

Letter to the Editor

Immunologic response and safety in birch pollen sublingual versus oral vestibule immunotherapy: A pilot study

To the Editor:

To date the efficacy of sublingual immunotherapy (SLIT) in the form of allergy immunotherapy tablets is well documented with a large number of clinical trials and it is accepted as an effective alternative to subcutaneous immunotherapy in the treatment of type I-mediated respiratory allergies in Europe, but is currently not likewise accepted in the United States.¹ The attention of many scientists and clinicians is directed toward how immunologic mechanisms mediate the effect of SLIT and improvement of its efficacy, while reducing adverse effects. Although the immunologic mechanisms of SLIT as well as subcutaneous immunotherapy remain to be elucidated in detail, the induction of regulatory T cells as well as a shift from an allergy-mediating T_H2 toward an allergy-preventing T_H1 immune response is propagated along with the induction of allergen-specific IgG.² Moreover, antibodies with IgE-blocking activities—quantified as the IgE-blocking factor—have been described recently within the allergen-specific IgG fraction, which are hypothesized to correlate with the clinical response to immunotherapy.³ The common link between these immune reactions are professional antigen-presenting cells such as dendritic cells (DCs) because these cells capture and process the allergen to induce an allergen-specific immune response after allergen presentation.² Recent published data suggest a pivotal role of resident oral mucosal DCs—such as oral Langerhans cells in SLIT.^{4,5} Moreover, it has been demonstrated that the number/density of oral DCs within the sublingual (S) region was significantly lower than in other regions such as the oral vestibule (V).⁶ Furthermore, V contains a potentially favorable ratio between oral Langerhans cells and mast cells—the latter contribute to the adverse events (AEs) in SLIT such as oral itching.⁶

The objective of this pilot trial was to generate initial data for the V route of allergen application—defined here as the pouch formed by lip and gingival mucosa—versus the S route in adult subjects suffering from allergic rhinoconjunctivitis induced by birch pollen. It was conducted as a multicenter, parallel-group, 1:1 randomized noninferiority phase II trial including 4 centers in Germany; 71 subjects received 2500 specific treatment units/mL birch pollen extract (SLITone, ALK-Abelló, Hørsholm, Denmark; 500 STU per dose) for 36 weeks. In case of successful trial outcome, the superiority of group V versus group S was tested at each control visit. Demographic data and inclusion criteria are depicted in Table E1 in this article's Online Repository at www.jacionline.org. The primary end point was immunologic changes from baseline (specific IgE-blocking factor^{3,7}) against an extract of *Betula verrucosa* after 36 (24, 12, 8, and 4) weeks of treatment. The primary end point was determined after imputing missing visits by using the last measurement upon treatment—the so-called posttreatment value—as outcome that was evaluated according to the trial protocol. Secondary end points were immunologic changes from baseline in specific IgE and IgG₄ against the same extract after 36 (24, 12, 8, and 4) weeks of treatment. Safety secondary end point was incidences of local reactions

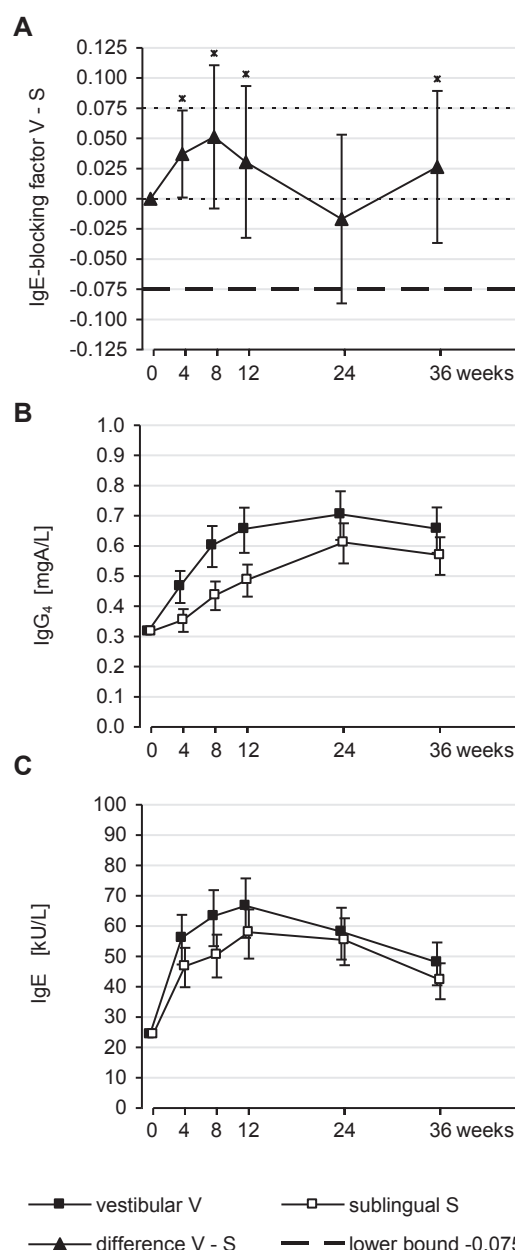


FIG 1. Differences (V – S) in levels of specific IgE-blocking factor against *Betula verrucosa* between oral vestibule (V) and sublingual (S) administration and 95% CIs determined at different time points during the treatment period of 36 weeks (A). Testing for noninferiority was simultaneously significant for all time points except for week 24 using $\delta = -0.075$ as the equivalence limit. Geometric means and standard errors (SE) of specific IgG₄ (B) and IgE (C).

related to the administration route. The study was conducted in compliance with the principles of ICH-E6 and Good Clinical Practice. The study was approved by local ethical committees and the Paul Ehrlich Institut (EudraCT no. 2009-014580-39).

Full analysis sets were used for analysis, statistics are described in the Methods section in this article's Online Repository at www.jacionline.org, and flow of patients through the study and the

number of missing blood samples is shown in Fig E1 in this article's Online Repository at www.jacionline.org.

Numerically higher levels of IgE-blocking factor against *B verrucosa* were seen after V administration compared with S administration at all visits except for week 24. The lower limits of the 95% CI of the difference V – S were –0.075 or more except for week 24. Therefore, noninferiority could be concluded posttreatment and at week 36, respectively. However, estimating the error rate according to Bonferroni-Holm, noninferiority could be derived at all control visits except for week 24. Interestingly, the superiority of V versus S could be shown at week 4 with respect to the IgE-blocking factor, but none of the later measurements (Fig 1). On investigating the outcome of secondary parameters, the levels of IgG₄ as well as IgE were found to be comparable. A weak nonsignificant trend of higher levels of IgG₄ in the early phase of treatment, especially up to week 12, could be seen in the V group (Fig 1). Further analysis of secondary end points included the safety profile of S versus V. During the entire treatment phase (S vs V: 84.2% vs 84.8%), within 30 minutes after first administration (42.1% vs 48.5%), on the entire day of first administration (65.8% vs 60.6%), and with respect to local reactions related to the administration route (57.9% vs 54.5%), no statistically significant differences in the incidences of AEs were observed (Table E1).

These first clinical data about V application of SLIT solutions indicate that the V region might represent an alternative allergen application site in respect to immunologic response. Although overall superiority in respect to primary immunologic parameters could not be demonstrated in this pilot study, significantly higher levels of IgE-blocking factor at week 4 may indicate a faster immunologic response within V compared with S allergen administration.

It has been described recently that DCs are located in significantly higher numbers in the V region than in the S region,⁶ and it is tempting to speculate that this could result in a faster response of IgE-blocking factor. In general, passive resorption is considered as the major mucosal crossing factor for peptides within the oral mucosa.⁸ In contrast to the S region, no major salivary ducts drain into the V region so that dilution of allergen solution during application is kept to a minimum and may therefore not be able to impair allergen resorption.⁹ Moreover, the V region forms a pocket surrounded by buccal and gingival mucosa and the teeth alignment, preventing allergens from being swallowed too early especially when using liquid solution.

In this pilot study, noninferiority, based on the level of specific IgE-blocking factor of V compared with S application, could be observed posttreatment and at week 36. Furthermore, no significant differences in the occurrence of AEs between the 2 application sites were observed. However, further studies are needed to evaluate safety and clinical efficacy before V applications can be considered in clinical practice.

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REFERENCES

- Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005;60:4-12.
- Allam JP, Novak N. Local immunological mechanisms of sublingual immunotherapy. *Curr Opin Allergy Clin Immunol* 2011;11:571-8.
- Shamji MH, Ljørring 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.
- Allam JP, Würtzen PA, Reinartz M, Winter J, Vrtala S, Chen KW, et al. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-beta 1 and IL-10-producing properties. *J Allergy Clin Immunol* 2010;126:638-45.e1.
- Mascarell L, Lombardi V, Louise A, Saint-Lu N, Chabre H, Moussu H, et al. Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4⁺ T cells. *J Allergy Clin Immunol* 2008;122:603-9.e5.
- Allam JP, Stojanovski G, Friedrichs N, Peng W, Bieber T, Wenzel J, et al. Distribution of Langerhans cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? *Allergy* 2008; 63:720-7.
- Durham SR, Emminger W, Kapp A, de Monchy JG, Rak S, Scadding GH, et al. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J Allergy Clin Immunol* 2012;129:717-25.e5.
- Senel S, Kremer M, Nagy K, Squier C. Delivery of bioactive peptides and proteins across oral (buccal) mucosa. *Curr Pharm Biotechnol* 2001;2:175-86.
- Amano O, Mizobe K, Bando Y, Sakiyama K. Anatomy and histology of rodent and human major salivary glands: overview of the Japan Salivary Gland Society-sponsored workshop. *Acta Histochem Cytochem* 2012;45:241-50.

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METHODS

As Full Analysis Set all randomized patients who received SLIT at least once were evaluated. In the Full Analysis Set, the primary end point was determined after imputing missing visits by means of Last Observation Carried Forward (ie, by using the individually last measurement upon treatment—the so-called post treatment value—as outcome). The further tests after 36, 24, 12, 8, and 4 weeks were based on observed cases. Noninferiority was tested in the prespecified order “posttreatment, week 36, week 24, week 12, week 8, week 4,” with a prespecified noninferiority margin of $\delta = -0.075$.

The trial outcome was classified as successful if a significant noninferiority could be demonstrated on step 1 (posttreatment) of the ordered test. In case of successful trial outcome, tests of superiority of group V versus group S were carried out 1-tailed at the level 2.5% at each control visit. The sample size calculation was based on the noninferiority test of the primary pharmacodynamics end point. From previous studies, an SD of approximately 0.15 for changes from baseline in the IgE-blocking factor after a longer period of treatment was derived. Using the well-founded concept of “equivalence

limit = $\sigma/2$,” $\delta = -0.075$ was chosen as the prespecified noninferiority limit of changes from baseline in the IgE-blocking factor.

Using this noninferiority limit, 0.1 as SD in the own homogeneous material, and $1 - \beta = 0.8$ as test power, 28 patients per group should terminate the study to verify noninferiority of V versus S at the level of $\alpha = 0.025$. Taking into account isolated dropouts, 32 patients per group (16 patients per center) were planned for recruitment. In the subsequent tests of superiority, a standardized treatment difference of 0.50 could be proven to be significant on the 1-sided 2.5% level. This was regarded as adequate power of this pilot study.

Because of the hierarchically ordered sequence of confirmatory tests, no additional adjustment for multiplicity was required. However, in case of an “interruption” in the sequence of significant test results, the type I error is not controlled as soon as the interruption occurs. But in this situation, a posteriori Bonferroni methods are indicated to estimate the multiple error rate. The generally applicable procedure of Bonferroni-Holm was used to complete the noninferiority tests over time, that is, the tests for noninferiority were simultaneously significant except for week 24.

Flow of patients through the study:

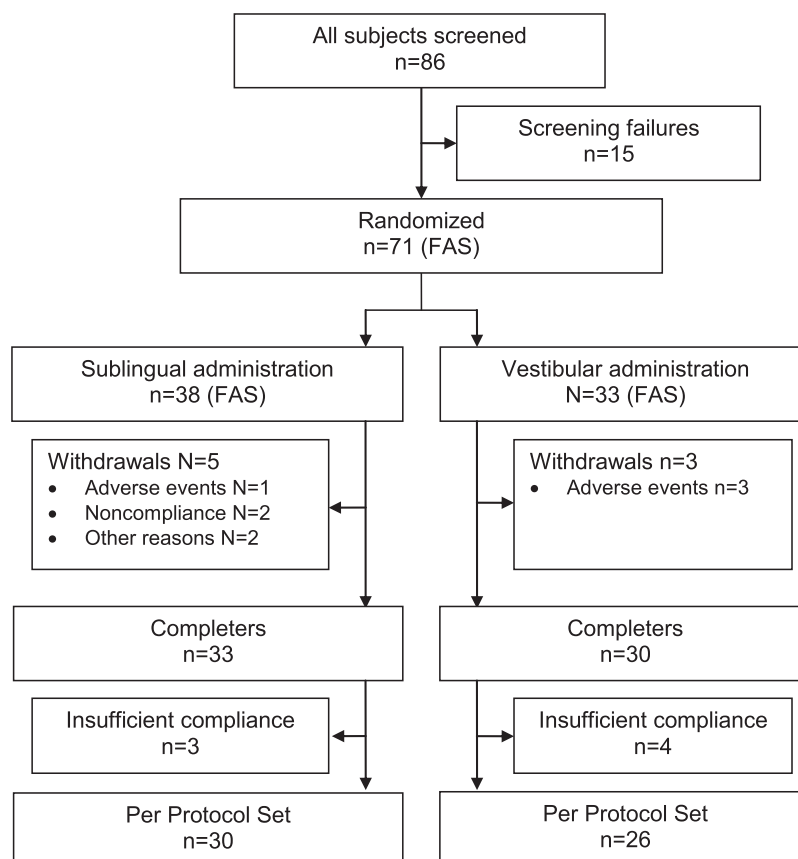


FIG E1. Thirty-eight patients were randomly allocated to sublingual (*S*) administration and 33 to oral vestibule (*V*) administration and, therefore, comprised the Full Analysis Set (*FAS*); 5 patients in the *S* group and 3 patients in the *V* group discontinued treatment before the end of the planned treatment period of 36 weeks, resulting in 33 patients with *S* administration and 20 patients with *V* administration who completed the study. Three patients in the *S* group and 4 patients in the *V* group were excluded from the per protocol analysis because of insufficient compliance, resulting in 30 patients with *S* administration and 26 patients with *V* administration who could be analyzed in the per protocol set. Blood samples were missing in the *S* group for visits 2, 3, and 4 in 3 patients, respectively, and for visit 5 in 6 patients and for visit 6 in 3 patients, and in the *V* group for visit 2 in 1 patient and visits 3, 4, and 5 in 3 patients, respectively.

TABLE E1. Demographic data on patients

Characteristic	Sublingual	Vestibular	All patients
No. of subjects	38	33	71
Age (y)			
Median	41.0	40.0	41.0
Range	18-60	19-63	18-63
Sex, n (%)			
Male	18 (47.4)	14 (42.4)	32 (45.1)
Female	20 (52.6)	19 (57.6)	39 (54.9)
Ethnic origin, n (%)			
White	37 (97.4)	31 (93.9)	68 (95.8)
Other	1 (2.6)	2 (6.1)	3 (4.2)
Height (cm)			
Median	175	170	173
Range	154-197	159-192	154-197
Weight (kg)			
Median	77	70	74
Range	51-140	50-100	50-140
Body mass index (kg/m ²)			
Median	24.1	23.6	24.0
Range	19.6-45.1	18.7-33.7	18.7-45.1
Duration of rhinoconjunctivitis (y), median	17	12	15
Bronchial asthma, n (%)	12 (31.6)	10 (30.3)	22 (31.0)
Specific immunotherapy in the history, n (%)	11 (28.9)	11 (33.3)	22 (31.0)
Specific IgE against <i>Betula verrucosa</i> (kU/L), median	28.80	26.19	27.28

Note. Eligible for the trial were male and female patients aged 18 to 65 years with a clinical history of birch pollen–induced allergic rhinoconjunctivitis of 2 years or more requiring treatment during the birch pollen season who had a positive skin prick test (SPT) to *Betula verrucosa* (wheal diameter ≥ 3 mm) and a positive specific IgE against *Betula verrucosa* (IgE class ≥ 2). Patients with uncontrolled or severe asthma ($FEV_1 < 70\%$ of predicted value in spite of adequate pharmacologic treatment) and a previous treatment by immunotherapy with birch or a cross-reacting tree pollen allergen within the previous 5 years were excluded.