

Current Topics

Recent Advances in Research on the Mechanisms and Regulation of Allergic Diseases

Review

State of the Art: Development of a Sublingual Allergy Immunotherapy Tablet for Allergic Rhinitis in Japan

Katsuyo Ohashi-Doi,^{*,a} Kaare Lund,^b Yuko Mitobe,^a and Kazuhiro Okamiya^a^aTorii Pharmaceutical Co., Ltd.; Tokyo 103–8439, Japan: and^bPapermill Medical; Copenhagen 2200, Denmark.

Received February 3, 2019

Allergic rhinitis (AR) caused by house dust mite (HDM) and Japanese cedar pollen (JCP) represents a significant, expanding health problem in Japan. Allergic symptoms often have a severe impact on the QOL such as sleep disturbance and reduced school and work performance. In addition to the classical symptoms, AR is known to be a risk factor for the development of allergic asthma, a potentially life-threatening condition. Allergy immunotherapy (AIT) is a well-documented, safe, effective treatment option for respiratory allergic disease. It has been demonstrated that AIT can provide relief from clinical symptoms and that AIT has the potential to provide long-term post-treatment effect. Although the mechanism of AIT is not fully understood, it can actively modulate protective allergen-reactive pathways of the immune system and alter the natural course of disease. Unlike pharmacotherapy, AIT addresses the basic immunological mechanisms that are responsible for the development and persistence of allergic conditions. Currently two main routes of AIT administration are commonly available, subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT). Both SCIT and SLIT are clinically effective, and SLIT is particularly well tolerated, with a lower risk of systemic allergic reactions compared with SCIT. To date, SLIT tablets have been developed for a range of different allergies including HDM and JCP and are the best-documented AIT treatment form. Here we introduce the current status of development of a SLIT tablet in Japan for AR, examine the clinical aspects and mechanism of action of AIT, and discuss the future directions of SLIT.

Key words allergy immunotherapy; immune tolerance; sublingual immunotherapy tablet; Japanese cedar; house dust mite

1. INTRODUCTION

Allergic rhinitis (AR) is a common, chronic allergic disease and due to high and increasing prevalence rates has become a global health problem.¹⁾ In affected individuals, AR often has a severe impact on the QOL, including sleep disturbance, work impairment, and learning disabilities.²⁾ In addition to the discomfort associated directly with AR symptoms, it is a risk factor for the development of allergic asthma, an important aspect that should be taken into account when considering available AR treatment options.^{3,4)} In terms of health economics, AR is a burden not only from a societal perspective where reduced work capacity and absence from work contribute to the associated costs of the disease but also from a healthcare perspective where medication, hospitalization, and frequent use of primary healthcare resources in general are important cost drivers.⁵⁾

Exposure to indoor and outdoor allergens is a major risk factor for AR. In Japan, allergic patients who suffer from perennial and/or seasonal AR are commonly poly-sensitized towards two major allergen sources, house dust mite (HDM) and Japanese cedar pollen (JCP). The most widely distributed HDM species that causes allergic symptoms worldwide are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. There is a large degree of cross-reactivity between the

two species, and HDM AR patients are most often sensitized to both *D. pteronyssinus* and *D. farinae*.

JCP pollinosis was first reported in 1963 and is now the main cause of seasonal AR in Japan from February to May. An epidemiological study has shown that the prevalence of JCP-induced pollinosis has increased by 10% annually in recent years, and currently AR afflicts an estimated 40% of the Japanese population.⁶⁾ Pharmacotherapy has been the major treatment of choice for patients with JCP-induced AR, but only 35% of AR patients are satisfied with antihistamine treatment, with the most frequent complaints being insufficient effect and daytime somnolence.⁷⁾

Allergy immunotherapy (AIT) is an established treatment option for allergic respiratory diseases.^{8–12)} AIT treatment consists of repeated sublingual or subcutaneous administration of allergens to gradually establish and increase immunological tolerance to the sensitizing allergens. Unlike pharmacotherapy, AIT addresses the basic immunological mechanisms of allergic disease and activates protective allergen-reactive pathways of the immune system. Therefore, AIT can provide relief from the clinical symptoms of AR and allergic asthma (AA)^{8–10)} and is the only known treatment option with the potential to provide long-term post-treatment benefit and alter the natural course of the allergic disease.^{12–14)} Furthermore, AIT has the ability to prevent both the onset of new allergic

*To whom correspondence should be addressed. e-mail: Katsuyo.doi@torii.co.jp

Table 1. Summary of Allergy Immunotherapy Product Development with Regulatory Approval in Japan

Allergen	Treatment	Product	Maintenance dose (JAU)	Indication
HDM	SCIT	Allergen extract	—	AR/AA
	SLIT drops	—	—	—
	Tablet	MITICURE® ACTAIR®	10000 57000	AR AR
JCP	SCIT	Allergen extract	—	AR
	SLIT drops	CEDARTOREN®	2000	AR
	Tablet	CEDARCURE®	5000	AR

sensitization and the development of co-morbidities of allergic rhinitis like AA.¹⁵⁾

2. ALLERGY IMMUNOTHERAPY

The concept of AIT recently celebrated its 100-year anniversary, based on the first scientific publication by Dr. L. Noon in 1911.^{16,17)} The paper by Noon described how subcutaneous inoculation with a grass pollen extract in patients suffering from seasonal hay fever provided resistance to subsequent conjunctival provocation with grass pollen extract and thereby established the principle of AIT. The main administration routes of AIT are subcutaneous injections (subcutaneous immunotherapy, SCIT) and sublingual administration (sublingual immunotherapy, SLIT). To date, SLIT in the form of tablets (SLIT tablets) and liquid formulations (SLIT drops) are available in Japan (Table 1). In general, SLIT tablets are currently available as freeze-dried or compressed formulations of allergen extracts^{8–10,12)} and SLIT drops are available as aqueous solutions of allergen extract, typically formulated with glycerin.^{18,19)} Both SLIT tablets and drops are administered under the tongue and held there until swallowed or spit out.

In 1998, the WHO position paper on immunotherapy classified AIT as a treatment form with the highest level of evidence and made recommendations regarding the composition and dosing of SCIT products.²⁰⁾ SCIT was shown to successfully reduce symptoms of AR and AA and lead to long-term tolerance against allergen exposure.^{21,22)} However, major drawbacks of SCIT include repeated painful injections and a certain risk of anaphylaxis connected with the subcutaneous injection regimen, as well as the necessity of repeated hospital or physician visits. As a consequence of these factors, sales of SCIT products in Japan have decreased steadily from the 1980s. In the WHO position paper, SLIT was proposed as a possible alternative to SCIT,²⁰⁾ and in a World Allergy Organization (WAO) position paper in 2009, SLIT was stated to be as effective as SCIT but with a better safety profile.²³⁾ The Japanese Rhinologic Society presented evidence-based recommendations concerning SLIT in 2012.²⁴⁾ Subsequently, there has been a steady increase in accumulated evidence of the clinical efficacy and tolerability of SLIT, and SLIT tablets in particular, for the treatment of both AR and AA.^{8–10,12)} A major milestone for SLIT tablet treatment was reached when the use of HDM SLIT as an add-on treatment for HDM-induced AA was included in the international Global Initiative for Asthma (GINA) global strategy for asthma management and prevention.⁴⁾

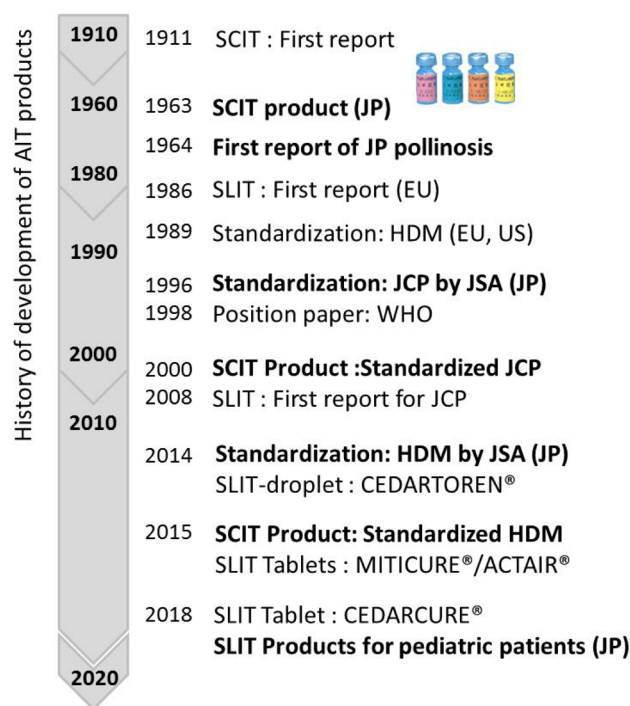


Fig. 1. History of Development of AIT Products
(Color figure can be accessed in the online version.)

3. CLINICAL EFFICACY

3.1. SCIT In Japan, SCIT was introduced in the early 1960s as a treatment for rhinitis and/or asthma caused by allergic sensitization (Fig. 1). The manufacture and use of non-standardized crude house dust extract for SCIT began in 1963 and continued until 2015, when standardized HDM allergen extract was developed.^{22,25)} Non-standardized JCP extract was launched in 1969 and used in clinical practice until 2000, when a method for JCP extract standardization was developed by the Japanese Allergology Society (JSA).²⁶⁾ SCIT with JSA-standardized HDM and JCP extracts has demonstrated reduced clinical symptoms and medication scores as well as improvement in the QOL.^{22,27)} Nevertheless, no large clinical trial has been performed in Japanese allergic patients with a standardized SCIT product.

3.2. SLIT Drops The clinical efficacy of SLIT drops using aqueous formulations of extracts of various allergen sources was demonstrated as the drops improved clinical symptoms compared to placebo in seasonal and perennial AR in both children and adults.^{28,29)} In Japan, two large clinical JCP SLIT trials (phases II and III) were conducted in 2008 and 2011, respectively.^{18,30)} These trials demonstrated statistically significant reductions in total nasal symptom and medication scores (TNSMS) compared to placebo with daily JCP SLIT drop treatment¹⁸⁾ (Fig. 2). Based on these results, the first Japanese SLIT drop product, Cedartolen, was registered in October 2014 as indicated for Japanese cedar pollinosis in adults and adolescents (≥ 12 years old). Cedartolen is based on a liquid formulation of JSA-standardized JCP allergen extract with a maintenance dose of 2000 Japanese Allergy Units (JAU).

Even though SLIT-drops are clinically effective against allergic symptoms, the maintenance of dose (2000 JAU) was

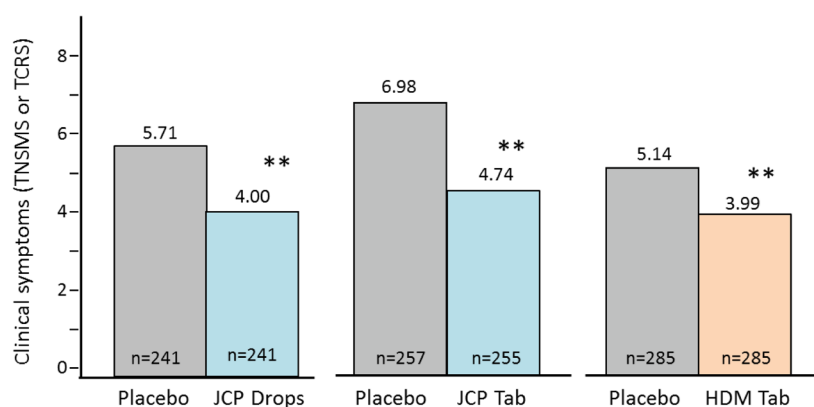


Fig. 2. Effects of SLIT Drops and SLIT Tablets on Clinical Symptoms in Allergic Patients

Effects of SLIT drops and SLIT tablets (JCP and HDM) on clinical symptoms in AR patients. Data are expressed as mean values. The statistical significance was determined using the *t*-test. ** $p < 0.01$ vs. placebo group. JCP drops: Cedartolen®; JCP tablets: CEDARCURE®; HDM tablets: MITICURE®. (Color figure can be accessed in the online version.)

chosen only as the maximum concentration based on SCIT products, without any dose-finding study. SLIT-drops (liquid formulations of allergen extracts) should be stored in a cold place (*i.e.*, 2–8°C) to ensure the stability of the formulation. SLIT tablets are therefore a more patient-friendly concept without any temperature control requirement.

3.3. SLIT Tablets Several comprehensive SLIT tablet clinical development programs for a number of different allergen sources including JCP, HDM, grass pollen, ragweed, and tree pollen have been conducted,^{8–12,31–34} which provided the regulatory basis for marketing authorizations (European Medicines Agency (EMA), U.S. Food and Drug Administration (FDA), and Pharmaceuticals and Medical Devices Agency (PMDA)) for a number of SLIT tablet products in different geographical regions including Europe, North America, and Japan. The trials included in these programs were all large, randomized, double-blind, placebo-controlled (DBPC) trials, and SLIT tablets are now the most thoroughly documented form of AIT.^{8–12}

In Japan, HDM SLIT tablets (Miticure and Actair) have been available since 2015.^{8,18} Although the nominal strengths of HDM SLIT tablets differ according to the major allergen content (Miticure: 10000 JAU; Actair: 57000 JAU) as assigned by the JSA,²⁵ both types of tablet showed clinical efficacy and good tolerability in Japanese patients with HDM-induced AR^{8,35} (Fig. 2). The apparent discrepancy between the clinically efficacious strengths of the two tablets may be due to different formulations since Miticure is based on a freeze-dried formulation and Actair is manufactured as a compressed tablet. Experimental comparisons between the two tablets have demonstrated a striking difference in their respective abilities to release allergen into solution: Whereas full allergen release from the freeze-dried tablet is achieved within a few seconds, a much longer period of time is needed for release of the same amount of allergen from the compressed tablet under identical experimental conditions.³⁶

Finally, a JCP SLIT tablet based on the same efficient freeze-dried formulation as Miticure was developed in 2018. In a large phases II/III trial, the clinical efficacy of three doses (2000, 5000, and 10000 JAU) of the JCP SLIT tablet was examined in 5- to 64-year-old patients.³¹ The optimal dose was determined to be 5,000 JAU, which is 2.5-fold higher than the maintenance dose (2000 JAU) of the SLIT-drops Cedartolen.

The dose of 5000 JAU was selected as the nominal strength of the JCP SLIT tablets now marketed in Japan under the name Cedarcure (Fig. 2).

During the initial development of SLIT tablets, large pivotal trials established their efficacy and tolerability in adult populations^{12,37} and in some cases in populations comprising both adults and adolescents,^{8,9} but large trials in pediatric populations have been lacking.

Recently, however, randomized DBPC trials that included pediatric patients have been conducted in Japan.^{11,31,38} Two of these trials investigated the effect and tolerability of SLIT tablets in the treatment of HDM-induced AR in patients aged between 5 and 17 years old. One in the form of a freeze-dried 10000-JAU HDM SLIT tablet,¹¹ and the other in the form of a compressed 300 index of reactivity (IR) (57000 JAU) HDM SLIT tablet.³⁸ A third trial was a phase II/III investigation of the clinical efficacy and tolerability of a freeze-dried JCP SLIT tablet in JCP pollinosis in a mixed pediatric/adult population comprising patients 5–64 years of age.³¹ In all three trials, the SLIT tablet treatments were well tolerated in all age-groups, but the two tablet types seem to differ with regard to efficacy. The treatment effect-size (improvement of clinical symptoms compared to placebo) obtained with the freeze-dried 10000-JAU HDM SLIT tablet were 21 and 26% in the pediatric population (5–11 years old) and the adolescent population (12–17 years old), respectively,¹¹ similar to the effects previously reported for the same HDM SLIT-tablets in a Japanese adult population.⁸ In the JCP SLIT tablet trial, the treatment effect-size was 23% in the pediatric (5–11-year-old) subgroup.³¹ For the compressed 300-IR/57000-JAU HDM SLIT tablet, the treatment effect-size in 5- to 16-year-old patients was 13%.³⁸

4. MECHANISM OF ACTION OF AIT

4.1. Allergic Reaction When an allergic individual who is sensitized to one or more allergen sources is exposed to airborne allergenic particles, *e.g.*, in the form of pollen grains, HDM fecal pellets, mold, or animal dander, individual allergen molecules and other, nonallergenic proteins adhere to the airway mucosa. The amounts of allergen required to elicit allergic symptoms are minute. Subjects sensitized to, *e.g.*, grass pollen, may react strongly to exposure levels that correspond

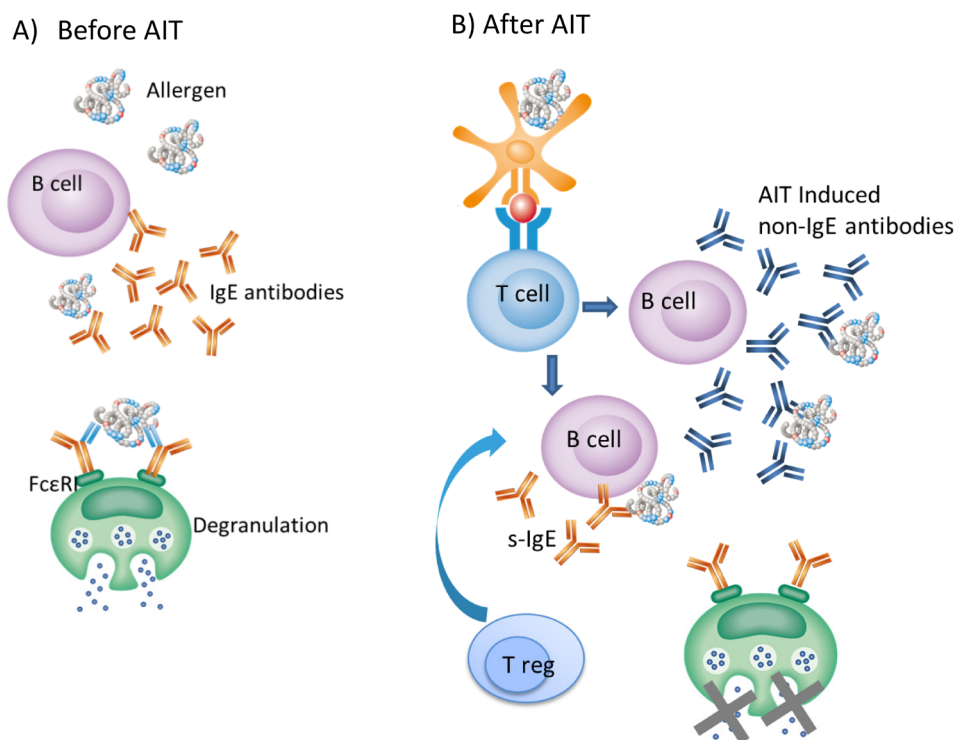


Fig. 3. Immunologic Responses in Allergy Patients

A) Before AIT. Patients become sensitized when exposed to allergen, and B cells start to produce IgE antibodies. IgE antibodies attach to the surface of effector cells (*i.e.*, mast cells, basophils). Exposure to allergen leads to cross-linking of the cell-bound IgE, followed by degranulation and rapid release of allergic mediators from effector cells. Inflammatory cells infiltrate into the tissue, and inflammatory reaction occurs in the late phase (6–10 h). B) After AIT. After AIT treatment, T cells instruct B cells to produce non-IgE-antibodies (IgG *etc.*). IgG antibodies do not cause degranulation of mast cells but compete with IgE for allergen binding. IgG and other allergen-specific non-IgE isotype antibodies block allergen binding to IgE, obstruct cross-linking of cell-bound IgE, and prevent allergen-mediated degranulation and release of allergic mediators from effector cells. During AIT, T cells develop into Treg cells that reduce IgE antibody production from B cells. (Color figure can be accessed in the online version.)

to as little as 10 pollen grains/m³.³⁷⁾ After being released from the allergenic particle on the mucosal surface, allergen molecules subsequently bind and cross-link to allergen-specific immunoglobulin E (IgE) antibodies that are attached to the surface of effector cells (mast cells and basophils) through the high-affinity transmembrane IgE receptor FcεRI. Through intracellular signaling pathways, the allergen-mediated FcεRI cross-linking leads to cellular degranulation and immediate release of histamine, leukotrienes, and other mediators that have been performed and stored in the effector cell granules. These mediators are directly responsible for the characteristic local symptoms of AR and rhinoconjunctivitis such as sneezing, runny nose, and red eyes (Fig. 3A). In parallel, allergen is taken up and presented by local antigen-presenting cells, leading to the activation of allergen-specific Th2 helper cells (Th2 cells). Proinflammatory cytokines, *e.g.*, interleukin (IL)-4, IL-13, and chemokines released by allergen-activated Th2 cells, are responsible for the further influx of mast cells and basophils as well as additional pro-inflammatory cell types such as eosinophils, neutrophils, and macrophages into the airways. In combination, allergen-mediated triggering of the immediate allergic reaction and activation of the later cellular responses contribute to the maintenance and amplification of airway inflammation.

Recently, group innate lymphoid group 2 (ILC2s) has focused on the implications for allergic type 2 inflammatory responses. ILC2s produce Th2 cytokines (IL-5, IL-13, *etc.*) that are involved in the allergic response. In human studies, peripheral blood ILC2s levels were increased 4 h after nasal

allergen challenge.³⁹⁾ ILC2s levels were increased in AR patients during the pollen season, indicating that ILC2s activation is induced by allergen, especially in the early phase of the allergic response. Although a very low number of ILC2s may be present in local tissue, Th2 activation by initiating ILC2s plays an important role in the immune response in allergic diseases. Interestingly, the suppression of ILC2s was found in AR patients with SCIT during the grass pollen season compared with untreated patients, suggesting that ILC2s might contribute to clinical and immunological tolerance with AIT during allergen exposure.⁴⁰⁾

4.2. Immunological Response Independent of the administration form, the aim of AIT is to reestablish immunological tolerance to allergen molecules and achieve a persistent beneficial clinical effect after discontinuation of treatment.⁴¹⁾ The immunological mechanism of successful AIT remains to be established in full but includes modulations of different subsets of allergen-specific T cells as well as the induction of a protective allergen-specific non-IgE antibody response. A recurring observation of AIT is treatment-induced immune deviation where the ratio of allergen-specific Th1:Th2 cells increases, driven primarily by a reduction in Th2 cells rather than an increased Th1 response.⁴²⁾ It was suggested that in the initial phase of AIT, the persistent stimulation of allergen-specific Th2 cells becomes transiently unresponsive and assumes a regulatory-like phenotype associated with IL-10 production.⁴³⁾ Continued allergen stimulation beyond the desensitization phase may lead to deletion of allergen-specific Th2 cells, which may result in longer-lasting AIT-induced pe-

ripheral tolerance and, in turn, persisting clinical effects.⁴³⁾ In addition to Th2 anergy and deletion, cell-mediated local and peripheral suppression of the allergen-specific Th2 response during AIT also involves the activity of allergen-specific T-regulatory cells (Tregs), including inducible IL-10-producing Treg cells.^{44,45)} However, owing to the refinement of experimental techniques and an increasing number of studies, multiple subsets of Tregs have now been identified, comprising cell types with both positive and negative influences on allergen tolerance, respectively.⁴⁶⁾

An additional possible contributor to immunological tolerance to inhaled allergens during AIT is the production of IgE-blocking antibodies which occurs as a result of treatment (Fig. 3B). Modulation of allergen-specific IgE antibodies as well as of allergen-specific antibodies of the non-IgE isotype (e.g., IgG1, IgG4, IgA) is a robust marker of AIT and follows a reproducible pattern.⁴⁷⁾ Increased HDM-specific IgG4 was observed after 52-week treatment with SLIT tablets (Fig. 4). During AIT, the group mean of allergen-specific IgE increased transiently in the early stages of treatment and then decreased to pretreatment levels over a period of months to a year. In contrast, the mean levels of allergen-specific IgG4 and allergen-specific antibodies of other non-IgE isotypes continue to increase for the duration of the treatment or may reach a steady plateau.^{47,48)}

Unlike IgE, the allergen-specific non-IgE antibodies do not cause effector cell degranulation in the presence of allergen. Rather, the increased concentrations of allergen-specific non-IgE antibodies induced by AIT are able to compete with IgE for the binding of allergen and thereby reduce effector

cell activation. The IgE-blocking capacity of AIT-induced antibodies can be assessed and quantified using the *ex vivo* basophil activation test (BAT).^{49–51)} However, as the BAT test requires access to fresh blood samples, alternative tests have been developed which provide a measure of AIT-induced IgE-blocking antibody activity. These include the IgE-blocking factor (IgE-BF) test⁵²⁾ and the facilitated antigen presentation (IgE-FAP) or binding assay (IgE-FAB).^{53,54)} The IgE-BF and IgE-FAB assays in particular have been used to monitor the immunological effects of AIT in clinical trials.^{12,55)}

4.3. Exploring Biomarkers A large body of data from large, randomized, DBPC clinical trials has demonstrated beyond doubt that AIT is a clinically effective treatment for AR with or without AA. However, although immunological pathways involving both the cellular and humoral compartments of the immune system have been described in detail, no surrogate biomarkers predictive or indicative of the effects of intervention with AIT have been identified. It has been proposed that candidate biomarkers of AIT should include immunological changes (e.g., allergen-specific IgG levels, T-cell responses, and/or cytokine production) and modification of the end-organ specific response (e.g., local allergic inflammation by provocation test).⁵⁶⁾

More recently, the European Academy of Allergy and Clinical Immunology (EAACI) has published a position paper with a review of immunological and clinical data obtained from clinical AIT trials and an evaluation of individual candidate biomarkers of AIT⁵⁷⁾ (Table 2). The task force that developed the EAACI position paper identified potential biomarkers within seven separate domains: 1) IgE (including total IgE (tIgE), specific IgE (sIgE), and sIgE/tIgE ratio); 2) IgG (including sIgG1, sIgG4, and sIgE/sIgG4 ratio); 3) IgE-inhibitory factors (IgE-BF and IgE-FAB); 4) basophil activation (BAT); 5) cytokines and chemokines; 6) cellular markers (Treg cells, Breg cells, and dendritic cells); and 7) *in vivo* biomarkers (including nasal and chamber provocation tests).⁵⁷⁾ It is noteworthy that four of the seven domains comprise biomarkers related directly to AIT-induced humoral responses (IgE, IgG) or to the inhibitory effect of allergen-specific non-IgE antibodies (IgE-BF, IgE-FAB, BAT). This may be due to the relatively easy access to serum antibodies and to the fact that *in vitro* antibody assays are less biased by experimental conditions than *in vitro* and *ex vivo* cellular assays, but it could also indicate the mechanistic relevance of IgE-blocking antibody development. An overall recommendation of the task force was to explore the use of allergen-specific IgG4 as a biomarker for compliance, and the s-IgE/t-IgE ratio and IgE-FAB as candidate biomarkers of clinical outcome.⁵⁷⁾

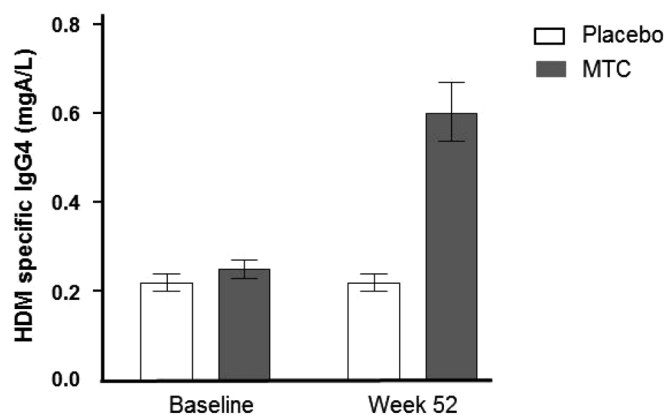


Fig. 4. Increase in Allergen-Specific IgG4 Antibodies after HDM SLIT Tablet Treatment

HDM (*D. farinae*)-specific IgG4 was measured using ImmnoCAP (Phadia). Data are expressed as geometric mean with 95% CI values. MTC: MITICURE® (10000 JAU).

Table 2. Currently Exploring Candidate Biomarker for AIT Products

Category	Detector	Index
Antibody	IgE	Sensitization
	IgG4	AIT induced antibodies
Functional assay	Basophil-activated inhibition (BAT)	Degranulation (histamine)
	IgE-blocking factor	Inhibitory effect on ALG-IgE complex
	Facilitated antigen presentation (FAP) inhibition	IgE-allergen binding
Others	Inflammatory cells (eosinophils)	Allergic reactions
	PBMCs/cytokines	Th1/Th2, balance

5. CONCLUSION AND FUTURE DIRECTIONS OF SLIT

Although allergy has become a global health problem with increasing prevalence worldwide, the awareness of the burden of disease for both individual patients and society at large is often not fully recognized. Allergic patients' access to AIT may require a long, tedious journey through the healthcare system where nonresponsiveness or unsatisfactory response to symptomatic medication must be established before AIT is prescribed. Given the potential of AIT to prevent further (neo)sensitization and prevent or delay the progression from AR to AA, more focus on the benefits and easier access to AIT for eligible patients is desirable. In Japan, the passing of the Basic Act on Allergic Diseases Measures and Equalization of Medical Care in Allergic Diseases throughout Japan in 2014 was a strong statement and call for action to raise awareness of the need to improve the quality of and access to the treatment of allergic disease.⁵⁸⁾

Recently, the unmet needs of AIT have been discussed by an international panel of experts who identified 10 different domains of improvement for clinical trial design,⁵⁹⁾ two of which had already been addressed by Japanese researchers: Screening of AIT trial participants with a standardized allergen provocation test (domain ii in Pfaar *et al.*⁵⁹⁾ was an inclusion criterion in a large, randomized, DBPC HDM SLIT tablet trial.⁸⁾ In domain ix, Pfaar *et al.* recommended changes in national legislation that would allow easier, faster access to AIT for children without the delay caused by a mandatory 5-year adult clinical program.⁵⁹⁾ Although additional high-quality pediatric trials are needed, recent data by three Japanese groups have demonstrated the efficacy and tolerability of HDM SLIT tablets in pediatric populations^{11,31,38)} following 1-year adult SLIT tablet trials.

An area that is likely to receive more attention in the future is the identification and validation of clinical and immunological biomarkers. In recent years, considerable efforts have been invested in large, randomized, DBPC SLIT tablet trials across several species, and the data accumulating from these trials provide a unique opportunity to investigate and possibly identify objective biomarkers of AIT and SLIT tablet immunotherapy in particular. The amount of data coming from these trials is historically unsurpassed, and by mining the combined bulk of clinical and immunological data it may be possible to identify surrogate markers of clinical effect or markers predictive of responders and nonresponders/placebo responders, which may identify eligible patients on an objective basis prior to treatment initiation. Although research in new AIT products and treatment concepts will continue, gaining a further understanding of allergic respiratory diseases and the clinical and immunological effects of SLIT tablet treatment through focused biomarker research may be the next step toward more efficient, personalized allergy treatment.

Acknowledgments The authors would like to thank Dr. Akira Hasegawa and Mr. Yusuke Tanaka for expert technical assistance.

Author Contributions KOD and KL contributed substantially to the conception of the contents and drafting the manuscript. YM and KO improved the manuscript. All authors approved the manuscript prior to submission to the journal.

Conflict of Interest KOD, YM, and KO are employees of Torii Pharmaceutical Co., Ltd., Tokyo, Japan. KL is an advisor for research projects and medical writing at Torii Pharmaceutical Co., Ltd.

REFERENCES

- 1) Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss M. "The WAO White Book on Allergy (update 2013)." World Allergy Organization; 2013.: <www.worldallergy.org> accessed December 3, 2015.
- 2) Brożek JL, Bousquet J, Agache I, *et al.* Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines—2016 revision. *J. Allergy Clin. Immunol.*, **140**, 950–958 (2017).
- 3) Canonica GW, Cox L, Pawankar R, *et al.* Sublingual immunotherapy: World Allergy Organization position paper 2013 update. *World Allergy Organ J.*, **7**, 6 (2014).
- 4) GINA 2018: Global Initiative for Asthma. "Global Strategy for Asthma Management and Prevention, 2018." <www.ginasthma.org>.
- 5) Green W, Kleine-Tebbe J, Klimek L, Hahn-Pedersen J, Norgaard Andreasen J, Taylor M. Cost-effectiveness of SQ[®] HDM SLIT-tablet in addition to pharmacotherapy for the treatment of house dust mite allergic rhinitis in Germany. *Clinicoecon. Outcomes Res.*, **9**, 77–84 (2017).
- 6) Yamada T, Saito H, Fujieda S. Present state of Japanese cedar pollinosis: the national affliction. *J. Allergy Clin. Immunol.*, **133**, 632–639 (2014).
- 7) Konno A, Kubo N. Patient satisfaction with the second generation antihistamines in the treatment of pollinosis and improved awareness on medical consultation—choice of drugs based on a large-scale questionnaire survey in patients with pollinosis. *Prog. Med.*, **28**, 2285–2296 (2008).
- 8) Okubo K, Masuyama K, Imai T, Okamiya K, Stage BS, Seitzberg D, Konno A. Efficacy and safety of the SQ house dust mite sublingual immunotherapy tablet in Japanese adults and adolescents with house dust mite-induced allergic rhinitis. *J. Allergy Clin. Immunol.*, **139**, 1840–1848.e10 (2017).
- 9) Nolte H, Bernstein DI, Nelson HS, Kleine-Tebbe J, Sussman GL, Seitzberg D, Rehm D, Kaur A, Li Z, Lu S. Efficacy of house dust mite sublingual immunotherapy tablet in North American adolescents and adults in a randomized, placebo-controlled trial. *J. Allergy Clin. Immunol.*, **138**, 1631–1638 (2016).
- 10) Virchow JC, Backer V, Kuna P, Prieto L, Nolte H, Villesen HH, Ljorring C, Riis B, de Blay F. Efficacy of a house dust mite sublingual allergen immunotherapy tablet in adults with allergic asthma: A randomized clinical trial. *JAMA*, **315**, 1715–1725 (2016).
- 11) Masuyama K, Okamoto Y, Okamiya K, Azuma R, Fujinami T, Riis B, Ohashi-Doi K, Natsui K, Imai T, Okubo K. Efficacy and safety of SQ house dust mite sublingual immunotherapy-tablet in Japanese children. *Allergy*, **73**, 2352–2363 (2018).
- 12) Durham SR, Emminger W, Kapp A, de Monchy JG, Rak S, Scadding GK, Wurtzen PA, Andersen JS, Tholstrup B, Riis B, Dahl R. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J. Allergy Clin. Immunol.*, **129**, 717–725.e5 (2012).
- 13) Valovirta E, Petersen TH, Piotrowska T, *et al.* Results from the 5-year SQ grass sublingual immunotherapy tablet asthma prevention (GAP) trial in children with grass pollen allergy. *J. Allergy Clin. Immunol.*, **141**, 529–538.e13 (2018).
- 14) Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.*, **341**, 468–475 (1999).
- 15) Passalacqua G. Specific immunotherapy: beyond the clinical scores. *Ann. Allergy Asthma Immunol.*, **107**, 401–406 (2011).
- 16) Noon L. Prophylactic inoculation against hay fever. *Lancet*, **177**, 1572–1573 (1911).

- 17) Durham SR, Leung DY. One hundred years of allergen immunotherapy: time to ring the changes. *J. Allergy Clin. Immunol.*, **127**, 3–7 (2011).
- 18) Okamoto Y, Okubo K, Yonekura S, Hashiguchi K, Goto M, Otsuka T, Murata T, Nakao Y, Kanazawa C, Nagakura H, Okawa T, Nakano K, Hisamitsu M, Kaneko S, Konno A. Efficacy and safety of sublingual immunotherapy for two seasons in patients with Japanese cedar pollinosis. *Int. Arch. Allergy Immunol.*, **166**, 177–188 (2015).
- 19) Mösges R, Pasch N, Schlierenkamper U, Lehmacher W. Comparison of the biological activity of the most common sublingual allergen solutions made by two European manufacturers. *Int. Arch. Allergy Immunol.*, **139**, 325–329 (2006).
- 20) Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J. Allergy Clin. Immunol.*, **102**, 558–562 (1998).
- 21) Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.*, **117**, 319–325 (2006).
- 22) Fujisawa T, Shimoda T, Masuyama K, *et al.* Long-term safety of subcutaneous immunotherapy with TO-204 in Japanese patients with house dust mite-induced allergic rhinitis and allergic bronchial asthma: multicenter, open label clinical trial. *Allergol. Int.*, **67**, 347–356 (2018).
- 23) Canonica GW, Bousquet J, Casale T, *et al.* Sub-lingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy*, **64** (Suppl. 91), 1–59 (2009).
- 24) Masuyama K, Goto M, Takeno S, Ohta N, Okano M, Kamijo A, Suzuki M, Terada T, Sakurai D, Horiguchi S, Honda K, Matsune S, Yamada T, Sakashita M, Yuta A, Fuchiwaki T, Miyanohara I, Nakayama T, Okamoto Y, Fujieda S. Guiding principles of sublingual immunotherapy for allergic rhinitis in Japanese patients. *Auris Nasus Larynx*, **43**, 1–9 (2016).
- 25) Takai T, Okamoto Y, Okubo K, Nagata M, Sakaguchi M, Fukutomi Y, Saito A, Yasueda H, Masuyama K. Japanese Society of Allergology task force report on standardization of house dust mite allergen vaccines — secondary publication. *Allergol. Int.*, **64**, 181–186 (2015).
- 26) Yasueda H, Okuda M, Yoshida H, Ito K, Baba M, Iikura Y, Okuhira H, Inoue S, Sakaguchi M, Miyamoto A. Guidelines for standardization of allergens and cedar pollen allergen extract in Japan. *Arerugi*, **45**, 416–421 (1996).
- 27) Fujisawa T, Tanida H, Nagao M. Rush immunotherapy for Japanese cedar pollinosis. *Jpn. J. Pediatr. Allergy Clin. Immunol.*, **26**, 151–157 (2012).
- 28) Nelson H, Cartier S, Allen-Ramey F, Lawton S, Calderon MA. Network meta-analysis shows commercialized subcutaneous and sublingual grass products have comparable efficacy. *J. Allergy Clin. Immunol. Pract.*, **3**, 256–266.e3 (2015).
- 29) Creticos PS, Esch RE, Couroux P, Gentile D, D'Angelo P, Whitlow B, Alexander M, Coyne TC. Randomized, double-blind, placebo-controlled trial of standardized ragweed sublingual-liquid immunotherapy for allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.*, **133**, 751–758 (2014).
- 30) Okubo K, Gotoh M, Fujieda S, Okano M, Yoshida H, Morikawa H, Masuyama K, Okamoto Y, Kobayashi M. A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. *Allergol. Int.*, **57**, 265–275 (2008).
- 31) Gotoh M, Yonekura S, Imai T, Kaneko S, Horikawa E, Konno A, Okamoto Y, Okubo K. Long-term efficacy and dose-finding trial of Japanese cedar pollen sublingual immunotherapy tablet. *J. Allergy Clin. Immunol. Pract.*, **7**, 1287–1297.e4 (2019).
- 32) Nolte H, Hebert J, Berman G, Gawchik S, White M, Kaur A, Liu N, Lumry W, Maloney J. Randomized controlled trial of ragweed allergy immunotherapy tablet efficacy and safety in North American adults. *Ann. Allergy Asthma Immunol.*, **110**, 450–456 (2013).
- 33) Couroux P, Ipsen H, Stage BS, Damkjaer JT, Steffensen MA, Salapatek AM, Lund K, Wurtzen PA. A birch sublingual allergy immunotherapy tablet reduces rhinoconjunctivitis symptoms when exposed to birch and oak and induces IgG4 to allergens from all trees in the birch homologous group. *Allergy*, **74**, 361–369 (2019).
- 34) Mäkelä MJ, Gyllfors P, Valovirta E, Steffensen MA, Gronager PM, Savolainen J, Winther L. Immunotherapy with the SQ tree SLIT-tablet in adults and adolescents with allergic rhinoconjunctivitis. *Clin. Ther.*, **40**, 574–586 (2018).
- 35) Okamoto Y, Fujieda S, Okano M, Yoshida Y, Kakudo S, Masuyama K. House dust mite sublingual tablet is effective and safe in patients with allergic rhinitis. *Allergy*, **72**, 435–443 (2017).
- 36) Ohashi-Doi K, Kito H, Du W, Nakazawa H, Ipsen H, Gudmann P, Lund K. Bioavailability of house dust mite allergens in sublingual allergy tablets is highly dependent on the formulation. *Int. Arch. Allergy Immunol.*, **174**, 26–34 (2017).
- 37) D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. *Allergy*, **62**, 976–990 (2007).
- 38) Okamoto Y, Fujieda S, Okano M, Hida H, Kakudo S, Masuyama K. Efficacy of house dust mite sublingual tablet in the treatment of allergic rhinoconjunctivitis: A randomized trial in a pediatric population. *Pediatr. Allergy Immunol.*, **30**, 66–73 (2019).
- 39) Doherty TA, Broide DH. Airway innate lymphoid cells in the induction and regulation of allergy. *Allergol. Int.*, **68**, 9–16 (2019).
- 40) Lao-Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH. Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J. Allergy Clin. Immunol.*, **134**, 1193–1195 (2014).
- 41) Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J. Allergy Clin. Immunol.*, **140**, 1485–1498 (2017).
- 42) Bacher P, Scheffold A. The effect of regulatory T cells on tolerance to airborne allergens and allergen immunotherapy. *J. Allergy Clin. Immunol.*, **142**, 1697–1709 (2018).
- 43) Wambre E. Effect of allergen-specific immunotherapy on CD4+ T cells. *Curr. Opin. Allergy Clin. Immunol.*, **15**, 581–587 (2015).
- 44) Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. *J. Allergy Clin. Immunol.*, **121**, 1467–1472.e1 (2008).
- 45) Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J. Allergy Clin. Immunol.*, **120**, 707–713 (2007).
- 46) Boonpiyathad T, Sokolowska M, Morita H, Ruckert B, Kast JI, Wawrzyniak M, Sangasapaviliya A, Pradubongsa P, Fuengthong R, Thantiworasit P, Sirivichayakul S, Kwok WW, Ruxrungtham K, Akdis M, Akdis CA. Der p 1-specific regulatory T-cell response during house dust mite allergen immunotherapy. *Allergy*, **74**, 976–985 (2019).
- 47) Akdis CA, Akdis M. Advances in allergen immunotherapy: aiming for complete tolerance to allergens. *Sci. Transl. Med.*, **7**, 280ps6 (2015).
- 48) Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, Till SJ. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J. Allergy Clin. Immunol.*, **121**, 1120–1125.e2 (2008).
- 49) MacGlashan DW Jr. Basophil activation testing. *J. Allergy Clin. Immunol.*, **132**, 777–787 (2013).
- 50) Nopp A, Cardell LO, Johansson SG, Oman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy*, **64**, 811–814 (2009).
- 51) Würtzen PA, Lund G, Lund K, Arvidsson M, Rak S, Ipsen H. A double-blind placebo-controlled birch allergy vaccination study II:

- correlation between inhibition of IgE binding, histamine release and facilitated allergen presentation. *Clin. Exp. Allergy*, **38**, 1290–1301 (2008).
- 52) Johansen N, Gronager PM, Ipsen H, Stranzl T, Lund K. Characterisation of a new method for quantitative determination of house dust mite allergen specific IgE-blocking factor for monitoring allergy immunotherapy with SQ HDM SLIT-tablet. *Allergy*, **71** (Suppl. 102), 118–272 (2016).
- 53) Shamji MH, Wilcock LK, Wachholz PA, Dearman RJ, Kimber I, Wurtzen PA, Larche M, Durham SR, Francis JN. The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. *J. Immunol. Methods*, **317**, 71–79 (2006).
- 54) Shamji MH, Francis JN, Wurtzen PA, Lund K, Durham SR, Till SJ. Cell-free detection of allergen-IgE cross-linking with immobilized phase CD23: inhibition by blocking antibody responses after immunotherapy. *J. Allergy Clin. Immunol.*, **132**, 1003–1005.e4 (2013).
- 55) Shamji MH, Ljorring C, Francis JN, Calderon MA, Larche M, Kimber I, Frew AJ, Ipsen H, Lund K, Wurtzen PA, Durham SR. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy*, **67**, 217–226 (2012).
- 56) EMA, European Medicines Agency. CHMP/EWP/18504/06. *Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases*. November, 2008.
- 57) Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF, Kleine-Tebbe J, Bohle B, Chaker AM, Till SJ, Valenta R, Poulsen LK, Calderon MA, Demoly P, Pfaar O, Jacobsen L, Durham SR, Schmidt-Weber CB. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper. *Allergy*, **72**, 1156–1173 (2017).
- 58) Nishima S. Basic act on allergic diseases measures and equalization of medical care in allergic diseases throughout the Japan. *Arerugi*, **66**, 190–203 (2017).
- 59) Pfaar O, Alvaro M, Cardona V, Hamelmann E, Mosges R, Kleine-Tebbe J. Clinical trials in allergen immunotherapy: current concepts and future needs. *Allergy*, **73**, 1775–1783 (2018).