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REVIEW

Critical appraisal of Timothy grass pollen extract GRAZAX® in the management of allergic rhinitis

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Abstract: Allergic rhinitis is one of the most common diseases of adult and pediatric age, associated with grass pollen (GP) allergy in >50% cases, with a consistent impact on quality of life of affected patients. A grass allergen tablet, containing standardized extract derived from Timothy grass (*Phleum pratense*) pollen and ~15 µg major allergen *P. pratense* (rPhl p 5), may be the future of allergen-specific immunotherapy (IT) for GP allergy. The aim of this review was to critically evaluate the role of Timothy GP extract IT for the management of allergic rhinitis. For this purpose, we have tried to analyze potential mechanisms of action at the basis of Timothy GP extract, we have reviewed efficacy studies to establish potential benefits and clinical response, and we have also evaluated safety and tolerability profiles and patient focus perspective, such as quality of life, satisfaction and acceptability, and compliance to this IT.

Keywords: Timothy grass pollen extract, allergic rhinitis, Grazax, efficacy, safety, compliance

Introduction

In United States and Western Europe, up to 20% of the adult population is affected by allergic rhinoconjunctivitis (RC).[1] Allergic rhinitis (AR) is the most common chronic disease in pediatric age and one of the most common diseases affecting adults.[2] Grass pollen (GP) is associated with >50% of AR cases, being the most common cause of respiratory allergies.[3] GP allergy and AR may have a great impact on the quality of life (QL) of people affected.[4,5]

AR is an IgE-mediated disease caused by inflammation of the inside lining of the nose, elicited by allergen exposure. Nasal obstruction (the only symptom often reported in preschool children), rhinorrhea, postnasal drip, nasal itching, and sneezing characterize AR, which are classified according to symptom frequency as intermittent (<4 days/week or <4 weeks/year) or persistent (>4 days/week and >4 weeks/year) and according to the disease severity as mild (if not interfere with QL) or moderate/severe (bad symptoms that affect QL). Severe AR includes factors such as impairment of daily scholar and/or work activities, leisure, and sport; sleep disturbance; and exacerbation of coexisting asthma.[2,6]

For AR treatment, the update of Allergic Rhinitis and Its Impact on Asthma guidelines published in 2010 recommended new-generation oral H1-antihistamines, especially in adults with persistent AR and in children with intermittent or persistent AR, suggesting intranasal H1-antihistamines in adults and children with seasonal AR. Intranasal glucocorticosteroids were recommended for treatment of AR in adults and suggested in children, and they should be preferred to oral and intranasal H1-antihistamines, especially in persistent AR, and also to oral leukotriene receptor antagonists. Other medications suggested were oral leukotriene receptor antagonists, intranasal

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chromones, intranasal ipratropium bromide for treatment of rhinorrhea, a very short course of intranasal decongestant (≤5 days) if severe nasal obstruction, or a short course of oral glucocorticosteroids if AR is associated with uncontrolled moderate or severe nasal and/or ocular symptoms.[7]

A tailored approach for AR treatment is needed for each patient, considering disease severity and duration, patient preference, and medications' efficacy, availability, and cost. However, optimal pharmacotherapy may not be completely effective in some patients with moderate/severe AR.[6] In these cases, sublingual immunotherapy (SLIT) or subcutaneous immunotherapy (SCIT) should be proposed, according to the last guidelines published in 2015.[2]

Allergen-specific immunotherapy (IT) is the only treatment for AR that is able to modify this allergic disease, changing its natural history, providing long-term remission, and preventing its progression. Its therapeutic indications include patients aged >5 years with demonstrable IgE against clinically important allergens.[8,9] Based on results from SCIT, international clinical guidelines recommend its continuation for 3–5 years.[10] Clinical benefits persist for at least 3–6 years after IT termination.[11]

The main approach to IT of the past include SCIT, which is however penalized by a disadvantageous route of administration, associated with the sporadic risk of severe side effects such as systemic allergic reactions, which need specialist centers for their management. Instead, sublingual administration of SLIT gives an attractive solution especially for the pediatric population, in which safety is fundamental, allowing a home-based therapy.[12]

A grass allergen tablet (GAT), consisting of a rapidly dissolving oral lyophilisate for once daily sublingual administration, may be the future treatment for GP allergy, especially for the use in pediatric age. Indeed, the sublingual form makes it an efficacious SLIT treatment, easy to use, with a favorable safety profile and high compliance, also giving a better control of dosage than the drop-based administration.[12] The good oral bioavailability of GAT is related to the presence of the gelatin NF (fish source) between the ingredients; however, this component limits the use of GAT in people affected by fish allergy.

GRASTEK® (Merck & Co., Inc., Whitehouse Station, NJ, USA) and Oralair (Stallergenes, Antony, France) are GP allergen extract sublingual tablets, indicated as SLIT for the treatment of patients with GP allergy.

Oralair is a fast-dissolving sublingual desensitization tablet that consists of five purified and calibrated pollen extracts: Timothy grass (*Phleum pratense*), perennial ryegrass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), sweet vernal grass (*Anthoxanthum odoratum*), and orchard grass (*Dactylis glomerata*).[13]

Merck Sharp & Dohme Corp. has submitted a biologics license application to US FDA for GRASTEK®, a sublingual tablet for oromucosal delivery, comprised of extract from Timothy grass (*P. pratense*) pollen. This tablet is administered as SLIT in adults, adolescents, and children (≥5 years of age) for the treatment of AR with or without conjunctivitis due to sensitivity to Timothy or related GP. The dosage of the tablets proposed for adult use in the US is 2,800 bioequivalent allergy units or 75,000 standardized quality tablet (SQ-T) units of standardized extract derived from Timothy grass (*P. pratense*) pollen, containing ~15 µg of major allergen *P. pratense* (rPhl p 5). GRASTEK® should be initiated at least 8–12 weeks prior to and throughout the GP season (without any up-dosing period) and performed for 3 years to obtain a prolonged effect, with a first daily dose taken at the health care provider's office, and the remaining doses at home.[3,14,15]

GRASTEK® is marketed in Europe under the trade name GRAZAX® (ALK-Abellò A/S, Hørsholm, Denmark). A Marketing Authorization Application for GRAZAX® was filed by the mutual recognition procedure in the European Union, which was first approved in 2006. GRAZAX® is now marketed in 30 countries.[15,16]

GRAZAX® contains the same active pharmaceutical ingredient of that used for >20 years in SCIT, named Alutard SQ® GP, that consist in a standardized *P. pratense* allergen extract, characterized by allergenic compounds with extensive cross-reactivity with GP from different species.[3,17,18]

GRASTEK® and GRAZAX® are formulations of major *P. pratense* recombinant allergen rPhl p 5. Almost 90% of patients with GP allergy are sensitized vs group 5 GP allergens. The rPhl p 5A domain contains several IgE epitopes, characterized by an optimal configuration for activation of efficient effector cells. So, already 15 years ago, Flicker et al suggested that rPhl p 5A fragment (and the corresponding IgE-facilitated allergen binding [IgE-FAB]) could be a useful tool to investigate the structural requirements needed for highly efficient activation of effector cells, in order to develop a more allergen-specific IT. They demonstrated that rPhl p 5A was characterized by extremely high allergenic activity involved in basophil histamine release and by reaction with serum IgE in 76% of GP-allergic patients.[19]

The recombinant wild-type allergens and genetically modified hypoallergenic allergen derivatives can be used for IT, as for complex allergen sources with only one major allergen, if the significant allergens have been included

in the vaccine.[20] Although the first clinical IT study with recombinant allergens used two different hypoallergenic derivatives of Bet v 1 (the major birch pollen allergen),[21] successively *Phleum* recombinant allergens were also successfully used.[10,22–25]

The aim of this review was to give a critical appraisal of Timothy GP extract SLIT in the management of AR. Considering that Oralair was a mix of five GP extracts, we have focused our attention only on GRAZAX®/GRASTEK®.

A PubMed search indexed for MEDLINE was undertaken to identify studies in adults and children using the terms "Timothy Grass Pollen Extract", "Allergic Rhinitis", "Grazax", "Grastek", "Efficacy", "Safety", "Tolerability", and "Compliance" as key words, alone and in combination. The date of our last search was February 2015, covering a time period of approximately 25 years. Only articles in English were reviewed. References of selected article were examined for relevant articles.

Mechanism of action of Timothy GP extract

Allergy is a particular case of an inflammatory reaction, in which allergen is the antigen that penetrates into the host (the allergic subject), resulting in a Th2 differentiation of specific T-cells subsequent to the antigen presentation. Th2 cells trigger the IgE synthesis by producing IL-4 and IL-13, while they attract and activate eosinophil polymorphonuclear cells by producing IL-5. If persistent exposure or reexposure occurs, IgE binding with their high-affinity receptor (FcεRI), situated on mast cells, results in their degranulation and histamine and leukotriene release, characterizing the early allergic reaction phase. Moreover, also the low-affinity receptor CD23 is involved in the binding of IgE with B-cells and other antigen-presenting cells (APCs). Instead, eosinophils mediate the late-phase response and chronic allergic reaction, through basic proteins production and injury of epithelia. Specific IT plays a role at each step of the allergic reaction as IgE and IgG productions, mast cell and eosinophil homing, T-cell activation, and antigen presentation.[26]

The oral cavity is a naturally tolerogenic and noninflamed environment, in spite of constant exposure to several foreign proteins, thanks to monocytes and Langerhans cells, able to produce TGF-β and IL-10. SLIT, in optimal doses, exerts local actions in the oral mucosa and/or regional lymph nodes, in addition to modest systemic changes as SCIT, taking part in the induction of tolerance, avoiding new sensitizations, and inducing long-term remission after discontinuation.[27]

Pharmacokinetic studies showed that sublingual administered allergen extracts are long detained and not quickly absorbed by oral mucosa, captured by dendritic cells, and presented to T-cells after their migration in the draining lymph nodes. This aspect may be the key factor underlying SLIT mechanism of action for the short-term and long-term effects. The mechanism hypothesized for SLIT short-term efficacy (regarding ultrarush or coseasonal administration) involves a downregulation of mast cells, with consequent hyporeactivity at the peak of the pollen season. For the clinically established long-lasting effects, T-regulatory (Treg) cell activation seems to be involved: Treg cells differentiate from naïve T-cells after application of soluble antigens to the mucosa and exert a suppressive effect on both Th1 and Th2 responses.[28] Peripheral T-cell tolerance is characterized mainly by the generation of allergen-specific Treg cells, associated with suppressive proliferative and cytokine responses vs main allergens. Subsets of Treg cells include the innate CD4+ CD25+ Treg and the inducible type 1 Treg (Tr1) cells.[29]

Peripheral T-cell tolerance observed in allergen-specific IT is initiated by the increased production of IL-10 and TGF-β of antigen-specific T-cells. There are still doubts if antigen-specific T-cells involved (that express CD4 and CD25) are inducible Tr1 cells (which have upregulated CD25) or innate CD4+ CD25+ Treg cells (producer of suppressive cytokines). Anyway, upregulation of CD4+ CD25+ Treg cells seems to play a role in allergen-specific IT. Treg cells suppress allergic inflammation, inhibiting allergen-specific immune responses and inducing suppression of APCs, Th2 and Th1 cells, allergen-specific IgE and eosinophils, mast cells, and basophils; they also act with IgG4 and/or IgA induction and interact with resident tissue cells and remodeling.[29]

SCIT and SLIT act as immune system modifiers, thanks to their ability to competitively inhibit allergen–IgE interaction, through an altered regulation of IgE synthesis, combined with the induction of IgE-blocking antibodies. Further mechanisms of action involve a shift from Th2 to Treg cells and inhibition of facilitating antigen presentation of the specific T-cells.[3] On the other hand, the excellent tolerability of SLIT also benefits the absence of mast cells, eosinophils, basophils, and effectors cells[28] in the oral mucosa of allergic subjects.

Studies documented that GRAZAX® and Alutard SQ® *P. pratense* provide a strong and qualitatively comparable antibody response and a dose-depending amount of serum antibodies for GRAZAX®.[3,30]

According to World Allergy Organization position paper on SLIT published in 2013, SLIT administration is associated

with: early antigen-specific IgE increase and seasonal IgE blunting, persistent antigen-specific IgG4 increase and IgE-blocking activity, eosinophils inhibition and adhesion molecules reduction in target organs, an early increase (at 4–12 weeks) in peripheral phenotypic Treg and delayed (at 12 months) immune deviation in favor of Th1 responses, CD25+ FOXP+ phenotypic Treg cells detection in the sublingual mucosa, and alterations in dendritic cell markers that correlate with clinical response to treatment.[27]

Allergen-specific IgE are mainly involved in the physiopathology of allergic diseases, while indirect evidences indicate that IgG4 could not be sensitizing antibodies, but protective antibodies, suggesting that selective modulation of IgE vs IgG4 production may be of potential therapeutic interest.[31]

The elevation of specific IgG4 during specific IT was confirmed more recently, even if it was described a long time ago.[10,32–38] IgG4 are blocking antibodies that prevent the encounter of allergen and IgE bound on their receptor located on effector cells and APCs; they also avoid the binding of the allergen–IgE complexes on the low-affinity IgE receptor on B-cells, decreasing their ability to present the allergen to specific T-cells. This last aspect is supported by an important decrease in the IgE–B-cells binding to GP-allergic patients under IT vs placebo.[26,39]

The engagement by IgG4-allergen complexes of the B isoform of the FcγRIIB (the low-affinity IgG receptor) on mast cells represents another hypothesis for IgG4 involvement during IT, resulting in a deactivation signal through phosphorylation of immunoreceptor-based inhibition motifs activating intracellular phosphatases, counterbalancing the effect of immunoreceptor-based activation motifs present in the intracellular tail of FcεRiγ.[26,40]

A randomized, placebo-controlled trial was performed on 56 adults >18 months, utilizing sublingual biopsies for measurement of local T-cells, APCs, and IL-12 mRNA expression. This study demonstrated the improvement in all appraisals, which correlated with late skin response inhibition (P=0.003) and with serum IgG4/IgE ratio increase (P=0.05) after GP SLIT.[32]

A research based on high-dose GP SLIT showed increases in GP-specific IgA2, IgG and IgG4, and sublingual FOXP3+ Treg,[41] associated with elevation in serum inhibitory activity for IgE-FAB. Furthermore, long-term benefits observed for 2 years following 3 years of GP SLIT was associated with the persistent increase of allergen-specific IgG4 levels as well as of functional IgG-associated inhibitory activity for IgE-FAB.[1,27,42]

An interim analysis of a randomized, double-blind, placebo-controlled Phase III trial with 3 years of daily treatment with GRAZAX® vs placebo, followed by a follow-up period of 2 years, demonstrated progressive immunologic changes and high efficacy over 2 years of therapy. 351 adults with GP allergy, cause of moderate-to-severe RC, were treated with active (n=189) or placebo (n=162) tablets for an average of 22 months. In the active group, specific IgG4 levels significantly increased (23 times the baseline level) over the two seasons.[10]

Immunological changes were detected after only 1 month of SLIT treatment for GP-allergic RC patients, as demonstrated in this randomized, double-blind, placebo-controlled trial, performed on 78 patients randomly assigned to receive either GRAZAX® (active group) or placebo in a 2:1 ratio, in preseasonal (at least for 8 weeks before the GP season) and coseasonal administration. 50 active and 25 placebo patients completed the trial. A statistically significant increase of *P. pratense* IgG4, IgE, and IgE-blocking factor was observed in the active group from baseline to the start of the GP season vs placebo (P>0.001, P=0.017, and P=0.005, respectively).[43]

These data were also demonstrated for SCIT, with significant higher levels of IgG4 in the SCIT group vs control group (P<0.001) (in 46 GP-allergic and rhinitic adults, randomized 3:1 to receive a short course of SCIT with *P. pratense* vs control group without specific IT) and immediate and delayed cutaneous responses to grass mix and *P. pratense* significantly diminished after SCIT (P<0.001).[23]

On the other hand, 2 years of SCIT induce a >100-fold in increase in IgG4 levels,[39] suggesting that kinetics might be different for subcutaneous and sublingual route of administration. However, as clinical benefits with the GAT were similar to that reported for SCIT in GP-allergic patients, they do not seem to be directly dependent on the increased amount of IgG4 levels.[10,44]

After the finding of the Th2 model in the early 1990s, it was hypothesized that specific IT triggers a Th2 to Th1 switching of T-cell activation, inhibits eosinophils, mast cells, and IgE production, driving isotypic commutation toward IgG4. This hypothesis was confirmed demonstrating was confirmed demonstrating an IFN-γ producing T-cells increase detected upon pollen challenge in treated rhinitic patients[45] and IL-4 production in nontreated patients vs IFN-γ in ones desensitized to house dust mites or GP, induced by allergen stimulation.[26,46]

During IT, a relevant increase of IL-10 production was observed.[39] IL-10 is an immunosuppressive cytokine,

implicated in induction and preservation of tolerance. In the early 2000s, Treg, a small population of T-cells, able to produce immunosuppressive cytokines as IL-10 and/or TGF-β, were recognized as IL-10 source during specific IT. The most studied among Treg cells was the natural subset expressing CD4 and CD25 at a high level together with the transcription factor FOXP3. It is supposed that a Treg deficiency can be involved in allergy and other inflammatory diseases. CD4+ CD25+ cells were decreased and ineffective in grass[47] and other respiratory allergies, while during specific IT against GP,[48] an increase in Treg cell activation was shown. An increase of IL-10 positive cells was observed in the nasal mucosa of challenged GP-allergic patients after specific IT vs placebo,[39] paralleled with the late-phase skin response inhibition.[49] After specific IT, FOXP3+ CD4+ CD25+ T-cells[50] and TGF-β+ cells[51] were detected in the nasal mucosa. A few studies concerning the mechanisms of SLIT have suggested similarities with injective route.[26,52]

Jeannin et al hypothesized the existence of a selective control pathway of IgE vs IgG4 production, reporting the first evidence of a molecule that differentially regulates their production. They observed that IL-10 had a differential effect on IgE vs IgG4 production by peripheral blood mononuclear cells, decreasing ε transcript expression and IgE production induced by IL-4, when added during the first 3 days of in vitro culture. It suggested that IL-10 decreased IL-4-induced IgE switching. On the contrary, IL-10 potentiated IgE production, if was added later on B cells that were already IgE switched. Interestingly, whatever be the time of addition (with maximal effect when added during the first 3 days), IL-10 augmented IL-4-induced γ4 transcript expression and IgG4 production. It was supposed that IL-10 enhanced IgG4 production by potentiating IgG4 switching induced by IL-4, acting also with the increase of growth and/or differentiation of cells already IgG4 committed. Moreover, early downregulating IL-10 effect on the production of IgE was reversed by CD40 ligation.[31]

On the other hand, the ability to induce immunologic changes is well supported by the production of IL-10, as demonstrated by Ciprandi et al, that also provided the first evidence that SLIT improved early bronchial disease in patients with perennial AR, evaluating the possible association between IL-10 and forced expiratory flow (FEF) at 25–75%. After 3 years of SLIT, FEF(25%–75%) significantly (P=0.0131) increased in 9 patients of the SLIT group (80.5%±6.7%), and this increase was significantly associated with IL-10 production (P=0.0025), whereas FEF(25%–75%) significantly (P=0.0021) decreased in the 10 nontreated patients considered as control (60.8%±2.62%).[53]

Concerning mast cells, a reduction of the allergen-induced c-Kit positive cell infiltration of the nasal mucosa has been demonstrated after GP IT vs placebo, associated with the effects correlated to the IgG4/IgE rise.[26,54] Tissue infiltration mediated by mast cells is stimulated by IL-9, which needs the simultaneous activation of c-Kit. There are some evidences that IL-9 may be upregulated in the nasal mucosa during the pollen season and that IT, reducing the local expression of IL-9, can be associated with inhibition of seasonal increases of c-Kit+ mast cells in the nasal mucosa.[54] These results are supported by the following study that included 44 patients affected by seasonal AR and asthma, evaluated previously and 2 years following a double-blind trial with GP IT. c-Kit+ mast cells resulted in increase in the nasal mucosa during the pollen season (P=0.0001) as well as IL-9 mRNA-positive cells (P=0.1), correlating with nasal EG2+ eosinophils (r=0.47, P=0.05) and IL-5 mRNA-positive cells (r=0.54, P=0.02). Seasonal increases of c-Kit+ mast cells in the nasal mucosal were significantly inhibited by IT (P=0.001) and the seasonal expression of IL-9 mRNA-positive cells (P=0.06) vs placebo.[54]

About T-cells, specific IT acts on APCs.[26] Specific IT is constituted by purified and standardized extracts, that contain allergens and nonproteic components, called pathogen-associated molecular patterns, capable of binding to Toll-like receptors (TLR) on the surfaces of professional APCs and nonprofessional APCs (epithelial cells, B-cells, endothelial cells, etc); this results in induction of APC differentiation into cells inducing T-cell differentiation.[55] Regarding the sublingual route, a recent study showed that, in a model of ovalbumin-induced asthma, sublingual ovalbumin effect was enhanced by TLR-2 agonist, which was able to trigger dendritic cells to produce IL-10 and IL-12, stimulating T-cell-IL-10 and IFN-γ production.[56] A weekly injection of IT during pollen season is enough to obtain a persistent effect on AR.[26,57]

Regulatory dendritic cells and human effector lead to T-cell differentiation, phenotype, and function. In vitro assessment of human effector and regulatory dendritic cells was performed from human monocytes (at the messenger RNA and protein levels), before and after administration of GAT. The authors observed that complement component 1 and stabilin-1 resulted in increase in peripheral blood mononuclear cells from clinical responders vs nonresponders or placebo-treated patients, so they could be considered candidate biomarkers of IT early efficacy as markers of a regulatory innate immune response, predictive of clinical tolerance.[27,58]

Some studies also reported an increase in CD8+ T-cells and decreases in the CD4/CD8 T-cell ratio,[34] but a clear association with these immunological changes and clinical responses to SLIT is still unknown, as well as contrasting data are reported for potential involvement of inhibition of basophil activation induced by SLIT; so, further studies are needed about these issues.[27]

In particular, very few studies were performed to understand the mechanism at the basis of action of GRAZAX®. So, we supposed that mechanisms of action of Timothy GP extract can be similar to those aforementioned, as the route of administration is the same as that of other SLITs, and GRAZAX® gives a qualitatively similar antibody response compared with Alutard SQ® *P. pratense* utilized in SCIT. Although further investigations are clearly required, pharmacokinetic and pharmacodynamic studies are not possible for IT and allergen products, according to the European Medicines Agency, because plasma concentration of the active substance is not measurable; however, the immunological changes previously mentioned (modifications in allergen-specific IgG levels, T-cell responses, and cytokine production) and changes of the end-organ specific response (provocation tests) should be evaluated.[59]

Efficacy studies, safety, and tolerability profile

Currently, the allergen-specific IT is the only therapy for AR and conjunctivitis, which is able to modify the progression of the disease, providing also efficacy in subsequent years after discontinuation.[59,60]

The long-term effect of SCIT on RC is well known;[6] however, studies of alternative administration route have been encouraged, due to some inconvenience about SCIT, linked to the injection-related discomfort and the numerous hospital visits and associated with the potential risk of IgE-mediated severe systemic reactions.[11]

The route of administration of SLIT is a more convenient form of IT with reduced risk of systemic adverse events, an important feature when IT is considered in pediatric age; efficacy and safety are highly determinative factors. Early trials of oral administration of SLIT have failed to demonstrate clinical benefit, may be due to the prompt allergen inactivation within the gastrointestinal tract[61] and the use of the first enteric-coated oral formulations. Instead, subsequent studies have given promising results about efficacy, as confirmed by a review article published in 2002[62] as well as by a recent meta-analysis, which concluded that SLIT could be associated with a significant decrease of symptoms and antiallergic drug use compared to placebo.[63] A reduction and prevention of the development of more severe allergic diseases and new allergies are also important effects of SLIT therapy.[64,65]

Several large trials have shown the favorable risk–benefit profile of GRAZAX® in reducing RC symptoms in adults, supporting 75,000 SQ-T once daily as the effective dose of GAT.

GRAZAX® has effects on multiple allergic symptoms such as nasal (runny nose, blocked nose, sneezing, and itchy nose) and eye (gritty feeling/red/itchy eyes and watery eyes) symptoms, as demonstrated by Durham and Riis, who conducted a double-blind, placebo-controlled trial, including 634 participants with GP-induced RC. Patients were randomized 1:1 to GRAZAX® 75,000 SQ-T or placebo, receiving preseasonal (for at least 16 weeks before) and seasonal IT. Eye and nasal symptoms (from 22% to 44%) appeared significantly reduced in the treatment group vs placebo ($P<0.0001$).[4]

A large-scale, double-blind, placebo-controlled trial conducted in adult age (18–65 years), indicated a strong dose–response correlation and clinical efficacy of SLIT. A dose-related response was detected comparing the efficacy of GAT at different dosages (2,500, 25,000, or 75,000 SQ-T) vs placebo; the dose of 75,000 SQ-T was associated with the highest reductions in the RC symptom (16%) and medication scores (28%) ($P=0.0710$ and $P=0.0470$, respectively) as well as an improvement of QL ($P=0.006$), with an increase of 54% of the number of days being well ($P=0.041$). The observed immunological alterations were time- and dose dependent. The length of preseasonal treatment may influence the clinical outcome: SLIT with 75,000 SQ-T units, administered preseasonally for at least 8 weeks, was associated with a highly statistically significant reduction in RC symptoms (21%, $P=0.0020$) and medication scores (29%, $P=0.0120$) vs placebo. Only 18 participants (2%) withdrew from the trial for suspected adverse reactions.[11]

Dahl et al demonstrated GAT efficacy (starting SLIT 16 weeks prior to the pollen season) vs placebo in a randomized, placebo-controlled, double-blind study, showing a decrease of 30% ($P<0.0001$) in RC symptoms and of 38% in RC drug score ($P<0.0001$) in the treatment group. The authors did not report severe local or systemic reactions, while localized mild mouth itching and swelling were the main well-tolerated side effects, associated with SLIT withdrawal in <4% of patients.[66]

Similar results on the main side effects were reported by Malling et al[30] in a randomized controlled trial with GAT at dosage of 2,500, 25,000, or 75,000 SQ-T units, preseasonally

(8 weeks before) and seasonally (15 weeks) administered. Transient mouth, eyes, or throat itching was reported as the most frequent mild adverse reaction among 64.7% of patients who described adverse events. This study also showed a significant effect of SLIT on the immune system, with a time- and dose-related rise of *P. pratense*-specific serum IgG, IgA, IgE, and IgE-competing components during the first 8 weeks. In conclusion, the authors supported the safety and immunogenicity of a once daily dose of 75,000 SQ-T.

RC and asthma coexist in up to 80% of patients as a part of the same allergic disease; on the other hand, allergic RC is the major risk factor for asthma development.[67] Therefore, several trials were performed to demonstrate an important role of specific IT in avoiding the evolution from allergic to asthmatic disease.[68–70]

Moreover, the establishment of a safety dose of GRAZAX® is very critical, considering that a massive exposure to airborne GP can trigger asthma in susceptible allergic individuals.[70]

Dahl et al[5] confirmed its previous results in a multicenter randomized controlled trial on 114 GP-allergic subjects, supporting the efficacy of 75,000 SQ-T GAT in asthmatic patients with RC in the prevention of RC symptoms and reduction of medication use, and its safety regarding the trigger of asthma crises.

Calderon and Essendrop assessed a safe dose range of GRAZAX® in 43 patients with coexisting RC and asthma in a randomized (3:1), double-blind, placebo-controlled, dose escalation trial. Before the pollen season, four groups of 12 patients started SLIT at intervals of 1 week, for 28 days with daily doses of 75,000, 150,000, 300,000, or 500,000 SQ-T units or placebo. Only the 500,000 SQ-T group was composed of five active and two placebo. There were no withdrawal for adverse events and no asthmatic exacerbations. Dose-related local mouth or throat reactions were reported, requiring therapy only in 18% of cases. The frequency and type of adverse events possibly related to GRAZAX® administration were similar with previously observed data reported in allergic patients with or without asthmatic disease.[5,71] This study supported the safety of the highest dose (up to 500,000 SQ-T) of GAT in asthmatic patients with RC.[70]

Previous investigations have suggested that a preseasonal SLIT started 8–10 weeks before the pollen season may be adequate,[5,11,14] but subsequent studies have proposed a timing of 10–16 weeks as a best solution due to a difficult prediction of the exact start of the pollen season. On the other hand, the safe profile of GRAZAX® supported the elimination of the up-dosing phase, simplifying its administration to a one-dose-a-day treatment.

From the analysis of the data from three clinical trials based on 934 patients[5,11,66] emerged a longer duration of preseasonal treatment with GRAZAX® that influenced the clinical efficacy obtained within the GP season, in terms of reductions in RC symptom and medication scores.[14]

As international guidelines recommend an IT treatment for at least 3–5 years (based on SCIT studies' results),[72,73] associated with persistent clinical effects also in the years after its termination,[67,74] increasing data support similar outcomes for sublingual route.[69,75] Therefore, follow-up trials after a 3-year period of daily treatment demonstrated that GRAZAX® provided a similar efficacy to that observed during SLIT period (RC and medication score decrease) 1 year after[76] as prolonged clinical effects parallel with progressive immunologic changes >2 years after 3 years of therapy.[1,10] These results confirmed that SQ-standardized grass allergy tablets were capable of disease modification, giving long-term benefits.

Most of the studies on efficacy and safety of SLIT are conducted on adult patients and only a few large randomized, placebo-controlled, double-blind multicenter studies are available for pediatric population. On the other hand, conflicting data were reported about clinical efficacy in children with AR, with one meta-analysis that did not support particular clinical benefit in this population,[63] while two other meta-analyses sustained SLIT safety and efficacy in pediatric age.[77,78] SLIT safety in subjects aged 3–18 years is supported by the only mild adverse reactions reported, without serious systemic involvement.[77] However, only in one of the ten studies included in meta-analysis, the SLIT was administered in sublingual tablets instead of the drops.[77]

In summary, GRAZAX® has not quite been investigated in pediatric age.

Ibañez et al observed that GRAZAX® was well tolerated in a pediatric population. They conducted a randomized controlled trial obtained by combining the data from the two studies with identical protocols. Sixty children aged 5–12 years suffering from RC and GP allergy (with or without asthma) were recruited from five centers in two countries (three in Germany and two in Spain). In GRAZAX® group, an overall of 810 adverse effects were reported, mostly mild (71%) or moderate (27%) mouth or throat local reactions, resolved within days, as well as oral pruritus (62%), throat irritation (36%), mouth edema (31%), and ear pruritus (22%).[12]

Instead, Bufe et al, investigating grass tablet efficacy in 253 children (5–16 years old) vs placebo (SLIT started 8–23 weeks before GP season), observed that RC symptom, medication, and asthma symptom scores were statistically

significantly different between the treated children and placebo (differences in medians relative to placebo were, respectively, 24%, 34%, and 64% in favor of the first group). This study also confirmed the immunomodulatory response induced by GRAZAX® and the safety in pediatric population, reporting oral pruritus as the most common side effect, without severe adverse reactions.[79]

Blaiss et al demonstrated that self-administration of grass SLIT could be safely and effectively used in North American children ≥5 years old. In this trial, daily symptom score, daily medication score, and standardized Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) score vs placebo improved 25%, 81%, and 18%, respectively. Also immunological changes occurred, such as Phl p 5-specific IgG4 and IgE-blocking factor levels significantly higher at the peak and end of the GP season.[80]

Valovirta et al are conducting the first double-blind, randomized controlled trial to assess the preventive effect of GRAZAX® on asthma development. In 7 months of screening, they recruited 812 children (5–12 years) at 101 centers in eleven countries, with GP-induced RC, and not asthmatic, randomized 1:1 to receive GRAZAX® or placebo once daily for 3 years, and followed by a blinded observational period of 2 years.[81]

In conclusion, other studies conducted in adult population confirmed the previous results.[82–85]

Table 1 summarizes the main efficacy studies reported in this review.

Patient focus perspective

Patient compliance is needful to achieve an optimal therapeutic response with IT, improving QL and resulting in a reduction in drug costs. On the contrary, poor compliance may result in more medications added to treat patients, worsening the problem. Several factors could negatively influence compliance to allergen-specific IT: duration of treatment, early phase adverse reactions, and medication use outside the pollen season. A long-lasting home treatment as SLIT is self-managed at home by patients and parents, requiring an adequate compliance.[86]

Very few studies were performed only for evaluating patients' adherence to therapy.

In particular, two recent researches focused themselves on the usefulness of a device that could help patients to remember to take IT.

The first one evaluated if compliance with GRAZAX® could be increased by providing an electronic compliance device (CED) (Memozax; a tablet container with a programmable daily acoustic alarm), enrolling 261 grass allergic patients, randomized 1:1 to GRAZAX® using a CED (group A, n=122) or not (group B, n=139) for 1 year. This study demonstrated that treatment compliance was similar (83%) and, in general, good and not related to the use of CED, associated with a significant clinical improvement in 81% of patients.[86]

The second one reported the results of a subgroup analysis of a multicenter, randomized, controlled, open-label European study on the use of GAT with or without the help of a device, administering SLIT preseasonally (6–12 weeks before), seasonally (8–10 weeks during), and for up to 2 weeks after the season end. A total of 82% (58/71) of patients used the device sometimes or always, 79% (50/63) described the device easy to use, 46% (32/69) considered useful to easily remember to take IT, and 61% (43/71) confirmed the consideration to use the device again. This investigation supported the use of the CED as a medication reminder, considered easy to use.[87]

The QL in patients taking Timothy GP extract SLIT for RC is rarely taken into consideration as the main outcome. So, this aspect was extensively discussed in the paragraph in the "Efficacy studies, safety, and tolerability profile" section.

The following studies focused mainly on QL, based on RQLQ.

Rak et al examined QL in 855 patients who received preseasonal (8 weeks before) and seasonal administration of GRAZAX® (2,500, 25,000, or 75,000 SQ-T) vs placebo (giving loratadine or placebo if symptoms were present). The authors showed that GRAZAX® improved QL more than placebo as in patients who used loratadine (first group) as in ones who did not use this drug (second group) and over loratadine use alone (third group). The RQLQ score was greater with 75,000 SQ-T vs placebo at first and second seasonal visit (17%, P=0.006 and 20%, P=0.020 respectively) in the first group and in the second group (21%, P=0.021) at second seasonal control, while in the third group, RQLQ score was 26% (P=0.014) greater with 75,000 SQ-T than loratadine at second visit.[88]

Frølund et al demonstrated persistent and clinically significant improvements in QL induced by grass allergy SLIT tablet vs placebo in adult patients with RC poorly controlled by symptomatic medications, associated with increase of the effect with increasing GP exposure. The population included 157 active treated patients for 3 years vs 126 placebo ones, followed by follow-up period of 1 year. The overall RQLQ score for the whole GP season assessed during follow-up was significantly better in the SLIT treated patients (relative difference to placebo: 23%, P=0.004), with

Table 1 Summary of the main efficacy studies

Study	Patients	Type of study	Age, years (range)	Dosage	Length of preseasonal treatment	Therapy duration	Follow-up (years)	Main results
Durham et al[11]	855	RCT	18–65	2,500, 25,000, or 75,000 SQ-T (0.5 μg, 5 μg, or 15 μg of Phl p 5)	8 weeks	16 weeks (preseasonal + seasonal)	No	Well tolerated, it can reduce symptoms and improve QL
Durham et al[76]	257	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	4–8 months	3 years (preseasonal + seasonal)	1 year	Consistent clinical improvement and immunologic changes, sustained 1 year after treatment
Durham et al[1]	634	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	4–8 months	3 years (preseasonal + seasonal)	2 years	Efficacy was supported by long-lasting significant effects on the allergen-specific antibody response
Calderon and Essendrop[70]	43	RCT	18–65	75,000, 150,000, 300,000, or 500,000 SQ-T (15 μg, 30 μg, 60 μg, or 100 μg)	Not mentioned	28 days (seasonal)	No	Doses up to 500,000 SQ-T in patients with asthma and RC were safe and well tolerated
Dahl et al[66]	634	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	16 weeks	The average GP season lasted 57.8 days (range 16–86 days)	No	Efficacious and well tolerated
Dahl et al[5]	114	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	10–14 weeks (84±17 days)	The pollen season varied from 52 days to 60 days	No	Self-administration was safe, and the treatment did not impair asthma control
Dahl et al[10]	351	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	4–8 months	The average GP season lasted 59 days (range 30–116 days) and 22 months (preseasonal + seasonal)	No	Progressive immunologic changes and highly significant efficacy over 2 years of continued treatment
Bufe et al[79]	253	RCT	5–16	75,000 SQ-T (15 μg of Phl p 5)	7.9–23.4 weeks	The average GP season lasted 32.4 days (range 4–92 days)	No	Efficacious, well tolerated, and associated with an immunomodulatory response
Durham et al[84]	439	RCT	18–65	75,000 SQ-T (15 μg of Phl p 5)	16 weeks	23 weeks (preseasonal + seasonal)	No	Efficacious, safe, and well tolerated
Blaiss et al[80]	345	RCT	5–17	75,000 SQ-T (15 μg of Phl p 5)	16 weeks	23 weeks (preseasonal + seasonal)	No	Safety profile, efficacy

Abbreviations: GP, grass pollen; Phl p 5, phleum p 5; QL, quality of life; RCT, randomized controlled trial; RC, rhinoconjunctivitis; SQ-T, standardized quality tablet.

higher improvement during the peak of the pollen season (28%, P=0.001).[89]

These results show that SLIT with GAT reduces symptoms and improves QL in allergic RC patients.[88,89]

On the other hand, some researches included pharmacoeconomic analyses to evaluate cost-effectiveness of GRAZAX® based on the improvement of QL.

Canonica et al have showed that GRAZAX® is a cost-effective therapy in Southern Europe for GP-induced RC, best of standard care for efficacy endpoints included quality adjusted life years (QALYs) gained, obtaining a significantly less use of rescue medications and fewer hours missed from work. Its annual price was in the range of €1,500–€1,900, including the future costs of asthma in these results and excluding Spanish trial centers that experienced an exceptionally low pollen season.[90]

GRAZAX® was a cost-effective therapy also in patients with coexisting RC and asthma, as demonstrated by data from another prospective pharmacoeconomic analysis, part of a multinational clinical trial on GRAZAX® efficacy (79 patients) vs placebo (72 patients). A significantly higher QALY for the active group was reported, in contrast to levels of resource use and productivity loss higher in placebo group. The cost per QALY gained with GRAZAX® was highly cost-effective (£4,319). Price sensitivity analyses showed that it remained cost-effective up to a tablet price of £5.07.[91]

Also in pediatric age, GAT improves patient outcomes, generating an incremental cost per QALY gained of £12,168, below commonly accepted thresholds in the UK. Therefore, it is considered a cost-effective strategy for GP-induced RC in the UK pediatric population.[92]

Bachert et al supported the cost-effectiveness of GAT also in Northern European countries, for a tablet price <€6. The price of the tablet in Germany, was, for example, €2.95, with consequent yearly treatment cost of €358. The cost per QALY gained was similar in the seven countries (€12,930 to €18,263 for an annual cost of the GAT of €1,500). This study showed that GRAZAX® was cost-effective for an annual cost <€2,200.[93]

Conclusion

SLIT treatment with GP allergen extract sublingual tablets may become an important tool for the therapy of the GP-induced RC and asthma symptoms in adult as well as in the children, thanks to its efficacy and safety profile, associated with its easy and safe route of administration and lack of dose buildup requirement that improves patient compliance.

However, further investigations are needed especially in pediatric age to better characterize its efficacy and safety, as well as a deepening in knowledge of its potential mechanisms of action would be advisable and desirable. In vitro tests and in vivo challenges may be useful tools to allow objective measurements of treatment efficacy of immunomodulatory treatments like SLIT.

Disclosure

The authors declare that there is no conflict of interest in this work.

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