International Consensus on Allergen Immunotherapy II: Mechanisms, standardization, and pharmacoeconomics

Marek Jutel, MD,[a] Ioana Agache, MD,[b] Sergio Bonini, MD,[c] A. Wesley Burks, MD,[d] Moises Calderon, MD,[e] Walter Canonica, MD,[f] Linda Cox, MD,[g] Pascal Demoly, MD,[h] Antony J. Frew, MD, FRCP,[i] Robyn O'Hehir, FRACP, PhD,[j] Jörg Kleine-Tebbe, MD,[k] Antonella Muraro, MD, PhD,[l] Gideon Lack, MD,[m] Désirée Larenas, MD,[n] Michael Levin, MD,[o] Bryan L. Martin, MD,[p] Harald Nelson, MD,[q] Ruby Pawankar, MD,[r] Oliver Pfaar, MD,[s] Ronald van Ree, PhD,[t] Hugh Sampson, MD,[u] James L. Sublett, MD,[v] Kazunari Sugita, MD,[w] George Du Toit, MD,[x] Thomas Werfel, MD,[y] Roy Gerth van Wijk, MD,[z] Luo Zhang, MD,[aa] Mübeccel Akdis, MD,[w] and Cezmi A. Akdis, MD[w] *Wroclaw, Poland, Brasov, Romania, London and Brighton, United Kingdom, Chapel Hill, NC, Rome, Genova, and Padua, Italy, Fort Lauderdale, Fla, Montpellier, France, Melbourne, Australia, Berlin, Mannheim, and Hannover, Germany, Mexico City, Mexico, Cape Town, South Africa, Silver Spring, Md, Denver, Colo, Tokyo, Japan, Amsterdam and Rotterdam, The Netherlands, New York, NY, Louisville, Ky, Davos, Switzerland, and Beijing, China*

This article continues the comprehensive international consensus (ICON) statement on allergen immunotherapy (AIT). The initial article also recently appeared in the *Journal*. The conclusions below focus on key mechanisms of AIT-triggered tolerance, requirements in allergen standardization, AIT cost-effectiveness, and regulatory guidance. Potential barriers to and facilitators of the use of AIT are described in addition to future directions. International allergy specialists representing the European Academy of Allergy and Clinical Immunology; the American Academy of Allergy, Asthma & Immunology; the American College of Allergy, Asthma and Immunology; and the World Allergy Organization critically reviewed the existing literature and prepared this summary of recommendations for best AIT practice. The authors contributed equally and reached consensus on the statements presented herein. (J Allergy Clin Immunol 2016;137:358-68.)

Key words: *International consensus, allergy, immunotherapy, allergen vaccine, allergen standardization, pharmacoeconomics, cost-effectiveness, mechanisms, tolerance, marketing authorization, regulatory authorities, unmet needs*

This article represents the second part of the international consensus (ICON) document on allergen immunotherapy (AIT), an effort of the International Collaboration in Asthma, Allergy and Immunology that includes the European Academy of Allergy and Clinical Immunology; the American Academy of Allergy, Asthma & Immunology; the American College of Allergy, Asthma and Immunology; and the World Allergy Organization. There are other articles that outline international or national guidelines, positions, or consensus statements on the current knowledge on AIT. In this document we offer a critical appraisal of major evidence on AIT mechanisms, recommendations on allergen standardization (AS), regulatory issues, pharmacoeconomics, and barriers to and facilitators of future developments in AIT. The governing boards of the participating organizations approved the final draft.

Abbreviations used

AIT:	Allergen immunotherapy
AS:	Allergen standardization
BAU:	Bioequivalent allergen units
Breg:	Regulatory B
CUA:	Cost-utility analysis
D50:	Dilution of extract that on average produces a 50 mm erythema (sum of lengths and width)
DC:	Dendritic cell
EMA:	European Medicines Agency
EU:	European Union
FOXP3:	Forkhead box protein 3
ICER:	Incremental cost-effectiveness ratio
IHRP:	In-house reference preparation
ILC:	Innate lymphoid cell
ILC2:	Type 2 innate lymphoid cell
MA:	Marketing authorization
NPP:	Named-patient product
QALY:	Quality-adjusted life year
SCIT:	Subcutaneous immunotherapy
SLIT:	Sublingual immunotherapy
ST:	Standard treatment
TLR:	Toll-like receptor
Treg:	Regulatory T
VIT:	Venom immunotherapy

MECHANISMS OF IMMUNOTHERAPY

The allergen-specific immune response involves a series of complex mechanisms. These include the structural features and dose of allergen, the route and timing of its exposure, the existence of innate immune response stimulants within the allergen and micromilieu, and the genetic susceptibility of the host.[1,2] Effective AIT sequentially activates multiple mechanisms (Fig 1), ideally resulting in multifaceted clinical improvement. Depending on the AIT protocol, desensitization to allergen, allergen-specific immune tolerance, and suppression of allergic inflammation appear within hours. This is followed by allergen-specific regulatory T (Treg) and regulatory B (Breg) cell generation, regulation of allergen-specific IgE and IgG_4, and

establishment of immune tolerance (Fig 1, *A*). AIT in particular targets type II immune cells, including T_H2 cells, type 2 innate lymphoid cells (ILC2), and type 2 cytotoxic T cells, which produce IL-4, IL-5, and IL-13, which induce mast cell, basophil, and eosinophil activation, as well as IgE antibody production (Fig 1, *B*).[3,4]

From [a]the Department of Clinical Immunology, Wroclaw Medical University, and "ALL-MED" Medical Research Institute, Wroclaw; [b]the Faculty of Medicine, Transylvania University, Brasov; [c]the Second University of Naples and IFT-CNR, Rome, and Expert-on-Secondment European Medicines Agency, London; [d]the Department of Pediatrics, University of North Carolina, Chapel Hill; [e]the Section of Allergy and Clinical Immunology, Imperial College London, National Heart and Lung Institute, Royal Brompton Hospital, London; [f]the Allergy & Respiratory Diseases Clinic, DIMI University of Genoa, IRCCS AOU San Martino, Genova; [g]the Allergy and Asthma Center, Fort Lauderdale; [h]University Hospital of Montpellier-INSERM U657, Montpellier; [i]the Department of Respiratory Medicine, Royal Sussex County Hospital, Brighton; [j]the Department of Allergy, Immunology and Respiratory Medicine, The Alfred Hospital and Monash University, Melbourne; [k]Allergy & Asthma Center Westend, Berlin; [l]the Department of Mother and Child Health, Padua General University Hospital; [m]the Division of Asthma, Allergy and Lung Biology, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, and the Children's Allergy Unit, Guy's and St Thomas' NHS Foundation Trust, London; [n]Hospital Médica Sur, Mexico City; [o]the Division of Allergy, School of Child and Adolescent Health, Red Cross War Memorial Children's Hospital, Cape Town; [p]the Department of Allergy and Immunology, Walter Reed Army Medical Center, Silver Spring; [q]National Jewish Health, Denver; [r]the Department of Pediatrics, Nippon Medical School, Tokyo; [s]the Center for Rhinology and Allergology, Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Mannheim; [t]Academic Medical Center, Departments of Experimental Immunology and of Otorhinolaryngology, University of Amsterdam; [u]the Department of Pediatrics, Division of Allergy-Immunology, and the Jaffe Food Allergy Institute at the Icahn School of Medicine at Mount Sinai, New York; [v]the Department of Pediatrics, Section of Allergy and Immunology, University of Louisville School of Medicine, Louisville; [w]the Swiss Institute for Allergy and Asthma Research, University of Zurich, and Christine Kühne-Center for Allergy Research and Education, Davos; [x]the Department of Pediatric Allergy, Division of Asthma, Allergy & Lung Biology, King's College London, and MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London; [y]the Department of Dermatology and Allergy, Division of Immunodermatology and Allergy Research, Hannover Medical School; [z]Erasmus Medical Center, Rotterdam; and [aa]the Beijing Institute of Otolaryngology, Beijing.

Disclosure of potential conflict of interest: M. Jutel has received consultancy fees from Anergis and has received lecture fees from Allergopharma. S. Bonini has received travel support and is an expert on secondment at the European Medicine Agency. A. W. Burks is on the FARE and World Allergy Organization boards; is on the Murdoch Children's Research Institute Advisory Board; is an unpaid consultant for Dynavax Technologies, Perrigo Company (PBN Nutritionals), and Perosphere; has received consultancy fees from GLG Research (Gerson Lehrman Group), ActoGeniX, Genentech, Sanofi US Services, and Valeant Pharmaceuticals North America; is employed by the University of North Carolina; has patents US5558869, US55973121, US6441142, US6486311, US6835824, US7485708, and US7879977; has received payment for developing educational presentations from Current Views 2012; has stock/stock options in Allertein and Mastcell Pharmaceuticals; and has received travel support for various grand rounds and presentations. M. Calderon is on the boards for ALK-Abelló and HAL Allergy, has received consultancy fees from ALK-Abelló and Stallergenes, and has received lecture fees from ALK-Abelló, Stallergenes, Merck, and Allergopharma. L. Cox has received personal fees from Greer and is the lead author on one of the studies discussed in the correspondence (publication date 2011) but has had no relationship with the sponsoring company in the past 36 months. P. Demoly has received consultancy fees from ALK-Abelló, Circassia, Stallergenes-Greer, Allergopharma, DBV, Thermo Fisher Scientific, Chiesi, and Pierre Fabre Medicament and has received lecture fees from Menarini, MSD, and AstraZeneca. A. J. Frew is on the ALK-Abelló board and has received research support from NIHR (UK). D. Larenas is on the CMICA board with no financial gain; has received consultancy fees from MEDA, Pfizer, MIT, BI, Novartis Glenmark, and Chiesi; has received research support from Novartis, Pfizer, MEDA, UCB, GlaxoSmithKline, Astra Zeneca, Senosiain, Sanofi, MSD, TEVA, and Carnot; has received lecture fees from Novartis, Pfizer, MEDA, Astra Zeneca, Sanofi, MSD, TEVA, and Chiesi; has received payment for development of educational presentations from Glenmark; and has received travel support from Novartis, Pfizer, MEDA, AstraZeneca, Sanofi, MSD, and Chiesi. M Levin is on the boards for the Allergy Society of South Africa and the Allergy Foundation of South Africa; has received consultancy fees from Mylan, Pharma Dynamics, and INNOVA Pharma; has received research support from Mylan, the Medical Research Council of South Africa, Thermo Fisher, GlaxoSmithKline/Aspen, AstraZeneca, Cipla, Astellas, Pharma Dynamics, and Beiersdorf; and has received lecture fees from Cipla, Takeda Nycomed, Pharma Dynamics, and Novartis. H. Nelson has received research support from Circassia and has received consulting fees from Circassia, Merck, Pearl Therapeutics and AstraZeneca. O. Pfaar has received research grants for his institution from Allergopharma (Germany), ALK-Abelló (Denmark), Stallergenes (France), HAL-Allergy (Netherlands), Artu Biologicals (Netherlands), Allergy Therapeutics/Bencard (United Kingdom/Germany), Hartington (Spain), Lofarma (Italy), Novartis/Leti (Germany/Spain), GlaxoSmithKline (United Kingdom), Essex-Pharma (Germany), Cytos (Switzerland), Curalogic (Denmark), Roxall (Germany), Biomay (Austria), Thermo Fisher (United States), Circassia (United Kingdom), E.U (FP-7-Health-2013-Innovation 1), Biotech Tools (Belgium), and MEDA-Pharma (Sweden); has received personal payments as consultant for Allergopharma (Germany), Anergis (Switzerland), Bencard (Germany), HAL-Allergy (Netherlands), Novartis/LETI (Germany), MEDA-Pharma (Germany), ALK-Abelló (Denmark), Biotech Tools (Belgium), GfK Bridgehead (United Kingdom), NAVIGANT-consulting (United States), Sanofi (United States), Guidepoint Global Advisors (United States), Pohl-Boskamp (Germany), Stallergenes (France), and Mobile Chamber Experts (a GA2LEN partner, Germany); has received personal payments for lectures, book chapters, or educational presentations from ALK-Abelló (Denmark), Allergopharma (Germany), Stallergenes (France), HAL-Allergy (Germany/Netherlands), Allergy Therapeutics/Bencard (United Kingdom/Germany), Hartington (Spain), Lofarma (Italy), Novartis/Leti (Germany/Spain), GlaxoSmithKline (Germany), Roxall (Germany), Thermo-Fisher (Germany), MEDA-Pharma (Germany), Schattauer (Germany), Springer (Germany). and GlaxoSmithKline (Germany); has served as advisor and on the speakers' bureaus for some of the aforementioned companies; has received travel grants from HAL-Allergy (Netherlands), Allergopharma (Germany), the European Academy of Allergy and Clinical Immunology (EAACI), the German Society for Allergology and Clinical Immunology (DGAKI), and the German Respiratory Society (DGP); and is the current chairman of the Immunotherapy Interest Group (IT IG) of the EAACI and is secretary of the ENT section of the DGAKI. R van Ree has received research support from the European Commission; has received consultancy fees as the Scientific Advisory Board Chair for HAL Allergy BV; is Vice President of the EAACI; has received consultancy fees from Bayer CropScience; and has received lecture fees from Thermo Fisher Scientific. H. Sampson, as of November 1, 2015, is a part-time employee of DBV Technologies and serves as their Chief Scientific Officer; DBV Technologies is developing an epicutaneous patch for the treatment of peanut allergy and other disorders. G. Du Toit has received research support from the National Institute of Allergy and Infectious Diseases (NIAID; NO1-AI-15416 [contract] and UM1AI109565 [grant], covering salary); has received contribution to NIAID contract/grant from Food Allergy and Research Education (FARE); has received Contribution to the KCL Division of Asthma, Allergy & Lung Biology (of which Pediatric Allergy Research is a part) from MRC & Asthma UK Centre; has received a BRC award to Guy's and St Thomas' NHS Foundation from the UK Department of Health through NIHR; and has equity holding in FoodMaestro. R Gerth van Wijk has received consultancy fees from MSD, HAL, Crucell, ALK-Abelló, Novartis, and Emerade; has received research support from NOW, STW, Novartis, Biomay, and DBV; has received lecture fees from Allergopharma and Thermo Fisher; has received payment for manuscript preparation from Chiesi; and receives royalties from de Tijdstroom and Bohn, Stafleu, van Loghum. C. A. Akdis has received consultancy fees from Actelion, Aventis, Stallergenes, Allergopharma, and Circacia; is employed by the Swiss Institute of Allergy and Asthma Research, University of Zurich, Switzerland; has received research support from Novartis, PREDICTA (European Commission's Seventh Framework programme no. 260895), the Swiss National Science Foundation, MeDALL (European Commission's Seventh Framework Programme no. 261357), and the Christine Kühne-Center. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication October 23, 2015; Revised November 30, 2015; Accepted for publication December 8, 2015.

Corresponding author: Marek Jutel, MD, Department of Clinical Immunology, Wroclaw Medical University, Chalubinskiego 5, PL-50-368 Wrocław, Poland. E-mail: marek.jutel@umed.wroc.pl.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.12.1300

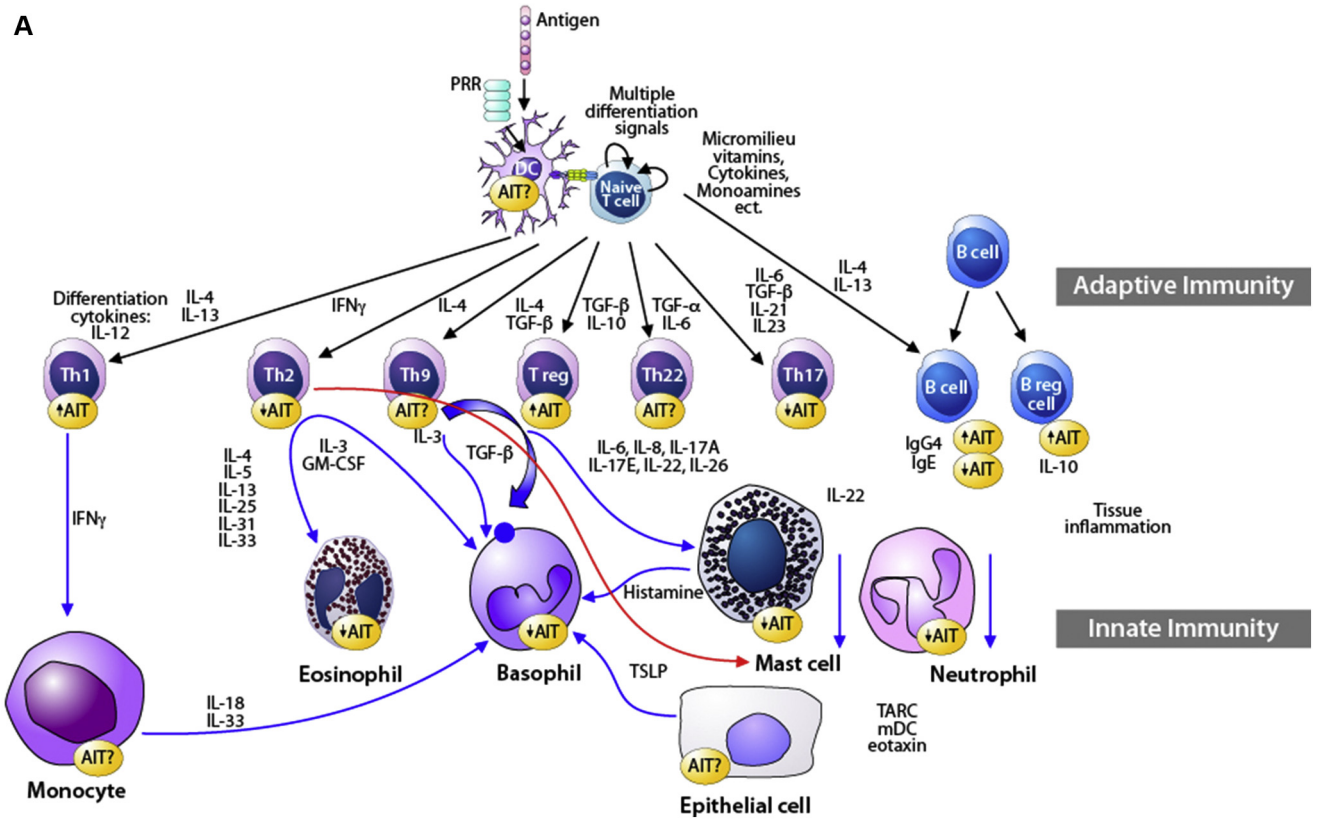

FIG 1. Cellular and molecular changes during AIT. **A,** Differentiation of naive T cells after allergen presentation in the presence of innate immune response substances that trigger pattern recognition receptors *(PRR)* and vitamins, monoamines that control cellular differentiation, and coexposed substances with the antigen and status of the cells and cytokines in the microenvironment is shown. Naive T cells can differentiate into T_H1, T_H2, T_H9, T_H17, and T_H22 T cells. Based on their respective cytokine profiles, responses to chemokines, and interactions with other cells, these T-cell subsets can contribute to general inflammation. The increase in T_H1 and Treg cell numbers plays a role in counterbalancing other effector cells. The balance between allergen-specific effector T cells (particularly T_H2 cells) and IL-10–producing Treg cells is decisive for the development or suppression of allergic inflammation. Treg cells and their cytokines suppress T_H2-type immune responses and contribute to the control of allergic diseases in several major ways. Similarly, induction of IL-10–producing Breg cells plays an essential role in suppression of IgE and induction of IgG_4. **B,** The suppression of peripheral ILCs, especially ILC2s, might contribute to T_H2 suppression and immunologic tolerance induced by AIT. *iNKT*, Invariant natural killer T; *TSLP*, thymic stromal lymphopoietin.

Early desensitization

The literature indicates that administration of AIT leads to very early decreases in the susceptibility of mast cells and basophils to degranulation in spite of the presence of increased allergen-specific IgE levels.[5] This effect appears to be similar to the one observed when these 2 immune cell types are rapidly desensitized in anaphylactic reactions to drugs.[6] Several mechanisms have been proposed to explain why mast cells and basophils become unresponsive to environmental proteins even in the presence of specific IgE. A number of studies have investigated the involvement of basophils in the very early induction of allergen tolerance and the so-called desensitization effect of venom immunotherapy (VIT).[7-9] Rapid upregulation of histamine type 2 receptors within the first 6 hours of the build-up phase of VIT was observed, which suppressed FcεRI-induced activation and mediator release of basophils,[7] and histamine receptor 2 has strong immune regulatory activities on T cells, dendritic cells (DCs), and basophils.[10] Overall, mast cells and basophils express many targets for future enhancement of the efficacy of AIT, as well as the development of novel biomarkers.[11,12]

T-cell tolerance

AIT induces a major change in allergen-specific T-cell subsets. The proportion of IL-4–secreting T_H2 cells decreases; meanwhile, IL-10–secreting inducible Treg cells specific for the same allergenic epitope increase in number and achieve function similar to the immune status observed in nonallergic healthy subjects. This appears to be one of the milestones in the development of peripheral tolerance to allergens.[1,13] A significant correlation exists between improvement of symptoms and the increase in inducible Treg cell numbers during immunotherapy.[14,15] Inducible Treg cells are composed of 2 sets: forkhead box protein 3 (FOXP3)–adaptive Treg cells and $FOXP3^-$ but IL-10–producing type 1 regulatory cells.[16] Studies investigating the role of different types of Treg cells during AIT have shown overlapping effects of

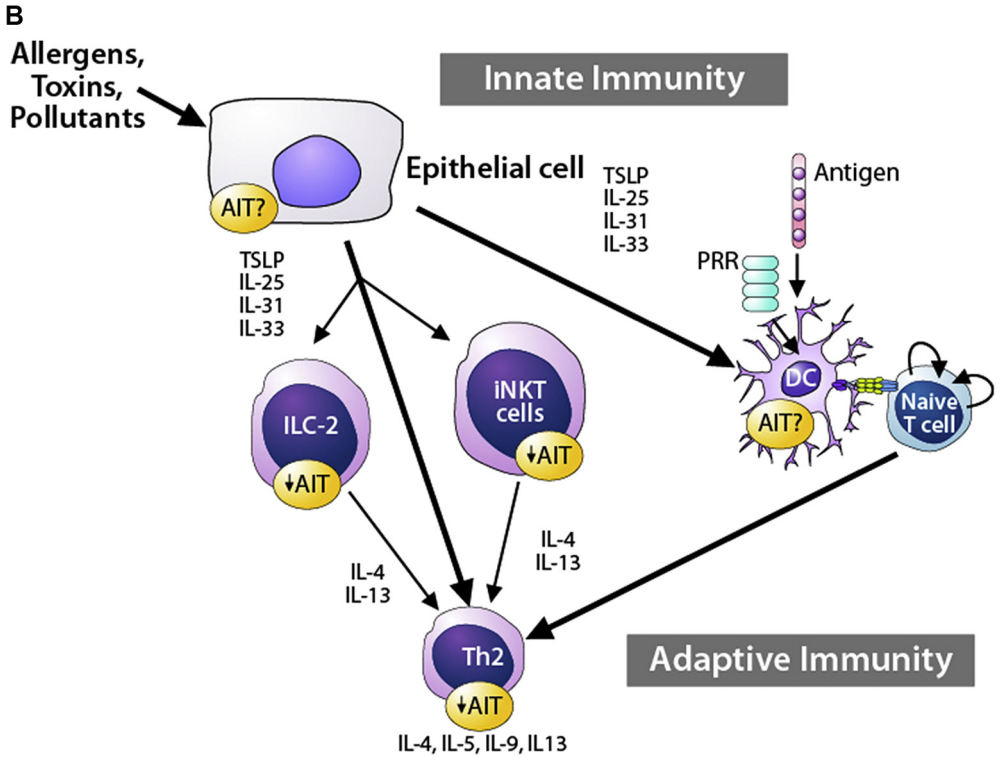

FIG 1. *(Continued).*

different Treg cell subsets for the induction of T-cell tolerance.[17,18] Secretion of IL-10 and TGF-β and expression of cytotoxic T lymphocyte antigen 4 and programmed death 1 protein on T-cell surfaces are also important for the suppressor activity of inducible Treg cells. Additionally, the runt homology domain transcription factors 1 and 3 both have an effect on TGF-β–mediated FOXP3 expression of inducible Treg cells in human subjects.

Various mechanisms can underlie AIT's induction of an allergen-specific Treg cell response.[19,20] It has been recently suggested that the target organ and site of immune tolerance induction during sublingual immunotherapy (SLIT) might be the tonsils.[21] This could hold true even in patients with tonsillectomy because the procedure removes only the pharyngeal tonsils while preserving the lingual and palatine tonsils. Plasmacytoid DCs with a high percentage of Treg cells were colocalized in human palatine and lingual tonsils. The ability of plasmacytoid DCs of human tonsil cells to generate $CD4^+CD25^+CD127^-FOXP3^+$ functional Treg cells further supports the tolerogenic function of DCs.[20] Similar to mechanisms of AIT, in high-dose antigen exposure of beekeepers, IL-10–secreting Treg cells inhibited proliferation of phospholipase A-specific effector T cells 7 days after the beginning of the bee venom season.[22] Blocking cytotoxic T lymphocyte antigen 4, programmed death 1, and IL-10 receptors inhibited this suppressive effect. Mouse models to mimic these effects are being developed, and prolonged desensitization schedules have been proposed to study immune tolerance–inducing activities.[23]

Another important recent study investigated the mechanisms underlying the way in which allergen tolerance can be broken in healthy subjects. The authors indicate stimulation of allergen-specific T cells with certain Toll-like receptors (TLRs), and proinflammatory cytokines can induce *in vitro* $CD4^+$ T-cell proliferation in peripheral lymphocytes. In this context stimulation of myeloid DCs with IL-1β, IL-6, TLR4, and TLR8 breaks allergen-specific $CD4^+$ T-cell tolerance.[24] Viral infections might play a role in immune tolerance–breaking roles through the abovementioned or other molecular mechanisms. Infection of the respiratory epithelium with rhinovirus can antagonize tolerance to inhaled antigen through combined induction of thymic stromal lymphopoietin, IL-33, and OX40 ligand.[25]

Box 1. Effective AIT triggers multiple mechanisms, which are sequentially activated (Fig 2)

AIT-induced immune tolerance controls:
- the acute phase of the allergic reaction and
- chronic events leading to inflammation and remodeling.

B-cell tolerance

The phenotypic expression of Breg cells plays a role in allergic disease. Distinct from IL-10–secreting DCs, IL-10–secreting allergen-specific Breg cells were shown to exist in bee venom–tolerant beekeepers and patients with bee venom allergy who had undergone VIT.[26] They were characterized as $CD73^-CD25^+CD71^+$ B cells, with a suppressive function on antigen-specific $CD4^+$ T cells and the capacity to produce specifically IgG_4. This work is supported by data showing that IL-10 overexpression in human B cells is sufficient to induce a regulatory role of B cells.[27] In addition to the direct role of Breg cells, Treg cell–derived IL-10 stimulates B cells to undergo class-switching toward production of IgG antibodies in the presence of IL-4, whereas IL-4 alone induces IgE production.[28] Human B cells can regulate $CD4^+$ T-cell plasticity to create flexibility

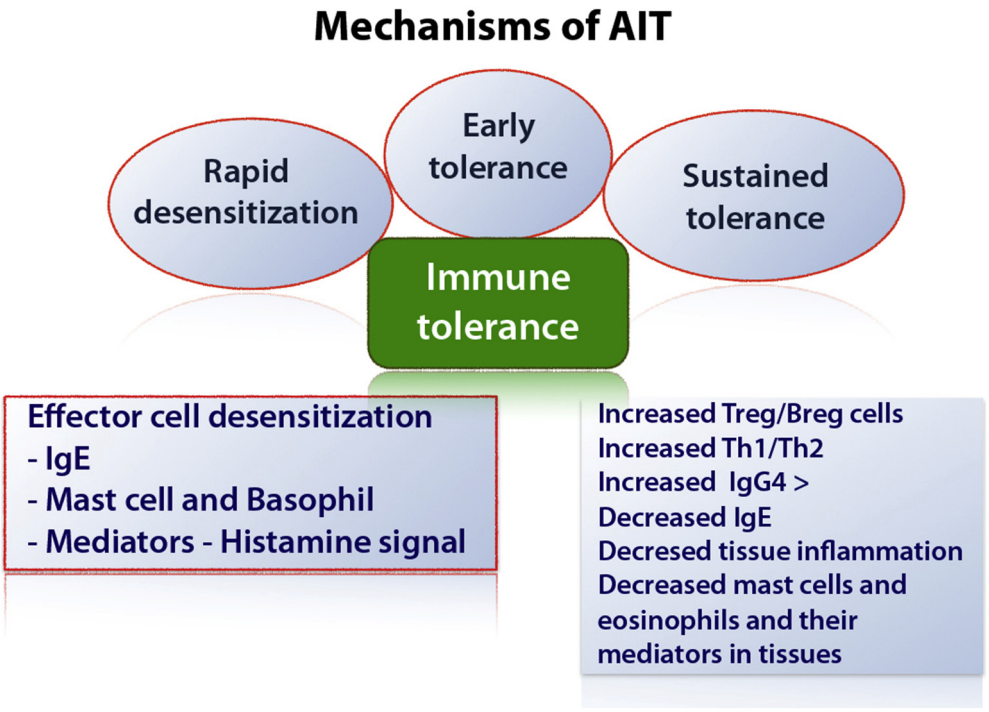

FIG 2. Rapid desensitization. Very early decreases in the susceptibility of mast cells and basophils to degranulation are observed. Mediators of anaphylaxis (histamine and leukotrienes) are released during AIT without inducing a systemic anaphylactic response. Several mechanisms have been proposed, such as upregulation of histamine type 2 receptors and decreased effector cell function, as reflected by a decrease in allergen-stimulated surface expression of CD63. Early changes in basophil sensitivity predict symptom relief with AIT. Immune tolerance involves the gradual increase in Treg and Breg cell numbers and tolerogenic antibody levels. Long-term tolerance induced by AIT involves changes in the memory T- and B-cell compartment, the T_H1/T_H2 shift, and the function of effector and structural cells.

in the effector T-cell response.[29] As a tolerogenic antibody, allergen-specific IgG_4 competes with allergen-specific IgE with the same specificity for allergen binding, thus preventing the release of mediators from mast cells and basophils. There is further possible formation of IgE-allergen-IgG_4 complexes that bind to both the FcγRIIb and FcεRI, inhibiting the IgE receptor.[30] IgG_4 antibodies of different specificities can exchange their immunoglobulin heavy chain through a process referred to as Fab arm exchange. This process leads to the formation of bispecific, functionally monovalent IgG_4 antibodies that are unable to cross-link allergens.[31] Furthermore, IgG_4 is unable to fix complement and has limited affinity for activating Fcγ receptors.[32] AIT is known to induce a transient increase in serum IgE levels in the early course of treatment, despite its protective clinical efficacy. The ratio of allergen-specific IgE to functional IgG_4 antibody might be useful in monitoring AIT because the IgE blocking activity of IgG_4 appears to correlate with clinical AIT outcome.[33,34]

Regulation of ILCs

ILC2s play a role in allergic responses through secretion of IL-5 and IL-13, and ILC2s can be studied in human peripheral blood.[3,4] ILC2s might have a role in the development of adaptive type 2 responses to local, but not systemic, antigen exposure.[35] ILC2s can also be demonstrated in induced sputum in children.[36] AIT has been shown to regulate ILCs, and seasonal increases in peripheral ILC2 numbers are inhibited by subcutaneous grass pollen immunotherapy.[37] Circulating ILC2 responses are increased in asthmatic patients but not in those with allergic rhinitis.[38] For further information, see Box 1 and Fig 2.

STANDARDIZATION OF ALLERGEN EXTRACTS

AS is a prerequisite to providing reagents for the diagnosis of and allergen-specific intervention in atopic diseases. Established methods for AS measure potency, ensure consistency in composition, and demonstrate stability. Molecular technologies have accelerated the characterization of allergen preparations, providing optimal reagents for advanced AS.[39]

AS and regulatory framework

European manufacturers use in-house reference preparations (IHRPs) and create their own allergen extract units accordingly.[40] The European Medicines Agency (EMA) recently adopted a guideline on production and quality of allergen products (http://www.gmp-compliance.org/guidemgr/files/GUIDELINE ON ALLERGEN PRODUCTS PRODUCTION AND QUALITY ISSUES.PDF). Homologous allergens are now based on sequence identity among allergenic proteins rather than taxonomic relationships between allergen sources. This guideline complements existing documents for development and marketing authorization (MA) of products for AIT in Europe. The US Food and Drug Administration provides guidance for US manufacturers. Vaccines standardized for potency in the United States include

Hymenoptera venoms (5 species), cat hair and pelt, dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), and pollen from 8 grass species and short ragweed. For each standardized extract, reference materials from the Center of Biologics Evaluation and Research are used to determine potency, forming the basis of IHRP calibration.

Biological AS *(in vivo)*

The Nordic method, which is commonly used in Europe, considers 10,000 biologically standardized units/mL to be equivalent to an allergen dose that elicits a wheal equal (in square millimeters) to that elicited by 10 mg/mL histamine dihydrochloride. *In vivo* testing consists of titrated skin prick tests with 5-fold allergen dilutions averaged in at least 20 moderately to highly sensitized allergic subjects. The intradermal dilution for the 50-mm sum of erythema that determines bioequivalent allergy units ($ID_{50}EAL$) method is used in the United States.[41] The dilution of extract that on average produces a 50-mm induration (sum of lengths and width [D50]) is assigned an arbitrary potency of 10,000 bioequivalent allergen units (BAU)/mL. Extracts with a mean D50 of 14, which falls between the 13th and 15th 3-fold serial dilution of the reference extract, are arbitrarily assigned the value of 100,000 BAU/mL. An extract with a mean D50 falling between the 11th and 13th dilutions is labeled 10,000 BAU/mL.

Biochemical and immunologic standardization *(in vitro)*

Various qualitative and quantitative biochemical methods provide information on extract composition.[42] Newer methods, such as mass spectrometry, can be expensive and technically challenging but can offer extremely powerful approaches for analysis of allergenic proteins, including detection of isoforms. Total potency is measured by IgE-binding inhibition or effector (ie, basophil) cell assays. Manufacturers usually combine different methods for AS and establish various in-process control measures for robust and reproducible allergen extract production.

Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification project and follow-up

A World Health Organization/International Union of Immunological Societies–initiated and European Union (EU)–funded (FP5) project for the Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification established comprehensive information on purified or recombinant forms of important major allergens (Bet v 1, Phl p 1, Phl p 5, Ole e 1, Der p 1, Der p 2, Der f 1, and Der f 2) and explored immunoassays for their quantification.[43,44] A follow-up project, which was supported by the Biological Standardization Program (BSP) of the European Directorate for the Quality of Medicines, performed a proficiency trial (BSP090) for ELISAs of Bet v 1 and Phl p 5a.[45-47] After approval by the European Pharmacopoeia Commission, these assays will become mandatory for allergen manufacturers in IHRP calibration. In 2012, both major allergens were introduced by the European Pharmacopoeia Commission as biological reference materials (http://crs.edqm.eu/db/4DCGI/View=Y0001565 and http://crs.edqm.eu/db/4DCGI/View=Y0001566), and the future will likely bring important additions.

PHARMACOECONOMICS AND COST-EFFECTIVENESS OF IMMUNOTHERAPY

The costs of allergic diseases are substantial, and AIT is a treatment modality that might alter the natural course of disease. In the long run of health economics, immunotherapy has the potential to result in cost savings because of decreased loss of workdays and lower drug costs, although it is not to be expected that the costs will be fully offset by savings in antiallergic medications during the first years of therapy. Economic studies have been published on the cost-effectiveness of immunotherapy, primarily from Europe and the United States.

Costs of AIT and standard treatment

Retrospective analyses have shown that subcutaneous immunotherapy (SCIT) affects health care expenditure.[48-50] In comparing costs before and after SCIT treatment among 3048 Medicaid-enrolled children with allergic rhinitis, SCIT produced a 12% reduction.[48] An 18-month period of SCIT resulted in associated costs that were reduced by 33% compared with those incurred by pediatric control subjects.[49] A prospective observational *Parietaria* species SCIT study revealed a cost reduction of 48% in the third year of treatment and of 80% 3 years after AIT concluded.[51] A ragweed immunotherapy trial of 2 years in asthmatic patients showed 30% reduction in medical costs in the immunotherapy versus placebo groups, but these savings did not offset the increased costs of immunotherapy.[52] A 1-year SLIT observational study showed a reduction in the costs of symptomatic drugs of 22% for patients with rhinitis and 34% for patients with rhinitis and asthma. When the costs of SLIT were included, the costs in the SLIT group were 73% higher.[53] Another SLIT house dust mite study in asthmatic patients compared 2 years of treatment with SLIT plus standard treatment (ST) with ST only, followed by 3 years of ST only. The savings in the fifth year amounted to 23%.[54]

Cost-effectiveness and cost-utility analyses

Economic analyses of both benefits of treatment and financial cost are important in addressing the question of whether one outweighs the other. Cost-effectiveness analysis studies express costs in monetary units and effects in physical units (eg, symptom-free days and occurrence of asthma exacerbations). Cost-utility analysis (CUA) evaluates the effects of treatment in terms of health-related quality of life (ie, quality-adjusted life years [QALYs]). An incremental cost-effectiveness ratio (ICER), which is defined as costs divided by benefits, can be calculated to estimate the costs of a certain gain. A gain of 1 QALY at a threshold of £20,000 to £30,000 is considered acceptable (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/191504/NICE_guide_to_the_methods_of_technology_appraisal.pdf).

Several cost-effectiveness analysis studies have demonstrated that SCIT and SLIT are economically advantageous.[55-58] A German study based on data from the literature in a decision tree model reached break even within a duration of 6 to 8 years and net savings at 10 years.[55] A French study, also based on a decision tree model, used the number of improved patients and the

number of asthmatic patients avoided as determination of outcome. ICERs were lower for SCIT (€583 and €597 for dust-mite and pollen allergy) than those for SLIT (€3938 and €824).[57]

The cost-effectiveness of SCIT was confirmed by 2 CUAs and those of SLIT by 4 CUAs derived from randomized clinical trials with sublingual grass pollen tablets.[52,54,59-61] Another CUA based on a *post hoc* analysis of 2 SLIT studies indicated that an ICER of less than the threshold of £20,000 could be achieved in patients with medium or high outcomes in their symptom scores.[62] One CUA evaluated treatment with different grass pollen products (Oralair [Stallergenes, Antony, France], Grazax [ALK-Abelló, Hørsholm, Denmark], and Alutard [ALK-Abelló] depot). From the German health care perspective (cost-utility ratio vs symptomatic treatment; incremental costs, QALYs, and willingness-to-pay) the analysis resulted in dominance of Oralair.[63]

Recently, a cost-effectiveness model was constructed based on MD data from the Rhinoconjunctivitis Quality of Life Questionnaire through meta-analyses and indirect comparison meta-analyses. Up to year 6, ICERs (cost per QALY) ranged from £28,650 (year 6) to £57,883 (year 3) for SCIT compared with ST and from £27,269 to £83,560 for SLIT compared with ST. Thus, with increasing time, both SCIT and SLIT were found to be approaching cost-effectiveness thresholds of £20,000 to £30,000.[64]

In conclusion, the majority of pharmacoeconomics studies support the viewpoint that AIT gives value for the money, with cost-effectiveness within 6 years of treatment initiation. However, heterogeneity in methodology limits interpretation of the studies. Data are obtained from small studies, retrospective databases, prospective observational studies, randomized trials, and literature searches. It is difficult to extrapolate the results from one health care setting to another, and there is considerable variation in cost-effectiveness across countries.[65] In addition, trials do not reflect real-life context, with noncompliance as a strong bias for economic analyses. Finally, many pharmacoeconomics studies have been sponsored by or associated with manufacturers. Large prospective and independent cost-effectiveness studies using a study design that provides a more realistic model are required. Moreover, there is a lack of economic data in other areas of the world outside Europe or the United States.

REGULATORY ISSUES

Although Noon[66] introduced AIT more than a century ago, a high degree of heterogeneity among countries on the regulatory aspects of this therapeutic option remains. In Europe the majority of products for AIT have been marketed for decades as named-patient products (NPPs), which are primarily responsible for meeting requirements of Good Manufacturing Practice.[67] Thus NPPs for AIT are commercially available and Good Manufacturing Practice compliant, even if they are "named patient," a term that refers to their prescription for a specific allergic patient.[42]

For these NPPs, information on clinical efficacy is not necessarily based on the documentation required by regulatory agencies for providing an MA, whereas numbers of adverse reactions are mainly assessed based on voluntary reports by producers, allergists, and patients.

In the last decade, the Directive 2001/20/EC and the amended Directive 2003/63/EC published important regulatory guidance, proposing central specifications for allergen products in both diagnostics and AIT.[42,67] Under these regulations, allergen products are classified as medicinal products. Given that they have the capacity to modify the immune system and because they are produced with an industrial process, they require an MA similar to all medicinal drugs. The EMA and national health authorities of the individual member states serve as regulatory agencies. Attaining an MA for allergen products is feasible through national or centralized procedures, as well as through mutual recognition.[42,67-69] In a national authorization the allergen product is only approved for marketing in the respective European country in which the application has been submitted. However, the approval can be expanded to other European member states in a "mutual recognition" procedure if the identical dossiers are submitted to these countries.[68]

Another possibility for EU-wide registration of medicinal products is the centralized procedure, in which the application dossier is initially submitted to the EMA as coordinating regulatory authority.[42,68] The EMA determines 2 representative European countries as rapporteur and corapporteur in reviewing and evaluating these dossiers. The central authorization allows MAs in all EU member states. The central procedure must be followed for an MA for recombinant allergen vaccines and other products based on biotechnological processes.[68] Other countries, such as the United States, currently follow a different set of procedures (Table I).[33,58,62,64,69]

The quality, safety, and clinical efficacy of allergen products under these authorization processes are required to be documented through a straightforward development plan, as outlined in the EMA guidance on the "Clinical development of products for specific immunotherapy for the treatment of allergic diseases" (CHMP/EWP/18504/2006; 2008). Applicants receive scientific advice from the EMA or from the national competent authorities on the preclinical and clinical phases of the development of the respective allergen products.[42] In addition to the development plan, the applicant must submit a pediatric investigational plan before an application for an MA can be submitted to the EMA (available at: http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500015814.pdf. 2009).

BARRIERS AND FACILITATORS FOR AIT

In spite of the facts that AIT represents a well-established, evidence-based therapy and that there has been great progress in both vaccine development and means of application in recent years, a number of key barriers and facilitators should be noted, as shown in Table II.[70,71]

FUTURE OF AIT

Recent advances in immunology and bioengineering enable ongoing modifications of AIT.[2,72] Still, the quality level of current evidence for these advances can be variable and includes conceptual studies in experimental models, proof-of-concept clinical studies with a limited number of subjects, and large-scale multicenter clinical studies (Box 2).

The most promising approaches to improve the efficacy and safety of vaccine-based AIT include bypassing IgE binding and targeting allergen-specific T and B cells with hypoallergenic recombinant allergen derivatives and immunogenic peptides, new adjuvants and stimulators of the innate immune response, fusion

TABLE I. Obtaining an MA in EU countries

National authorization	The allergen product is only approved for marketing in the respective European country in which the application has been submitted.	The approval can be expanded to other European member states in a "mutual recognition" procedure if the identical dossiers are submitted to these countries.[62]
EU-wide registration	The application is submitted to the EMA, which nominates 2 EU countries as rapporteur and corapporteur for review and evaluation.[33,62] The application contains a development plan documenting the quality, safety, and clinical efficacy of allergen products, as outlined in the EMA guidance (CHMP/EWP/18504/2006; 2008)[64] and a pediatric investigational plan.[58]	The central authorization allows an MA in all EU member states. The central procedure must be followed for recombinant allergen vaccines and other products based on biotechnological processes.[62]

TABLE II. Barriers and facilitators for better use of AIT

Barriers	
The application of AIT is limited in many areas because of the low awareness of AIT's potential.	There is worldwide acceptance and increased awareness that AIT reduces long-term costs and burden of allergies and potentially changes the natural course of the disease.
Regulations on AIT	Regulations on AIT have profound effects on allergy practice, allergen manufacturers, and research programs. Especially in the EU, allergy vaccines should undergo registration as all other drugs. There is a need for a standardized approach between regulatory agencies from different regions of the world.
Adherence to AIT	The demand of prolonged treatment over several years might impair patients' adherence.
Facilitators	
Evidence-based documentation	Standardization, validation, and consensus on clinical outcome measures for clinical trials Identification and validation of biomarkers for AIT monitoring Environmental exposure chambers as suitable surrogates for natural allergen exposure[70,71] Validated tools for assessing the effectiveness of AIT in real life: postmarketing studies
Guidelines and recommendations	Standardization of guidelines and recommendations at global and national societal levels
Better selection of patients	Diagnostic tools for better identification of the clinically relevant patient's sensitization profile for a proper vaccine selection Proper use of component-resolved diagnosis to identify potential responders and nonresponders
More convenient AIT regimens	Validation of different regimens (preseasonal and perennial), mode of updosing, duration of therapy, maximal dose, and cumulative dose in terms of efficacy and safety
Novel approaches	Confirmation of existing evidence of efficacy and safety of novel approaches in independent phase 3, double-blind, placebo-controlled trials
Pharmacoeconomics	More evidence on the overall cost-saving effects of AIT application Limit the high costs of current treatment and clinical development
Joint commitment	Coordinated actions among regulators, industry, and the scientific environment to find solutions that properly answer the health expectations of allergic patients

of allergens with immune modifiers and peptide carrier proteins, and new routes of vaccine administration.[24,73-75] Similar approaches are being undertaken in AIT for food allergy, and some progress has been made through the development of AIT encompassing 3 major forms of treatment: oral immunotherapy, SLIT, and epicutaneous immunotherapy.[76]

The cloning of allergen proteins and genetic engineering have enabled the production of vaccines that have well-defined molecular, immunologic, and biological characteristics, as well as modified molecular structure (allergen fragments, fusions, hybrids, and chimeras).[73,74] These approaches open the possibility of enhancing the tolerogenic T cell–dependent signal with administration of higher doses of preparation and a low risk of anaphylaxis. Clinical trials with recombinant allergen preparations primarily for grass pollen, birch pollen, and house dusts mites showed good clinical efficacy compared with placebo. However, because they do not show significantly better effects than natural extracts, the pharmaceutical industry has stopped development because of the problematic justification of the high costs of vaccine development and licensing.[77,78] Large multicenter clinical studies with peptide vaccines for cat and birch allergy are currently underway.

The application of more powerful adjuvants might be easier and economically justified. Detoxified LPS (monophosphoryl lipid A), CpG oligonucleotides, imidazoquinolines, and adenine derivatives, all of which activate innate immune response, are the most suitable candidates for allergy vaccination, with more effective induction of specific T_H1 differentiation.[79] Studies are being performed with 1,25-dihydroxyvitamin D3 as an additive to increase Treg cell responses by affecting DCs for their tolerogenic properties.[80] Novel research provides an enormous number of immune stimulators and methods for coupling with allergens; however, both proof-of-concept and controlled large clinical studies have yet to be performed.[73,74,79,80] Another approach

Box 2. Improving the efficacy and safety of vaccine-based AIT by targeting allergen-specific T and B cells and bypassing IgE binding

Hypoallergenic recombinant allergen derivatives and immunogenic peptides.

New adjuvants and stimulators of the innate immune response.

Fusion of allergens with immune modifiers and peptide carrier proteins.

New routes of vaccine administration. Combination of AIT with immune response modifiers, including anti-IgE (omalizumab).

Box 3. Consensus statement on AIT mechanisms and recommendations for standardization and pharmacoeconomics

1. AIT is an immune-mediated biological treatment, which acts through the complex interplay between Treg and Breg cells, blocking IgG_4 antibodies and tissue effector–mediated mechanisms.
2. Providing reagents for AIT requires application of modern biotechnological approaches for AS and vaccine preparation.
3. The majority of pharmacoeconomics studies demonstrate the cost-effectiveness of AIT within 6 years of treatment initiation.
4. Regulatory agencies classified AIT vaccines as medicinal products, which require an MA similar to medicinal products.
5. Better understanding of barriers and facilitators for AIT is essential for further developments in the field.
6. Recent progress in biotechnology and the understanding of the mechanism of AIT open the window for new opportunities for safer and more effective AIT.

includes allergen covalently coupled to carbohydrate-based particles for targeting DCs with enhanced adjuvanticity or the use of a carrier protein, such as the PreS domain of the hepatitis B virus fused to 2 nonallergenic peptides.[81] A good safety profile, a significant decrease in the risk of anaphylaxis, and improved rescue medication scores were also reported for the combination of AIT with immune response modifiers, including anti-IgE (omalizumab).[82,83]

In the treatment of allergic rhinitis and asthma, both SCIT and SLIT show efficacy in reducing symptom scores and medication use, improving quality of life, and inducing sustained disease-modifying effects based on changes in specific immunologic markers.[2] Work is ongoing for new routes of administration, such as the intralymphatic and epicutaneous routes.[84] In addition, extension of SLIT to other allergens in randomized phase 3 trials to develop new products is being pursued, as are schedules and efforts to shorten the duration of AIT.[85,86] Direct head-to-head studies comparing novel routes with SCIT are strongly needed.[84,87]

CONCLUSIONS

This portion of the international consensus document provides a comprehensive overview of AIT mechanisms, recommendations for standardization, and pharmacoeconomics. In addition, we have critically appraised barriers to and facilitators of further study and provided perspective on what waits on the AIT horizon (Box 3).

We thank Professor Stefan Vieths for critical reading of the manuscript.

REFERENCES

1. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol 2014;133:621-31.
2. 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;131:1288-96.e3.
3. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 2015;135:626-35.
4. Agache I, Sugita K, Morita H, Akdis M, Akdis CA. The complex type 2 endotype in allergy and asthma: from laboratory to bedside. Curr Allergy Asthma Rep 2015;15:29.
5. Uermosi C, Zabel F, Manolova V, Bauer M, Beerli RR, Senti G, et al. IgG-mediated down-regulation of IgE bound to mast cells: a potential novel mechanism of allergen-specific desensitization. Allergy 2014;69:338-47.
6. Romano A, Torres MJ, Castells M, Sanz ML, Blanca M. Diagnosis and management of drug hypersensitivity reactions. J Allergy Clin Immunol 2011;127(suppl):S67-73.
7. Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. J Allergy Clin Immunol 2012;130:1153-8.
8. Shamji MH, Layhadi JA, Scadding GW, Cheung DK, Calderon MA, Turka LA, et al. Basophil expression of diamine oxidase: a novel biomarker of allergen immunotherapy response. J Allergy Clin Immunol 2015;135:913-21.e9.
9. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. J Allergy Clin Immunol 2015;135:1249-56.
10. Ferstl R, Frei R, Schiavi E, Konieczna P, Barcik W, Ziegler M, et al. Histamine receptor 2 is a key influence in immune responses to intestinal histamine-secreting microbes. J Allergy Clin Immunol 2014;134:744-6.e3.
11. Harvima IT, Levi-Schaffer F, Draber P, Friedman S, Polakovicova I, Gibbs BF, et al. Molecular targets on mast cells and basophils for novel therapies. J Allergy Clin Immunol 2014;134:530-44.
12. Kaczorowski M, Jutel M. Human T regulatory cells: on the way to cognition. Arch Immunol Ther Exp 2013;61:229-36.
13. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy 2011;66:725-32.
14. Suarez-Fueyo A, Ramos T, Galan A, Jimeno L, Wurtzen PA, Marin A, et al. Grass tablet sublingual immunotherapy downregulates the TH2 cytokine response followed by regulatory T-cell generation. J Allergy Clin Immunol 2014;133:130-8, e1-2.
15. Lou W, Wang C, Wang Y, Han D, Zhang L. Responses of CD4(+) CD25(+) Foxp3(+) and IL-10-secreting type I T regulatory cells to cluster-specific immunotherapy for allergic rhinitis in children. Pediatr Allergy Immunol 2012;23:140-9.
16. Sugita K, Hanakawa S, Honda T, Kondoh G, Miyachi Y, Kabashima K, et al. Generation of Helios reporter mice and an evaluation of the suppressive capacity of Helios(+) regulatory T cells in vitro. Exp Dermatol 2015;24:554-6.
17. Mobs C, Ipsen H, Mayer L, Slotosch C, Petersen A, Wurtzen PA, et al. Birch pollen immunotherapy results in long-term loss of Bet v 1-specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies. J Allergy Clin Immunol 2012;130:1108-16.e6.
18. 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 2008;121:1467-72.e1.
19. Tsai YG, Lai JC, Yang KD, Hung CH, Yeh YJ, Lin CY. Enhanced CD46-induced regulatory T cells suppress allergic inflammation after *Dermatophagoides pteronyssinus*-specific immunotherapy. J Allergy Clin Immunol 2014;134:1206-9.e1.
20. Palomares O, Martin-Fontecha M, Lauener R, Traidl-Hoffmann C, Cavkaytar O, Akdis M, et al. Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-beta. Genes Immun 2014;15:511-20.
21. Palomares O, Rückert B, Jartti T, Kücüksezer UC, Puhakka T, Gomez E, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. J Allergy Clin Immunol 2012;129:510-20.

22. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. J Exp Med 2008;205:2887-98.
23. Fox EM, Torrero MN, Evans H, Mitre E. Immunologic characterization of 3 murine regimens of allergen-specific immunotherapy. J Allergy Clin Immunol 2015; 135:1341-51, e1-7.
24. Kucuksezer UC, Palomares O, Ruckert B, Jartti T, Puhakka T, Nandy A, et al. Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood. J Allergy Clin Immunol 2013;131:875-85.
25. Mehta AK, Duan W, Doerner AM, Traves SL, Broide DH, Proud D, et al. Rhinovirus infection interferes with induction of tolerance to aeroantigens through OX40 ligand, thymic stromal lymphopoietin, and IL-33. J Allergy Clin Immunol 2016;137:278-88.
26. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J Allergy Clin Immunol 2013;131: 1204-12.
27. Stanic B, van de Veen W, Wirz OF, Ruckert B, Morita H, Sollner S, et al. IL-10-overexpressing B cells regulate innate and adaptive immune responses. J Allergy Clin Immunol 2015;135:771-80.e8.
28. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy 2008;63: 1455-63.
29. de Wit J, Jorritsma T, Makuch M, Remmerswaal EB, Klaasse Bos H, Souwer Y, et al. Human B cells promote T-cell plasticity to optimize antibody response by inducing coexpression of T(H)1/T(FH) signatures. J Allergy Clin Immunol 2015; 135:1053-60.
30. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004;4:313-8.
31. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007;317:1554-7.
32. Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology 2002;105: 9-19.
33. Shamji MH, Ljorring C, Francis JN, Calderon MA, Larche M, Kimber I, et al. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. Allergy 2012;67:217-26.
34. Focke-Tejkl M, Weber M, Niespodziana K, Neubauer A, Huber H, Henning R, et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy. J Allergy Clin Immunol 2015; 135:1207-17, e1-11.
35. Gold MJ, Antignano F, Halim TY, Hirota JA, Blanchet MR, Zaph C, et al. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. J Allergy Clin Immunol 2014;133:1142-8.
36. Nagakumar P, Denney L, Fleming L, Bush A, Lloyd CM, Saglani S. Type 2 innate lymphoid cells in induced sputum from children with severe asthma. J Allergy Clin Immunol 2016;137:624-6.
37. 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 2014;134:1193-5.e4.
38. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J Allergy Clin Immunol 2014;134:671-8.e4.
39. Chapman MD, Briza P. Molecular approaches to allergen standardization. Curr Allergy Asthma Rep 2012;12:478-84.
40. Larenas-Linnemann D, Cox LS. Immunotherapy and Allergy Diagnostics Committee of the American Academy of Allergy, Asthma and Immunology. European allergen extract units and potency: review of available information. Ann Allergy Asthma Immunol 2008;100:137-45.
41. Turkeltaub PC. Biological standardization based on quantitative skin testing—the ID50 EAL method (intradermal dilution for 50 mm sum of erythema diameters determines the allergy unit). Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1987;169-73.
42. Kaul S, May S, Luttkopf D, Vieths S. Regulatory environment for allergen-specific immunotherapy. Allergy 2011;66:753-64.
43. van Ree R, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O, et al. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. Allergy 2008;63:310-26.
44. Chapman MD, Ferreira F, Villalba M, Cromwell O, Bryan D, Becker WM, et al. The European Union CREATE project: a model for international standardization of allergy diagnostics and vaccines. J Allergy Clin Immunol 2008;122: 882-9.e2.
45. Kaul S, Dehus O, Zimmer J, Vieths S. Validation of major allergen references and ELISAs—current state of the BSP090 project. Arb Paul Ehrlich Inst Bundesinstitut Impfstoffe Biomed Arzneim Langen Hess 2013;97:45-53.
46. Vieths S, Barber D, Chapman M, Costanzo A, Daas A, Fiebig H, et al. Establishment of recombinant major allergens Bet v. 1 and Phl p 5a as Ph. Eur. reference standards and validation of ELISA methods for their measurement. Results from feasibility studies. Pharmeur Bio Sci Notes 2012;2012:118-34.
47. Neske F, Schorner C, Buchheit KH, Costanzo A, Hanschmann KM, Himly M, et al. BSP090–the follow-up to CREATE. Arb Paul Ehrlich Inst Bundesinstitut Impfstoffe Biomed Arzneim Langen Hess 2009;96:12-20.
48. 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. J Allergy Clin Immunol 2008;121:227-32.
49. Hankin CS, Cox L, Lang D, Bronstone A, Fass P, Leatherman B, et al. Allergen immunotherapy and health care cost benefits for children with allergic rhinitis: a large-scale, retrospective, matched cohort study. Ann Allergy Asthma Immunol 2010;104:79-85.
50. Hankin CS, Cox L, Bronstone A, Wang Z. Allergy immunotherapy: reduced health care costs in adults and children with allergic rhinitis. J Allergy Clin Immunol 2013;131:1084-91.
51. Ariano R, Berto P, Tracci D, Incorvaia C, Frati F. Pharmacoeconomics of allergen immunotherapy compared with symptomatic drug treatment in patients with allergic rhinitis and asthma. Allergy Asthma Proc 2006;27:159-63.
52. Creticos PS, Reed CE, Norman PS, Khoury J, Adkinson NF Jr, Buncher CR, et al. Ragweed immunotherapy in adult asthma. N Engl J Med 1996;334:501-6.
53. Berto P, Frati F, Incorvaia C, Cadario G, Contiguglia R, Di Gioacchino M, et al. Comparison of costs of sublingual immunotherapy and drug treatment in grass-pollen induced allergy: results from the SIMAP database study. Curr Med Res Opin 2008;24:261-6.
54. Ariano R, Berto P, Incorvaia C, Di Cara G, Boccardo R, La Grutta S, et al. Economic evaluation of sublingual immunotherapy vs. symptomatic treatment in allergic asthma. Ann Allergy Asthma Immunol 2009;103:254-9.
55. Schadlich PK, Brecht JG. Economic evaluation of specific immunotherapy versus symptomatic treatment of allergic rhinitis in Germany. Pharmacoeconomics 2000;17:37-52.
56. Petersen KD, Gyrd-Hansen D, Dahl R. Health-economic analyses of subcutaneous specific immunotherapy for grass pollen and mite allergy. Allergol Immunopathol (Madr) 2005;33:296-302.
57. Berto P, Passalacqua G, Crimi N, Frati F, Ortolani C, Senna G, et al. Economic evaluation of sublingual immunotherapy vs symptomatic treatment in adults with pollen-induced respiratory allergy: the Sublingual Immunotherapy Pollen Allergy Italy (SPAI) study. Ann Allergy Asthma Immunol 2006;97: 615-21.
58. Omnes LF, Bousquet J, Scheinmann P, Neukirch F, Jasso-Mosqueda G, Chicoye A, et al. Pharmacoeconomic assessment of specific immunotherapy versus current symptomatic treatment for allergic rhinitis and asthma in France. Eur Ann Allergy Clin Immunol 2007;39:148-56.
59. Bachert C, Vestenbaek U, Christensen J, Griffiths UK, Poulsen PB. Cost-effectiveness of grass allergen tablet (GRAZAX) for the prevention of seasonal grass pollen induced rhinoconjunctivitis—a Northern European perspective. Clin Exp Allergy 2007;37:772-9.
60. Canonica GW, Poulsen PB, Vestenbaek U. Cost-effectiveness of GRAZAX for prevention of grass pollen induced rhinoconjunctivitis in Southern Europe. Respir Med 2007;101:1885-94.
61. Nasser S, Vestenbaek U, Beriot-Mathiot A, Poulsen PB. Cost-effectiveness of specific immunotherapy with Grazax in allergic rhinitis co-existing with asthma. Allergy 2008;63:1624-9.
62. Ruggeri M, Oradei M, Frati F, Puccinelli P, Romao C, Dell'Albani I, et al. Economic evaluation of 5-grass pollen tablets versus placebo in the treatment of allergic rhinitis in adults. Clin Drug Investig 2013;33:343-9.
63. Westerhout KY, Verheggen BG, Schreder CH, Augustin M. Cost effectiveness analysis of immunotherapy in patients with grass pollen allergic rhinoconjunctivitis in Germany. J Med Econ 2012;15:906-17.
64. Meadows A, Kaambwa B, Novielli N, Huissoon A, Fry-Smith A, Meads C, et al. A systematic review and economic evaluation of subcutaneous and sublingual allergen immunotherapy in adults and children with seasonal allergic rhinitis. Health Technol Assess 2013;17:vi, xi-xiv, 1-322.
65. Keiding H, Jorgensen KP. A cost-effectiveness analysis of immunotherapy with SQ allergen extract for patients with seasonal allergic rhinoconjunctivitis in selected European countries. Curr Med Res Opin 2007;23:1113-20.
66. Noon L. Prophylactic inoculation against hay fever. Lancet 1911;1:1572-3.
67. Bonini S. Regulatory aspects of allergen-specific immunotherapy: Europe sets the scene for a global approach. World Allergy Organ J 2012;5:120-3.

68. Lorenz AR, Luttkopf D, Seitz R, Vieths S. The regulatory system in Europe with special emphasis on allergen products. Int Arch Allergy Immunol 2008;147: 263-75.
69. Cox L, Jacobsen L. Comparison of allergen immunotherapy practice patterns in the United States and Europe. Ann Allergy Asthma Immunol 2009;103: 451-61, 95.
70. Rosner-Friese K, Kaul S, Vieths S, Pfaar O. Environmental exposure chambers in allergen immunotherapy trials: current status and clinical validation needs. J Allergy Clin Immunol 2015;135:636-43.
71. Nolte H, Maloney J, Nelson HS, Bernstein DI, Lu S, Li Z, et al. Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber. J Allergy Clin Immunol 2015;135: 1494-501.e6.
72. Casale TB, Stokes JR. Immunotherapy: what lies beyond. J Allergy Clin Immunol 2014;133:612-20.
73. Jutel M, Akdis CA. Novel immunotherapy vaccine development. Curr Opin Allergy Clin Immunol 2014;14:557-63.
74. Jutel M, Van de Veen W, Agache I, Azkur KA, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy and novel ways for vaccine development. Allergol Int 2013;62:425-33.
75. Sharma P, Gaur SN, Arora N. Immunotherapy with B cell epitopes ameliorates inflammatory responses in Balb/c mice. Clin Exp Immunol 2015;179:128-36.
76. Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. J Allergy Clin Immunol 2014;133: 318-23.
77. Jutel M, Solarewicz-Madejek K, Smolinska S. Recombinant allergens: the present and the future. Hum Vaccin Immunother 2012;8:1534-43.
78. Linhart B, Focke-Tejkl M, Weber M, Narayanan M, Neubauer A, Mayrhofer H, et al. Molecular evolution of hypoallergenic hybrid proteins for vaccination against grass pollen allergy. J Immunol 2015;194:4008-18.
79. Fili L, Cardilicchia E, Maggi E, Parronchi P. Perspectives in vaccine adjuvants for allergen-specific immunotherapy. Immunol Lett 2013;161:207-10.
80. Grundstrom J, Neimert-Andersson T, Kemi C, Nilsson OB, Saarne T, Andersson M, et al. Covalent coupling of vitamin D3 to the major cat allergen Fel d 1 improves the effects of allergen-specific immunotherapy in a mouse model for cat allergy. Int Arch Allergy Immunol 2012;157:136-46.
81. Linhart B, Narayanan M, Focke-Tejkl M, Wrba F, Vrtala S, Valenta R. Prophylactic and therapeutic vaccination with carrier-bound Bet v 1 peptides lacking allergen-specific T cell epitopes reduces Bet v 1-specific T cell responses via blocking antibodies in a murine model for birch pollen allergy. Clin Exp Allergy 2014;44:278-87.
82. Schneider LC, Rachid R, LeBovidge J, Blood E, Mittal M, Umetsu DT. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. J Allergy Clin Immunol 2013;132:1368-74.
83. Larenas-Linnemann D, Wahn U, Kopp M. Use of omalizumab to improve desensitization safety in allergen immunotherapy. J Allergy Clin Immunol 2014;133: 937-937.e2.
84. Kundig TM, Johansen P, Bachmann MF, Cardell LO, Senti G. Intralymphatic immunotherapy: time interval between injections is essential. J Allergy Clin Immunol 2014;133:930-1.
85. Creticos PS, Esch RE, Couroux P, Gentile D, D'Angelo P, Whitlow B, et al. Randomized, double-blind, placebo-controlled trial of standardized ragweed sublingual-liquid immunotherapy for allergic rhinoconjunctivitis. J Allergy Clin Immunol 2014;133:751-8.
86. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. J Allergy Clin Immunol 2014;133:121-9, e1-2.
87. von Moos S, Johansen P, Tay F, Graf N, Kundig TM, Senti G. Comparing safety of abrasion and tape-stripping as skin preparation in allergen-specific epicutaneous immunotherapy. J Allergy Clin Immunol 2014;134:965-7.e4.