative cells.
14. Measure cell expression of CD63high populations.

Table 3: Proposed 14-step shrimp basophil activation testing protocol

A positive BAT is defined when there is an increase in at least 5% of CD63-positive basophils being measured for at least one dilution of shrimp extract, in comparing with the baseline activation level in the negative control. (102) It is estimated that a sample size of 21 shrimp allergic patients would give 99% power, at a 2-sided type I error probability of 0.05, to detect a 5% of CD63-positive basophil difference in BAT. Sample size calculation were performed with the use of JMP software, version 16.0.0 (SAS Institute).

Summary and Conclusions:

There are two primary goals in this project. The first goal is to systematically review the status of AIT and consider all the essential aspects and factors regarding AIT, while the second one is to leverage the accumulated knowledge in AIT and food allergy to develop a BAT protocol for shrimp allergy. The establishment of a BAT protocol with the focus on shrimp allergy is particularly important as the data may be used to improve study outcomes in a National Institute of Allergy and Infectious Diseases/National Institute of Health funded multicenter shrimp OIT clinical trial consortium for which Houston Methodist Hospital is a proposed clinical trial site.

The workflow during this project utilizes multiple resources from the beginning of literature review to the resulting BAT protocol establishment. It truly demonstrates a stepwise approach in a way an individual with basic knowledge of allergy could reach the similar answer. The detailed review of AIT offers not only a guide to future OIT, but also shed light on the usage of BAT to further enhance the diagnosis accuracy and diminish the risks of OIT-related adverse reactions. For example, from the past experience of subcutaneous immunotherapy, it has been

demonstrated repeatedly that the immunotherapy, no matter the routes of administration, requires at least 3 to 5 years to modify the type 2 immune hypersensitivity, as both 2-year trials of oral and sublingual immunotherapy failed to reach long-term immune tolerance. (104-105) Furthermore, the testing biomarkers used for OIT trials, including IgG4 and IgG4/IgE ratio, are all derived from precedent AIT studies.

In recent years, BAT has become a powerful tool and biomarker that can be used to support allergy diagnoses, unravel mechanisms on heterogeneous allergic reactions, and monitoring the effects of AIT/OIT. However, challenges such as cut-off validation and methodology standardization remain for BAT before it can be adopted as a universal testing procedure. The project herein is to propose a specific shrimp BAT protocol. In the ensuing multicenter shrimp OIT clinical trial consortium, the comprehensive assessment of shrimp BAT protocol impact on OIT outcomes and its cost-effectiveness would be warranted.

CHAPTER-3

OTHER SCHOLARLY ACCOMPLISHMENT IN THE ALLERGY, IMMUNOLOGY, AND VACCINOLOGY

Allergy

Reference: Lin CH. Treatment of hypertension in patients with asthma. N Engl J Med. 2019;381(23):2278-2279.

In their review article, Christiansen and Zuraw summarize the current management of hypertension in patients with asthma. (106) However, the article does not mention the potential merits of the use of anticholinergic agents, which have less cardiovascular stimulation and interaction with drugs for hypertension than β2-agonists, as alternative or add-on medications to β2-agonists in such patients.

Anticholinergic bronchodilators have been recommended for patients with asthma who could not receive β2-agonists or for patients with asthma attacks induced by beta-blockers. (107-108) In addition, a long-acting anticholinergic bronchodilator has been shown to have a favorable cardiovascular safety profile and beneficial outcomes in both uncontrolled asthma despite standard treatment and mild asthma with an endotype of type 2 low inflammation. (109-111) In patients with asthma as well as hypertension and other coexisting conditions, physicians should consider anticholinergic bronchodilators within the context of precision medicine.

Immunology

Reference: Lin CH. Difelikefalin in hemodialysis patients with pruritus. N Engl J Med. 2020;382(21):2064-2065.

Fishbane et al. report favorable results with difelikefalin, a peripherally restricted and selective agonist of kappa opioid receptors, in treating patients undergoing hemodialysis who had moderate-to-severe pruritus. (112) However, the article does not address the importance of diabetes in the trial, even though more than half the trial participants had that diagnosis. Patients with a sole diagnosis of diabetes rarely present with either a localized or a more generalized form of pruritus. (113-115) Furthermore, since opioids continue to be alternatives to treat diabetic sensory neuropathy, difelikefalin may have synergistic effects, relevant not only for pruritus but also for other neuropathic symptoms in patients with diabetes, including paresthesia and pain. (116) Can Fishbane et al. clarify whether the results with difelikefalin for chronic kidney disease–associated pruritus are better in patients with diabetes than in those who do not have diabetes?

Vaccinology

Reference: Lin CH. BNT162b2 Covid-19 Vaccine in Adolescents. N Eng J Med. September 15, 2021. Epub ahead of print.

Frenck et al. report the outcomes of BNT162b2 vaccination in adolescents, and the Food and Drug Administration has authorized the expansion of Emergency Use Authorization for the BNT162b2 vaccine to include adolescents 12 to 15 years of age, with full approval of the vaccine

in persons 16 years of age or older. (117) However, because study protocols consistently separate the administration of the BNT162b2 vaccine from that of other, routine vaccines by a period of 14 to 28 days, questions remain regarding the immunogenicity and safety of coadministration of the BNT162b2 vaccine with other vaccines. (118) So far, no trial has been registered to evaluate the coadministration of the BNT162b2 vaccine and other routine vaccines in children. There has been one trial involving adults receiving pneumococcal vaccine coadministered with a booster dose of the BNT162b2 vaccine (ClinicalTrials.gov number, NCT04887948).

Although the Centers for Disease Control and Prevention allows the concomitant use of Covid-19 vaccines and other vaccines on the same day, no direct evidence supports such a recommendation. (119) Furthermore, the known immunogenicity and safety issues associated with vaccine coadministration or vaccine combinations, such as the coadministration of the pneumococcal and meningococcal conjugate vaccines being linked with immune interactions and the combination measles–mumps–rubella and varicella vaccine being linked with fever and febrile seizure, are seen in children. (120) Given the low hospitalization rate among children with Covid-19 (13 cases per million patients as of April 2021), are we in such an emergency as to skip vaccine coadministration studies of a new mRNA vaccine used in children? (121)

References

1. Ramachandran M, Aronson JK. John Bostock's first description of hayfever. J R Soc Med. 2011;104(6):237-40.
2. Lin CH, Alandijani S, Lockey RF. Subcutaneous versus sublingual immunotherapy. Expert Rev Clin Immunol. 2016;12(8):801-3.
3. Summary Health Statistics for Hay Fever: National Health Interview Survey, 2015, available at https://www.cdc.gov/nchs/fastats/allergies.htm
4. Summary Health Statistics for Asthma: National Health Interview Survey, 2015, available at https://www.cdc.gov/nchs/fastats/asthma.htm
5. Gupta RS, Warren CM, Smith BM, et al. Prevalence and severity of food allergies among us adults. JAMA Netw Open. 2019;2(1):e185630.
6. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol. 1998;102(4 Pt 1):558-62.
7. PALFORZIA [package insert]. Brisbane, CA: Aimmune Therapeutics, Inc.
8. Kamdar TA, Peterson S, Lau CH, Saltoun CA, Gupta RS, Bryce PJ. Prevalence and characteristics of adult-onset food allergy. J Allergy Clin Immunol Pract. 2015;3(1):114-5.e1.
9. Sicherer SH, Muñoz-furlong A, Sampson HA. Prevalence of seafood allergy in the United States determined by a random telephone survey. J Allergy Clin Immunol. 2004;114(1):159-65.

10. Blumenthal MN, Fine L. Definition of an Allergen (Immunobiology). In: Lockey RF, Ledford DK (Eds.) Allergens and Allergen Immunotherapy: Subcutaneous, Sublingual and Oral. 5th ed. CRC Press (Taylor & Francis Group), New York, NY; 2014:1–525.
11. Carter MC, Ruiz-esteves KN, Workman L, Lieberman P, Platts-mills TAE, Metcalfe DD. Identification of alpha-gal sensitivity in patients with a diagnosis of idiopathic anaphylaxis. Allergy. 2017
12. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113(5):832-6.
13. Kim SH, Ye YM, Palikhe NS, Kim JE, Park HS. Genetic and ethnic risk factors associated with drug hypersensitivity. Curr Opin Allergy Clin Immunol. 2010;10(4):280-90.
14. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: a practice parameter third update. J Allergy Clin Immunol. 2011;127(1 Suppl):S1-55.
15. Golden DB, Demain J, Freeman T, Graft D, Tankersley M, Tracy J, et al. Stinging insect hypersensitivity: A practice parameter update 2016. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2017; 118(1):28-54.
16. Sampath V, Sindher SB, Zhang W, Nadeau KC. New treatment directions in food allergy. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2018; 120(3):254-262.

17. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol. 2008;100(3 Suppl 3):S1-148.
18. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clinical and translational allergy. 2013; 3(1):3.
19. Bodtger U. Prognostic value of asymptomatic skin sensitization to aeroallergens. Current opinion in allergy and clinical immunology. 2004; 4(1):5-10.
20. Crobach MJ, Hermans J, Kaptein AA, Ridderikhoff J, Petri H, Mulder JD. The diagnosis of allergic rhinitis: how to combine the medical history with the results of radioallergosorbent tests and skin prick tests. Scandinavian journal of primary health care. 1998; 16(1):30-6.
21. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008; 63 Suppl 86:8-160.
22. 2018 GINA Report, Global Strategy for Asthma Management and Prevention, available at http://ginasthma.org/2018-gina-report-global-strategy-for-asthma-management-and-prevention/
23. Craig T, Sawyer AM, Fornadley JA. Use of immunotherapy in a primary care office. American family physician. 1998; 57(8):1888-94, 1897-8.
24. Hankin CS, Cox L, Lang D, Levin A, Gross G, Eavy G, et al. Allergy immunotherapy among Medicaid-enrolled children with allergic rhinitis: patterns of care, resource use, and costs. The Journal of allergy and clinical immunology. 2008; 121(1):227-32.

25. Cox LS, Hankin C, Lockey R. Allergy immunotherapy adherence and delivery route: location does not matter. The journal of allergy and clinical immunology. In practice. ; 2(2):156-60.

26. Larsen JN, Broge L, Jacobi H. Allergy immunotherapy: the future of allergy treatment. Drug Discov Today. 2016;21(1):26-37.

27. Frew AJ, Smith HE. Allergen-specific Immunotherapy. In: O'Hehir RE, Holgate ST, Sheikh A. Middleton's Allergy Essentials. Elsevier; 2016.

28. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol. 2003;33(5):1205-14.

29. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. World Allergy Organ J. 2015;8(1):17.

30. Randhawa IS, Junaid I, Klaustermeyer WB. Allergen immunotherapy in a patient with human immunodeficiency virus: effect on T-cell activation and viral replication. Ann Allergy Asthma Immunol. 2007;98(5):495-7.

31. Sánchez-morillas L, Reaño martos M, Iglesias cadarso A, Pérez pimiento A, Rodríguez mosquera M, Domínguez lázaro AR. Vasculitis during immunotherapy treatment in a patient with allergy to Cupressus arizonica. Allergol Immunopathol (Madr). 2005;33(6):333-4.

32. Branco-ferreira M, Clode MH, Palma-carlos AG. Distal digital vasculitis induced by specific immunotherapy. Allergy. 1998;53(1):102-3.

33. Phanuphak P, Kohler PF. Onset of polyarteritis nodosa during allergic hyposensitization treatment. Am J Med. 1980;68(4):479-85.
34. Bunnag C, Dhorranintra B. A preliminary study of circulating immune complexes during allergen immunotherapy in Thai patients. Asian Pac J Allergy Immunol. 1989;7(1):15-21.
35. Ross RN, Nelson HS, Finegold I. Effectiveness of specific immunotherapy in the treatment of allergic rhinitis: an analysis of randomized, prospective, single- or double-blind, placebo-controlled studies. Clin Ther. 2000;22(3):342-50.
36. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, et al. International consensus on allergy immunotherapy. J Allergy Clin Immunol. 2015;136(3):556-68.
37. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. J Allergy Clin Immunol. 2013;131(5):1288-96.e3.
38. Helbling A, Reimers A. Immunotherapy in fungal allergy. Curr Allergy Asthma Rep. 2003;3(5):447-53.
39. Dhami S, Kakourou A, Asamoah F, Agache I, Lau S, Jutel M, et al. Allergen immunotherapy for allergic asthma: A systematic review and meta-analysis. Allergy. 2017;72(12):1825-1848.
40. Lin SY, Azar A, Suarez-Cuervo C, Diette GB, Brigham E, Rice J, et al. The Role of Immunotherapy in the Treatment of Asthma. Comparative Effectiveness Review No. 196 (Prepared by the Johns Hopkins University Evidence-based Practice Center under Contract No.290-2015-00006-I). AHRQ Publication No. 17(18)-EHC029-EF. Rockville, MD:

Agency for Healthcare Research and Quality. March 2018. Posted final reports are located on the Effective Health Care Program search page. DOI: https://doi.org/10.23970/AHRQEPCCER196

41. European Medicines Agency. Guideline on allergen products: production and quality issues. London;2008 EMEA/CHMP/BWP/304831/2007. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003333.pdf
42. Roberts G, Pfaar O, Akdis CA, Ansotegui IJ, Durham SR, van Wijk RG, et al. 2017. EAACI GUIDELINES ON ALLERGEN IMMUNOTHERAPY ALLERGIC RHINOCONJUNCTIVITIS. In: Antonella Muraro, Graham Roberts (ed.). Allergen Immunotherapy Guidelines Part 2: Recommendations.Zurich: European Academy of Allergy and Clinical Immunology.
43. Halken S, Larenas-Linnemann D, Roberts G, Calderón MA, Angier E, Pfaar O, et al. EAACI GUIDELINES ON ALLERGEN IMMUNOTHERAPY PREVENTION OF ALLERGY. In: Antonella Muraro, Graham Roberts (ed.). Allergen Immunotherapy Guidelines Part 2: Recommendations.Zurich: European Academy of Allergy and Clinical Immunology.
44. Zolkipli Z, Roberts G, Cornelius V, Clayton B, Pearson S, Michaelis L et al. Randomized controlled trial of primary prevention of atopy using house dust mite allergen oral immunotherapy in early childhood. J Allergy Clin Immunol 2015;136:1541-1547.
45. Garcia BE, Gonzalez-Mancebo E, Barber D, Martin S, Tabar AI, Diaz de Durana AM et al. Sublingual immunotherapy in peach allergy: monitoring molecular sensitizations and reactivity to apple fruit and Platanus pollen. J Investig Allergol Clin Immunol 2010;20:514-520.

46. Szepfalusi Z, Bannert C, Ronceray L, Mayer E, Hassler M, Wissmann E et al. Preventive sublingual immunotherapy in preschool children: first evidence for safety and protolerogenic effects. Pediatr Allergy Immunol 2014; 25:788-795.
47. Pifferi M, Baldini G, Marrazzini G, Baldini M, Ragazzo V, Pietrobelli A et al. Benefits of immunotherapy with a standardized Dermatophagoides pteronyssinus extract in asthmatic children: a three-year prospective study. Allergy 2002;57:785-790.
48. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Randomized controlled open study of sublingual immunotherapy for respiratory allergy in reallife: clinical efficacy and more. Allergy 2004; 59:1205- 1210.
49. Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002;109:251-256.
50. Limb SL, Brown KC, Wood RA, Eggleston PA, Hamilton RG, Adkinson NF, Jr. Long-term immunologic effects of broad-spectrum aeroallergen immunotherapy. Int Arch Allergy Immunol 2006;140:245-251.
51. Dominicus R. 3-years' long-term effect of subcutaneous immunotherapy (SCIT) with a high-dose hypoallergenic 6-grass pollen preparation in adults. Eur Ann Allergy Clin Immunol 2012; 44:135-140.
52. Valovirta E, Berstad AK, de BJ, Bufe A, Eng P, Halken S et al. Design and recruitment for the GAP trial, investigating the preventive effect on asthma development of an SQ-standardized grass allergy immunotherapy tablet in children with grass pollen-induced allergic rhinoconjunctivitis. Clin Ther 2011;33:1537-1546.

53. Valovirta E, Petersen TH, Piotrowska T, Laursen MK, Andersen JS, Sorensen HF et al. Results from the 5-year SQ grass SLIT-tablet asthma prevention (GAP) trial in children with grass pollen allergy. J Allergy Clin Immunol 2017. pii: S0091-6749(17)31088-6.
54. Nielsen L, Johnsen CR, Mosbech H, Poulsen LK, Malling HJ. Antihistamine premedication in specific cluster immunotherapy: a double-blind, placebo-controlled study. J Allergy Clin Immunol 1996;97:1207-13, Ib.
55. Hejjaoui A, Ferrando R, Dhivert H, Michel FB, Bousquet J. Systemic reactions occurring during immunotherapy with standardized pollen extracts. J Allergy Clin Immunol 1992;89:925-33, III.
56. Portnoy J, Bagstad K, Kanarek H, Pacheco F, Hall B, Barnes C. Premedication reduces the incidence of systemic reactions during inhalant rush immunotherapy with mixtures of allergenic extracts. Ann Allergy 1994;73:409-18, Ib.
57. Grier TJ, LeFevre DM, Duncan EA, Esch RE. Stability of standardized grass, dust mite, cat, and short ragweed allergens after mixing with mold or cockroach extracts. Ann Allergy Asthma Immunol 2007;99:151-60, LB.
58. Esch RE. Allergen immunotherapy: what can and cannot be mixed? J Allergy Clin Immunol 2008;122:659-60, IV.
59. Metzger WJ, Turner E, Patterson R. The safety of immunotherapy during pregnancy. J Allergy Clin Immunol 1978;61:268-72, III.
60. Shaikh WA. A retrospective study on the safety of immunotherapy in pregnancy. Clin Exp Allergy 1993;23:857-60, III.

61. Schwartz HJ, Golden DB, Lockey RF. Venom immunotherapy in the Hymenoptera-allergic pregnant patient. J Allergy Clin Immunol 1990;85: 709-12, III.
62. Glovsky MM, Ghekiere L, Rejzek E. Effect of maternal immunotherapy on immediate skin test reactivity, specific rye I IgG and IgE antibody, and total IgE of the children. Ann Allergy 1991;67:21-4, III.
63. Flicker S, Marth K, Kofler H, Valenta R. Placental transfer of allergen-specific IgG but not IgE from a specific immunotherapy-treated mother. J Allergy Clin Immunol 2009;124:1358-60, e1. NR
64. Roberts G, Hurley C, Turcanu V, Lack G. Grass pollen immunotherapy as an effective therapy for childhood seasonal allergic asthma. J Allergy Clin Immunol 2006;117:263-8, Ib.
65. Rodriguez Perez N, Ambriz Moreno Mde J. Safety of immunotherapy and skin tests with allergens in children younger than five years. Rev Alerg Mex 2006; 53:47-51, III.
66. Finegold I. Immunotherapy: when to initiate treatment in children. Allergy Asthma Proc 2007;28:698-705, IV
67. Frew AJ, Powell RJ, Corrigan CJ. Durham systemic reaction. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117:319-25, Ib.
68. Kohno Y, Minoguchi K, Oda N, Yokoe T, Yamashita N, Sakane T, et al. Effect of rush immunotherapy on airway inflammation and airway hyperresponsiveness after bronchoprovocation with allergen in asthma. J Allergy Clin Immunol 1998;102:927-34, IIa

69. Fox R.W., Lockey R.F. (2007) Allergen Immunotherapy. In: Lieberman P., Anderson J.A. (eds) Allergic Diseases. Current Clinical Practice. Humana Press
70. Nelson BL, Dupont LA, Reid MJ. Prospective survey of local and systemic reactions to immunotherapy with pollen extracts. Ann Allergy 1986;56:331-4, III.
71. Prigal SJ. A ten-year study of repository injections of allergens: local reactions and their management. Ann Allergy 1972;30:529-35, III.
72. Tankersley MS, Butler KK, Butler WK, Goetz DW. Local reactions during allergen immunotherapy do not require dose adjustment. J Allergy Clin Immunol 2000;106:840-3, III.
73. Coop CA, Tankersley MS. Patient perceptions regarding local reactions from allergen immunotherapy injections. Ann Allergy Asthma Immunol. 2008;101(1):96-100.
74. Kelso JM. The rate of systemic reactions to immunotherapy injections is the same whether or not the dose is reduced after a local reaction. Ann Allergy Asthma Immunol. 2004;92(2):225-7.
75. Tankersley MS, Butler KK, Butler WK, Goetz DW. Local reactions during allergen immunotherapy do not require dose adjustment. J Allergy Clin Immunol. 2000;106(5):840-3.
76. Roy SR, Sigmon JR, Olivier J, Moffitt JE, Brown DA, Marshall GD. Increased frequency of large local reactions among systemic reactors during subcutaneous allergen immunotherapy. Ann Allergy Asthma Immunol. 2007;99(1):82-6.

77. Van Metre TE Jr, Rosenberg GL, Vaswani SK, Ziegler SR, Adkinson NF. Pain and dermal reaction caused by injected glycerin in immunotherapy solutions. J Allergy Clin Immunol 1996;97:1033-9, IIa.

78. Calabria CW, Coop CA, Tankersley MS. The GILL study: glycerin-induced local reactions in immunotherapy. J Allergy Clin Immunol. 2008;121(1):222-6.

79. Cox L, Larenas-linnemann D, Lockey RF, Passalacqua G. Speaking the same language: The World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System. J Allergy Clin Immunol. 2010;125(3):569-74, 574.e1-574.e7.

80. Epstein TG, Liss GM, Murphy-berendts K, Bernstein DI. AAAAI/ACAAI surveillance study of subcutaneous immunotherapy, years 2008-2012: an update on fatal and nonfatal systemic allergic reactions. J Allergy Clin Immunol Pract. 2014;2(2):161-7.

81. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol. 1987;79(4):660-77.

82. Reid MJ, Lockey RF, Turkeltaub PC, Platts-mills TA. Survey of fatalities from skin testing and immunotherapy 1985-1989. J Allergy Clin Immunol. 1993;92(1 Pt 1):6-15.

83. Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections. J Allergy Clin Immunol. 2006;117(1):169-75.

84. Bernstein DI, Wanner M, Borish L, Liss GM. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990-2001. J Allergy Clin Immunol. 2004;113(6):1129-36.

85. Epstein TG, Liss GM, Murphy-berendts K, Bernstein DI. Risk factors for fatal and nonfatal reactions to subcutaneous immunotherapy: National surveillance study on allergen immunotherapy (2008-2013). Ann Allergy Asthma Immunol. 2016;116(4):354-359.e2.
86. Cox LS, Sanchez-borges M, Lockey RF. World Allergy Organization Systemic Allergic Reaction Grading System: Is a Modification Needed?. J Allergy Clin Immunol Pract. 2017;5(1):58-62.e5.
87. Nielsen L, Johnsen CR, Mosbech H, Poulsen LK, Malling HJ. Antihistamine premedication in specific cluster immunotherapy: a double-blind, placebo-controlled study. J Allergy Clin Immunol. 1996;97(6):1207-13.
88. Berchtold E, Maibach R, Muller U. Reduction of side effects from rushimmunotherapy with honey bee venom by pretreatment with terfenadine. Clin Exp Allergy 1992;22:59-65, Ib.
89. Reimers A, Hari Y, Muller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine: a double-blind, placebo-controlled trial. Allergy 2000;55:484-8, Ib.
90. Brockow K, Kiehn M, Riethmuller C, Vieluf D, Berger J, Ring J. Efficacy of antihistamine pretreatment in the prevention of adverse reactions to Hymenoptera immunotherapy: a prospective, randomized, placebo-controlled trial. J Allergy Clin Immunol 1997;100:458-63, Ib.
91. Wohrl S, Gamper S, Hemmer W, Heinze G, Stingl G, Kinaciyan T. Premedication with montelukast reduces local reactions of allergen immunotherapy. Int Arch Allergy Immunol 2007;144:137-42, Ib.

92. Lin MS, Tanner E, Lynn J, Friday GA Jr. Nonfatal systemic allergic reactions induced by skin testing and immunotherapy. Ann Allergy 1993;71:557-62, III.

93. Rank MA, Oslie CL, Krogman JL, Park MA, Li JT. Allergen immunotherapy safety: characterizing systemic reactions and identifying risk factors. Allergy Asthma Proc 2008;29:400-5, III.

94. DaVeiga SP, Caruso K, Golubski S, Lang DM. A retrospective survey of systemic reaction from allergen immunotherapy. J Allergy Clin Immunol 2008;121(suppl): S124, III.

95. Scranton SE, Gonzalez EG, Waibel KH. Incidence and characteristics of biphasic reactions after allergen immunotherapy. J Allergy Clin Immunol. 2009;123(2):493-8.

96. Sampson HA, Muñoz-furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol. 2006;117(2):391-7.

97. Lieberman P, Nicklas RA, Randolph C, Oppenheimer J, Bernstein D, Bernstein J, et al. Anaphylaxis--a practice parameter update 2015. Ann Allergy Asthma Immunol. 2015;115(5):341-84.

98. Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the diagnosis and management of food allergy in the UnitedStates: report of the NIAID-sponsored expert panel. J Allergy Clin Immunol 2010; 126(6 Suppl):S1–S58.

99. Vickery BP, Vereda A, Casale TB, et al. AR101 Oral Immunotherapy for Peanut Allergy. N Engl J Med. 2018;379(21):1991-2001

100. Chu DK, Wood RA, French S, et al. Oral immunotherapy for peanut allergy (Pace): a systematic review and meta-analysis of efficacy and safety. The Lancet. 2019;393(10187):2222-2232.

101. Eberlein B. Basophil activation as marker of clinically relevant allergy and therapy outcome. Front Immunol. 2020;11:1815.

102. Santos AF, Alpan O, Hoffmann H. Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice. Allergy. Published online February 27, 2021:all.14747.

103. Hemmings O, Kwok M, McKendry R, Santos AF. Basophil activation test: old and new applications in allergy. Curr Allergy Asthma Rep. 2018;18(12):77.

104. Chinthrajah RS, Purington N, Andorf S, et al. Sustained outcomes in oral immunotherapy for peanut allergy (POISED study): a large, randomised, double-blind, placebo-controlled, phase 2 study. Lancet. 2019;394(10207):1437-1449.

105. Scadding GW, Calderon MA, Shamji MH, et al. Effect of 2 years of treatment with sublingual grass pollen immunotherapy on nasal response to allergen challenge at 3 years among patients with moderate to severe seasonal allergic rhinitis: the grass randomized clinical trial. JAMA. 2017;317(6):615-625.

106. Christiansen SC, Zuraw BL. Treatment of hypertension in patients with asthma. N Engl J Med 2019;381:1046-1057.

107. Expert panel report 3: guidelines for the diagnosis and management of asthma. Bethesda, MD: National Heart, Lung, and Blood Institute, August 2007. (NIH publication no. 07-4051.)

108. Fanta CH. Asthma. N Engl J Med 2009;360:1002-1014.

109. Dusser D, Ducharme FM. Safety of tiotropium in patients with asthma. Ther Adv Respir Dis 2019;13.

110. Kerstjens HAM, Engel M, Dahl R, et al. Tiotropium in asthma poorly controlled with standard combination therapy. N Engl J Med 2012;367:1198-1207.

111. Lazarus SC, Krishnan JA, King TS, et al. Mometasone or tiotropium in mild asthma with a low sputum eosinophil level. N Engl J Med 2019;380:2009-2019.

112. Fishbane S, Jamal A, Munera C, Wen W, Menzaghi F. A phase 3 trial of difelikefalin in hemodialysis patients with pruritus. N Engl J Med 2020;382:222-232.

113. Weisshaar E, Szepietowski JC, Dalgard FJ, et al. European S2k guideline on chronic pruritus. Acta Derm Venereol 2019;99:469-506.

114. Wahid Z, Kanjee A. Cutaneous manifestations of diabetes mellitus. J Pak Med Assoc 1998;48:304-305.

115. Yamaoka H, Sasaki H, Yamasaki H, et al. Truncal pruritus of unknown origin may be a symptom of diabetic polyneuropathy. Diabetes Care 2010;33:150-155.

116. Vinik AI. Diabetic sensory and motor neuropathy. N Engl J Med 2016;374:1455-1464.

117. Frenck RW Jr, Klein NP, Kitchin N, et al. Safety, immunogenicity, and efficacy of the BNT162b2 Covid-19 vaccine in adolescents. N Engl J Med 2021;385:239-250.

118. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603-2615.

119. Centers for Disease Control and Prevention. COVID-19 vaccines: interim clinical considerations for use of COVID-19 vaccines currently approved or authorized in the United States. 2021 (https://www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html. opens in new tab).

120. Hall E, Wodi AP, Hamborsky J, Morelli V, Schillie S, eds. Epidemiology and prevention of vaccine-preventable diseases. 14th ed. Atlanta: Centers for Disease Control and Prevention, 2021.

121. Havers FP, Whitaker M, Self JL, et al. Hospitalization of adolescents aged 12-17 years with laboratory-confirmed COVID-19 — COVID-NET, 14 states, March 1, 2020–April 24, 2021. MMWR Morb Mortal Wkly Rep 2021;70:851-857.