

What Do We Really Know About Intralymphatic Immunotherapy?

L. Klimek, MD, PhD^{1,*}

G. Senti, MD²

H. J. Hoffmann, PhD³

T. Kündig, MD⁴

Address

^{*,1}Center for Rhinology and Allergology, An den Quellen 10, D-65183, Wiesbaden, Germany

Email: ludger.klimek@Allergiezentrum.org

²Clinical Trials Center, University Hospital Zurich, Zurich, Switzerland

³Department of Clinical Medicine, Department of Respiratory Diseases and Allergy, Aarhus University, Aarhus, Denmark

⁴Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

Published online: 15 September 2018

© Springer Nature Switzerland AG 2018

This article is part of the Topical Collection on *Specific Immunotherapy*

Keywords Intralymphatic immunotherapy · ILIT · Allergen-specific immunotherapy · AIT · Allergic rhinitis

Abstract

Purpose of review Allergen-specific immunotherapy (AIT) is the only causal treatment method of IgE-mediated allergies that may lead to long-term symptom amelioration even after the end of treatment, positively interfere with the course of disease, and improve the immunological situation of the patient. While AIT in general requires treatment periods over 3 up to 5 years, intralymphatic immunotherapy (ILIT) needs only three ultrasound-guided injections of low allergen doses into inguinal lymph nodes with 4-week time interval making the entire treatment possible within 2 months.

Recent findings The number of published ILIT trials is continuously increasing and it has been mainly used for the indication of allergic rhinitis using commercially available grass pollen and birch extracts and *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, dog or cat allergens, and moreover a recombinant MAT-Fel d 1 vaccine and autologous semen from a patient with post-orgasmic illness syndrome.

Summary ILIT is a very promising AIT technique that could widely improve patient treatment. However, there is not enough convincing evidence for a routine use of ILIT and no authorized commercial allergen extracts exist for this approach, so far.

Dose-escalation studies and prospective DBPC efficacy trials need to be performed in diverse allergens like insect venom. Moreover, pediatric populations have not been present in former ILIT studies.

Introduction

Allergen-specific immunotherapy (AIT) is the only causal treatment method of IgE-mediated allergies. AIT may confer long-term symptom amelioration even after the end of treatment, positively interfere with the course of disease, and improve the immunological situation of the patient [1–3].

The effectiveness of AIT for allergic rhinitis treatment has been demonstrated in many clinical studies and systematic overviews of systematic reviews summarize the results (different allergen preparations, doses, and application routes) [4, 5].

AIT administers gradually increasing allergen doses to the allergic individual thereby altering the immune response to the allergen [1].

Routes used for allergen application in AIT include—but are not limited to [2, 6]—sublingual administration (SLIT) [7], subcutaneous injections (SCITs) [8, 9], oral application (OIT), epicutaneous application (EPIT), intradermal application (IDIT) [10], local nasal administration (LNIT) [11], and intralymphatic injections (ILIT) [12••, 13, 14•, 15, 16, 17•].

The effectiveness and the efficacy of the diverse approaches in AIT depend largely on the composition and formulation of the applied allergens or allergen extracts [18, 19] as well as on the possible use of adjuvants [20].

With the growing knowledge of the immune response and the underlying mechanism of action of AIT, there was a constant evolution of therapeutic regimens and type of therapeutic extracts, application, and adjuvants added [21].

For SCIT, non-modified aqueous or physically coupled (semidepot) allergen extracts are commercially available and chemically modified extracts (allergoids) as semidepot extracts. For the large range of allergens, efficacy data from clinical trials are available in varying numbers and quality. Some of these allergen extracts have been used for intralymphatic injection by applying the antigen directly into an inguinal lymph node; this has been named intralymphatic immunotherapy (ILIT).

Intralymphatic immunotherapy

ILIT consists merely of three ultrasound-guided injections of low allergen doses into inguinal lymph nodes with a 1-month time interval. Thus, the entire treatment can be performed within 2 months.

In comparison, SCIT with commonly marketed allergen extracts requires up to 70 injections and doctor visits over up to 5 years, something that only 5% of eligible patients judge worth the inconvenience [8].

The immunological basis for ILIT might be found in the immunogenicity of antigens that are largely ignored in the periphery, whereas antigens in a lymph node are strongly immunogenic. The chances for an antigen to meet a specific T or B cell are orders of magnitudes higher in a lymph node, because of the enormous density of T and B cells [22]. ILIT has been proven to enhance the immune response when using various types of vaccines, i.e., proteins, short peptides, mRNA, naked DNA, bacteria, immunostimulatory complexes (ISCOMS), and dendritic cells [23].

Besides ILIT considerably reducing the resource overhead for the patient, the physician, and the reimbursing party, the relatively short treatment duration avoids using multiple batches of vaccine or treatment interruptions due to supply chain bottlenecks. If a single batch of vaccine is not on stock for a whole treatment cycle, the immunotherapy will not be started until supply is resumed, thus avoiding interruptions, premature terminations, or unsatisfactory outcome. Another advantage of the short treatment duration, i.e., 2 months vs. 3 to 5 years, appears to be better treatment adherence of patients. In their first clinical trial, Senti et al. [11] observed that the 58 patients starting the three intralymphatic injections also finished the three injections and thus, 100% of patients who started the treatment also finished the treatment. In contrast, of those patients randomized into the conventional SCIT arm of the study, only 32 of the 54 patients who started the treatment also finished the full 3 years.

So far, ILIT has mainly been used for the indication of allergic rhinitis (AR) or allergic rhinoconjunctivitis (ARC). Commercially available grass pollen and birch extracts have been used in most of the clinical trials with ILIT, but also extracts of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and dog or cat allergens.

One trial used a recombinant major cat dander allergen Fel d 1 (MAT-Fel d 1) vaccine in patients suffering from cat dander.

In a case report of a patient with post-orgasmic illness syndrome, ILIT was used with the autologous semen of that patient [24].

None of the ILIT trials in humans have used any adjuvant in addition to alum.

Technique of ILIT

ILIT is currently administered to the lymph nodes of the inguinal area, specifically to those lymph nodes in the upper outer quadrant of the hiatus saphenus, i.e., the lymph node along the vena circumflexa ileum superficialis. These lymph nodes should be preferred for three reasons: (i) these lymph nodes are located relatively superficially, on average only approximately 5 mm beneath the skin surface, even in overweight patients. In contrast, the larger lymph nodes along the vena saphena magna are considerably deeper below the skin surface and therefore more difficult to palpate, find with ultrasound and to inject. (ii) Due to differences in orthostatic pressure, lymph production from the abdominal area is less than lymph production from the legs. Lymph nodes with less lymph flow should be preferred for injection, as the goal of ILIT is to produce a high concentration of allergen around antigen-presenting dendritic cells in order to pulse them with allergen as efficiently as possible. High lymph flow washes the allergen out of the lymph node into circula-

tion, reduces allergen concentration, and may lead to an allergic response. (iii) The accompanying vein (V. circumflexa ileum superficialis) is relatively small, thus minimizing the risk for accidental intravenous injection. In contrast, the lymph nodes along the vena saphena magna are along a very sizable vein.

In practical terms, the lymph nodes in the upper outer quadrant around the hiatus saphenus can typically be palpated rather easily, making access to these lymph nodes unproblematic even in obese patients. Using ultrasound, the lymph node can then be exactly located, as the paracortex of the node, i.e., the area with lymph follicles, is hypoechogenic in comparison to the surrounding adipose tissue. Allergen should be deposited in the paracortex of the lymph node. We recommend injecting a volume of 100 µl of allergen preparation. When using an alum-absorbed preparation, the alum will produce a marked echo effect in the ultrasound, providing a visual control of the success of targeting the lymph node. Also, we recommend use of the Doppler mode to exclude close proximity of larger blood vessels. Last but not the least, like before any injection of allergen, aspiration should be performed in order to minimize the risk for accidental intravascular injection. The density of blood vessels in lymph nodes is not particularly high and not higher than that of the surrounding adipose tissue, i.e., where conventional SCIT is administered.

ILIT is not painful. Neither lymph nodes, nor adipose tissues are strongly innervated. The only (minimal) pain the patients feels is the needle penetrating through the dermis. Injection of the lymph node itself cannot be felt. Therefore, the "pain" of ILIT is identical to the "pain" of SCIT. Senti et al. have asked patients to record the "pain" of the ILIT injection. Patients rated the intralymphatic injection significantly less painful than the venous blood draw they had during the same study visit [11].

Clinical trials with intralymphatic immunotherapy

The number of published ILIT trials is continuously increasing. In 2015, Senti et al. [14•] reported about the accumulated scientific experience in ILIT up to 2014 on a total of four clinical trials. Since then, several more studies have been published.

The first ILIT study had been performed by Senti et al. and was published in 2008 [11]. A total of 165 patients with grass pollen-induced allergic rhinoconjunctivitis (ARC) were randomly assigned to receive either 3 intralymphatic injections with 1-month intervals (cumulative allergen dose: 3000 SQ-U of Alutard SQ) or 54 subcutaneous injections of the grass pollen extract over 3 years (cumulative allergen

dose: 4,031,540 standardized quality units [SQ-U] of Alutard SQ, ALK-Abelló, Hørsholm, Denmark). The nasal response to grass allergen in specific nasal allergen challenges decreased similarly in both groups. Symptoms of ARC skin prick test reactivity were reduced after ILIT. Side effects of ILIT were restricted to mild pain at the site of injection that was less than that of venous puncture. Systemic adverse events were only mild and less if compared to those under SCIT.

The same group published a randomized double-blinded ILIT trial in patients with cat allergies [13] with a modular antigen transporter vaccine with the recombinant major cat dander allergen Fel d 1 (MAT-Fel d 1). ILIT with MAT-Fel d 1 if compared with placebo had fewer adverse events and a stronger reduced reaction in nasal allergen challenges with cat allergen. ILIT with MAT-Fel d 1 also stimulated regulatory T cell responses and increased cat allergen-specific IgG₄ levels, with both correlating positively with interleukin (IL)-10 production.

The immunological data of this trial has been worked up in detail by Zaleska et al. [25].

In a double-blinded placebo-controlled clinical trial in 36 patients with grass or birch pollen-induced allergic rhinoconjunctivitis, ILIT was found to be effective and safe resulting in a marked reduction of seasonal allergic symptoms [16].

Patterson et al. published a randomized, double-blinded, placebo-controlled, parallel group pilot study in 15 adolescents in the USA with bothersome nasal, ocular, and/or respiratory symptoms correlating with the timing of the grass pollen season and grass pollen sensitization [26]. They concluded ILIT to be efficacious and safe and recorded notably low adverse reactions.

All ILIT trials so far noted a clinical improvement except for the one reported by Witten et al. [27], which showed immunological changes but no clinical improvement and symptoms even tended to worsen.

By analyzing the differences between their study and all other successful trials, it was obvious that in the Witten study, the interval between ILIT-injections was shorter than in all other trials, i.e., 2 weeks instead of 4 weeks. This may explain the missing clinical effect by a compromised memory B cell formation and affinity maturation [28, 29].

It is well accepted in vaccine immunology that both these processes require periods where antigen levels are low, so that there is competition for the antigen in germinal centers that can positively select high-affinity B cells [28]. This is reasonable immunological explanation for the differences in this study if compared to all others.

The first ILIT open-label clinical trial [13] was re-analyzed for methodological effects in measuring and analyzing the efficacy of immunotherapies of grass pollen allergy [30]. The conclusions included a need for methodological recommendations, as the choice of analytical methods affects the outcome.

Recently, Hellkvist et al. performed a study using intralymphatic immunotherapy with only three lymph node injections using a combination of grass and birch allergens [31].

A significant reduction in the reactivity to nasal challenges with grass pollen allergen was found in comparison to pre-treatment findings. In addition, safety of ILIT was excellent with no therapy-related major adverse events occurring in any patient [31].

Schmid et al. [17•] performed an open trial using ILIT in seven patients with grass pollen allergic rhinoconjunctivitis using three inguinal lymph node injections at monthly intervals.

Symptom-medication scores of these patients were reduced as well as the reaction in nasal allergen challenges and titrated skin prick test, whereas no effect on quality of life was found. ILIT also induced an increase in the serum level of grass allergen-specific plasmablasts expressing isotypes other than IgE.

Lee et al. report on a heterogenic group of 11 patients with allergic rhinitis to *Dermatophagoides farinae* (Df), *Dermatophagoides pteronyssinus* (Dp), dog, or cat that scored their allergic symptoms during exposure to house dust, dogs, or cats in daily life using visual analogue scales (VAS) ranging from 0 to 100 mm when they were exposed to their respective allergen before and 4 months and 1 year after ILIT. The VAS for symptoms rhinorrhea, sneezing, itchy nose, itchy eyes, nasal obstruction, and postnasal drip during were alleviated 4 months and 1 year after the first day of ILIT [32]. However, this was not a controlled study.

Post-orgasmic illness syndrome (POIS) is a rare disease in men characterized by local allergic symptoms and transient flu-like illness that nearly always occurs after masturbation, during sexual intercourse, or spontaneous ejaculation and lasts for 2 to 7 days. Men with POIS become ill rather immediately after ejaculation. Two subtypes are distinguished: primary and secondary.

Kim et al. reported on a Korean man with POIS in whom the existence of semen-specific immunoglobulin E was confirmed using immunoglobulin E immunoblotting and enzyme-linked immunosorbent assay and who could successfully be treated by ILIT [24].

An earlier publication reported on two patients with POIS receiving hypsensitization therapy composed of multiple subcutaneous injections of autologous semen that resulted in a gradual decrease of symptoms. However, this required frequent subcutaneous injections and preceding masturbations to obtain the autologous semen.

In contrast, the patient treated by Kim et al. with ILIT showed a faster improvement of symptoms after only few intralymphatic injections [24].

ILIT in veterinary indications

ILIT has been successfully performed in various animal species, such as mice, rats, dogs, horses, ponies, cows, and monkeys, as reviewed previously [33].

ILIT is now used to treat allergic airway diseases in dogs, cats, and horses [34] and also in canine atopic dermatitis [35, 36] and other animal allergies [37].

Fischer et al. reported the results of a study on canine atopic dermatitis after ILIT was applied to 20 dogs with atopic dermatitis using allergens injected 3 up to 7 times into the popliteal lymph node every 4 weeks. A good clinical response was seen in 12 of these dogs (60%). However, without control group and unblinded, this study could not validate the efficacy of ILIT against canine atopic dermatitis.

In a translational research study, Martínez-Gómez et al. reported the results of murine experiments performed in female mice that were immunized three times at 2-week intervals by injection with the major bee venom allergen phospholipase A₂ ($n = 5$) or the major cat allergen recombinant Fel d 1 ($n = 4$) by ILIT into the inguinal lymph nodes. Allergen-specific IgG and T cell responses in addition to the production of IgG_{2a} were increased.

Biodistribution studies suggested that the delivery of antigens into lymph nodes is more effective than delivery into the subcutis [38].

Veterinary studies were performed by the group of Jonsdottir et al. who reported on the results of a pilot study of ILIT against allergic dermatitis of horses caused by the bites of midges (*Culicoides* spp.) [39]. Twelve horses were vaccinated three times with 10 µg of each of the four recombinant *Culicoides nubeculosus* allergens. Six horses were injected intralymphatically and six subcutaneously; no horse in both group showed allergic adverse reactions. Both treatment regimens demonstrated similar positive effects.

The same group performed another study on horses using intralymphatically injected allergens in aluminum hydroxide (alum) or in alum/monophosphoryl lipid A, also demonstrating that horses were protected from allergic skin reactions [40].

Conclusion

Allergen-specific immunotherapy (AIT) is a disease-modifying immunologically based treatment option for IgE-mediated allergies like allergic rhinoconjunctivitis and allergic asthma. From the different delivery options for AIT, subcutaneous and sublingual immunotherapies are well documented—at least on the level of certain well-documented products.

A reason, why AIT is used in less than 10% of patients where it could be used according to internationally accepted indications [41] might be due to the fact that current treatment schedules using SCIT or SLIT are very time-consuming and can take 3 years of treatment or even more [3].

For other ways of immunotherapy application like epicutaneous, bronchial, nasal, and oral application, no authorized products are marketed, so far.

Both SCIT and SLIT can reduce not only symptoms associated with the disease and medication need, but also affect the progression of the allergic disease, in a certain population of patients even permanently.

New application routes for the delivery of allergens are currently being evaluated in clinical trials and may become important ways of delivery in the future. ILIT is one of these promising application routes. Moreover, new immune-modifying agents like virus-like particles and other adjuvants may change allergen immunotherapy in the future. The results from the hypothesis-driven randomized controlled trials will become available that hopefully can clarify the clinical value of these immune-modifying ATI strategies.

Despite the predominantly favorable reports of clinical trials and the observed immunologic changes, intralymphatic immunotherapy is currently still in a proof of concept status. Yet, there is not enough convincing evidence for a routine use of ILIT in treating allergic conditions. Commercial extracts based on current European and international regulations for classification, composition, and quality requirements of allergen extracts need to be developed. Clinical trials with allergen products for intralymphatic immunotherapy need to be performed delivering data on gradual dose-escalation studies and DBPC efficacy trials.

Another unmet need is the use of more diverse allergens. While grass or birch pollen and cat dander have been investigated on larger numbers of patients, there

are many more seasonal and perennial allergens accessible to AIT.

A shorter and effective immunotherapy for insect venom hypersensitivity would be most welcome.

Optimizing the vaccines could be another option, while the commercial vaccines tested so far all were adsorbed to alum hydroxide without other adjuvants.

ILIT continues to be underexplored in pediatric patients and further investigation in this population is mandatory. With the availability of widely accepted outcome measures, clinical trials will become better comparable and internationally accepted readouts should be used also in ILIT trials [24].

In the clinical trials with ILIT so far, inconsistent endpoints with non-uniform methods of assessing endpoints, inconsistent and mainly short follow-up periods, and only moderate overall statistical power are found.

Compliance with ethical standards

Conflict of interest

Dr. Kündig reports grants from AlleCure Corp. USA, grants from ImVision AG, during the conduct of the study. In addition, Dr. Kündig has patents on intralymphatic immunotherapy where he is named as inventor. These patents, however, are owned by the University of Zurich. Dr. Klimek reports grants and personal fees from ALK-Abelló, Denmark; personal fees from MEDA, Sweden; grants and personal fees from Novartis, Switzerland; grants and personal fees from Allergopharma, Germany; grants and personal fees from Bionorica, Germany; personal fees from Boehringer Ingelheim, Germany; grants and personal fees from GSK, Great Britain; grants and personal fees from Lofarma, Italy; grants from Biomay, Austria; grants from HAL, Netherlands; grants from LETI, Spain; grants from Roxall, Germany; and grants from Bencard, Great Britain, outside the submitted work. Dr. Senti declares that she has no conflict of interest. Dr. Hoffmann declares that he has no conflict of interest.

Human and animal rights and informed consent

This article does contain studies with human subjects performed by GS, TK and HJH.

These studies have been performed according to the Declaration of Helsinki in its actual version and a positive vote of the responsible Ethical Committee was given before start of the study.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Brehler R, Klimek L, Vogelberg C, Werfel T, Pfaar O, Hamelmann E. Evidence vs. efficacy in allergen-specific immunotherapy: considerations using the example of tradable products in Germany. *Allergo J Int*. 2016;25:38–43.
 2. Pfaar O, Bonini S, Cardona V, Demoly P, Jakob T, Jutel M, et al. Perspectives in allergen immunotherapy: 2017 and beyond. *Allergy*. 2018;73(Suppl 104):5–23.
 3. Pfaar O, Bachert C, Bufe A, Buhl R, Ebner C, Eng P, et al. Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases: S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (OGAI), the Swiss Society for

- Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). *Allergo J Int.* 2014;23(8):282–319.
4. Nurmatov U, Dharni S, Arasi S, Roberts G, Pfaar O, Muraro A, et al. Allergen immunotherapy for allergic rhinoconjunctivitis: a systematic overview of systematic reviews. *Clin Transl Allergy.* 2017;7:24.
 5. Hoffmann HJ, Valovirta E, Pfaar O, Moingeon P, Schmid JM, Skaarup SH, et al. Novel approaches and perspectives in allergen immunotherapy. *Allergy.* 2017;72(7):1022–34.
 6. Klimek L, Kundig T, Senti G. Allergen specific immunotherapy for rhinitis allergica: new applications. *Hno.* 2015;63(5):343–51.
 7. Jin JJ, Li JT, Klimek L, Pfaar O. Sublingual immunotherapy dosing regimens: what is ideal? *J Allergy Clin Immunol Pract.* 2017;5(1):1–10.
 8. Klimek L, Brehler R, Hamelmann E, Kopp M, Ring J, Treudler R, et al. Development of subcutaneous immunotherapy in over 100 years: from beginning to immunologically-oriented therapy concepts. Part 1: Immunological mechanisms, efficacy and indications. *Allergo J Int.* 2018 (in press).
 9. Klimek L, Brehler R, Hamelmann E, Kopp M, Ring J, Treudler R, et al. Development of subcutaneous immunotherapy in over 100 years: from beginning to immunologically-oriented therapy concepts. Part 2: preventive aspects and innovations. *Allergo J Int.* 2018 (in press).
 10. Rotiroti G, Shamji M, Durham SR, Till SJ. Repeated low-dose intradermal allergen injection suppresses allergen-induced cutaneous late responses. *J Allergy Clin Immunol.* 2012;130(4):918–924 e1.
 11. Passalacqua G, Canonica GW. Local nasal specific immunotherapy for allergic rhinitis. *Allergy, Asthma, Clin Immunol: Off J Can Soc Allergy Clin Immunol.* 2006;2(3):117–23.
 - 12.●● Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci U S A.* 2008;105(46):17908–12.
- This publication describes the first application of ILIT in men.
13. Senti G, Cramer R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy Clin Immunol.* 2012;129(5):1290–6.
 - 14.● Senti G, Kundig TM. Intralymphatic immunotherapy. *World Allergy Organ J.* 2015;8(1):9.
- Excellent review of ILIT.
15. Hylander T, Larsson O, Petersson-Westin U, Eriksson M, Kumlien Georen S, Winqvist O, et al. Intralymphatic immunotherapy of pollen-induced rhinoconjunctivitis: a double-blind placebo-controlled trial. *Respir Res.* 2016;17:10.
 16. Hylander T, Latif L, Petersson-Westin U, Cardell LO. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. *J Allergy Clin Immunol.* 2013;131(2):412–20.
 - 17.● Schmid JM, Nezam H, Madsen HH, Schmitz A, Hoffmann HJ. Intralymphatic immunotherapy induces allergen specific plasmablasts and increases tolerance to skin prick testing in a pilot study. *Clin Transl Allergy.* 2016;6:19.
- This study provides new insights into possible immunological mechanisms of ILIT.
18. Akdis CA, Akdis M. Advances in allergen immunotherapy: aiming for complete tolerance to allergens. *Sci Transl Med.* 2015;7(280):280ps6.
 19. Klimek L, Bachmann MF, Senti G, Kundig TM. Immunotherapy of type-1 allergies with virus-like particles and CpG-motifs. *Expert Rev. Clin Immunol.* 2014;10(8):1059–67.
 20. Klimek L, Schmidt-Weber CB, Kramer MF, Skinner MA, Heath MD. Clinical use of adjuvants in allergen immunotherapy. *Expert Rev. Clin Immunol.* 2017;13(6):599–610.
 21. Klimek L, Pfaar O, Bousquet J, Senti G, Kundig T. Allergen immunotherapy in allergic rhinitis: current use and future trends. *Expert Rev. Clin Immunol.* 2017;13(9):897–906.
 22. Kundig TM, Bachmann MF, DiPaolo C, Simard JJ, Battegay M, Lothar H, et al. Fibroblasts as efficient antigen-presenting cells in lymphoid organs. *Science.* 1995;268(5215):1343–7.
 23. Senti G, Johansen P, Kundig TM. Intralymphatic immunotherapy: from the rationale to human applications. *Curr Top Microbiol Immunol.* 2011;352:71–84.
 24. Klimek L, Bergmann KC, Biedermann T, Bousquet J, Hellings P, Jung K, et al. Visual analogue scales (VAS): measuring instruments for the documentation of symptoms and therapy monitoring in cases of allergic rhinitis in everyday health care: position Paper of the German Society of Allergology (AeDA) and the German Society of Allergy and Clinical Immunology (DGAKI), ENT Section, in collaboration with the working group on Clinical Immunology, Allergology and Environmental Medicine of the German Society of Otorhinolaryngology, Head and Neck Surgery (DGHNO-KHC). *Allergo J Int.* 2017;26(1):16–24.
 25. Zaleska A, Eiwegger T, Soyer O, van de Veen W, Rhyner C, Soyka MB, et al. Immune regulation by intralymphatic immunotherapy with modular allergen translocation MAT vaccine. *Allergy.* 2014;69(9):1162–70.
 26. Patterson AM, Bonny AE, Shiels WE 2nd, Erwin EA. Three-injection intralymphatic immunotherapy in adolescents and young adults with grass pollen

- rhinoconjunctivitis. *Ann Allergy Asthma Immunol.* 2016;116(2):168–70.
27. Witten M, Malling HJ, Blom L, Poulsen BC, Poulsen LK. Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy? *J Allergy Clin Immunol.* 2013;132(5):1248–1252 e5.
 28. Kundig TM, Johansen P, Bachmann MF, Cardell LO, Senti G. Intralymphatic immunotherapy: time interval between injections is essential. *J Allergy Clin Immunol.* 2014;133(3):930–1.
 29. Malling HJ, Witten M, Poulsen LK. Reply: To *J Allergy Clin Immunol.* 2014;133(3):931–932.
 30. Graf N, Dinkel B, Rose H, Hothorn LA, Gerhard D, Johansen P, et al. A critical appraisal of analyzing nasal provocation test results in allergen immunotherapy trials. *Rhinology.* 2014;52(2):137–41.
 31. Hellkvist L, Hjalmarsson E, Georen SK, Karlsson A, Lundkvist K, Winqvist O, et al. Intralymphatic immunotherapy with two concomitant allergens, birch and grass - a RDBPC trial. *J Allergy Clin Immunol.* 2018.
 32. Lee SP, Jung JH, Lee SM, Joe E, Kang IG, Kim ST, et al. Intralymphatic immunotherapy alleviates allergic symptoms during allergen exposure in daily life. *Allergy Asthma Immunol Res.* 2018;10(2):180–1.
 33. Senti G, Johansen P, Kundig T. Intralymphatic immunotherapy: from the rationale to human applications. In: Valenta R, Coffman R, editors. *Vaccines against Allergies.* Berlin: Springer-Verlag Berlin; 2011. p. 71–84.
 34. Mueller RS, Jensen-Jarolim E, Roth-Walter F, Marti E, Janda J, Seida AA, et al. Allergen immunotherapy in people, dogs, cats and horses - differences, similarities and research needs. *Allergy.* 2018.
 35. Fischer N, Rostaher A, Favrot C. Intralymphatic immunotherapy: an effective and safe alternative route for canine atopic dermatitis. *Schweiz Arch Tierheilkd.* 2016;158(9):646–52.
 36. Timm K, Mueller RS, Nett-Mettler CS. Long-term effects of intralymphatic immunotherapy (ILIT) on canine atopic dermatitis. *Vet Dermatol.* 2018;29(2):123–e49.
 37. Kim ST, Park SH, Lee SM, Lee SP. Allergen-specific intralymphatic immunotherapy in human and animal studies. *Asia Pac Allergy.* 2017;7(3):131–7.
 38. Martinez-Gomez JM, Johansen P, Erdmann I, Senti G, Cramer R, Kundig TM. Intralymphatic injections as a new administration route for allergen-specific immunotherapy. *Int Arch Allergy Immunol.* 2009;150(1):59–65.
 39. Jonsdottir S, Hamza E, Janda J, Rhyner C, Meinke A, Marti E, et al. Developing a preventive immunization approach against insect bite hypersensitivity using recombinant allergens: a pilot study. *Vet Immunol Immunopathol.* 2015;166(1–2):8–21.
 40. Jonsdottir S, Svansson V, Stefansdottir SB, Schupbach G, Rhyner C, Marti E, et al. A preventive immunization approach against insect bite hypersensitivity: intralymphatic injection with recombinant allergens in alum or alum and monophosphoryl lipid A. *Vet Immunol Immunopathol.* 2016;172:14–20.
 41. Weschenfelder AK, Klimek L, Mulleneisen N, Renz H, Wehrmann W, Werfel T, et al. Course of respiratory allergy by treatment strategy based on German routine data. *Allergo J Int.* 2017;26(6):195–203.