Lou et al. World Allergy Organization Journal (2020) 13:100458
http://doi.org/10.1016/j.waojou.2020.100458

WORLD ALLERGY ORGANIZATION JOURNAL

Open Access

Artemisia Annua sublingual immunotherapy for seasonal allergic rhinitis: A multicenter, randomized trial

Hongfei Lou[a,b,1], Xueyan Wang[c,1], Qingyu Wei[d], Changqing Zhao[e], Zhimin Xing[f], Qinna Zhang[g], Juan Meng[h], Shaoqiang Zhang[i], Huifang Zhou[j], Ruixia Ma[k], Hua Zhang[l], Hui Liu[m], Weiguo Xue[n], Chengshuo Wang[a,b]** and Luo Zhang[a,b,o]*

ABSTRACT

Background: *Artemisia annua* is the most common outdoor aeroallergen throughout Northern China; however, no multicenter study has investigated sublingual immunotherapy (SLIT) as a treatment option for *Artemisia annua*-induced allergic rhinitis (AR). The aim of this study was to evaluate the efficacy and safety of an innovative SLIT for *Artemisia annua*-related AR.

Methods: This was a randomized, double-blind, placebo-controlled, multicenter, phase 3 clinical trial conducted in China (NCT XXX). A total of 702 *Artemisia annua*-sensitized eligible patients were randomized in a ratio of 2:1 to receive *Artemisia annua*-SLIT or placebo. The treatment lasted 32 weeks; including 5-weeks up-dosing phase and 27-weeks maintenance phase. The primary endpoint was the daily combined score of medication and rhinoconjunctivitis symptom (CSMRS), and secondary endpoints were daily total nasal symptom score (dTNSS) and daily rescue medication score (dRMS) during peak pollen period. Safety of treatment was evaluated according to adverse events (AEs) experienced.

Results: Mean daily CSMRS was significantly improved during the peak pollen period in the SLIT group compared with the placebo group (1.46 ± 0.47 vs 1.88 ± 0.42, $P < 0.0001$ in full analysis set [FAS]; 1.49 ± 0.52 vs 1.95 ± 0.46, $P < 0.0001$ in per protocol set [PPS]); representing a 22.3% and 23.6% reduction, respectively, relative to placebo. In specifically *Artemisia annua* monosensitized patients, mean daily CSMRS reductions were demonstrated as 24.1% and 27.0% in the FAS and PPS populations, respectively, when comparing the active treatment to placebo treatment. Similarly, SLIT decreased dTNSS in peak pollen period by 19.0% in FAS and 22.3% in PPS, respectively, relative to placebo. In coincidence, dRMS in peak pollen period was reduced by 22.0% in FAS and 26.0% in PPS. 65.8% patients in SLIT group experienced treatment-related AEs, none of which was serious.

[a]Department of Otolaryngology Head and Neck Surgery, Beijing TongRen Hospital, Capital Medical University, Beijing, 100730, China
*Corresponding author. Department of Otolaryngology Head and Neck Surgery, Beijing TongRen Hospital, Capital Medical University, No.1, DongJiaoMinXiang, DongCheng District, Beijing 100730, PR China. E-mail: dr.luozhang@139.com
**Corresponding author. Department of Otolaryngology Head and Neck Surgery, Beijing TongRen Hospital, Capital Medical University, No.1, Dongjiaominxiang, DongCheng District, Beijing, 100730, PR China. Email: wangcs830@126.com

[1] These authors contributed equally to this work.
Full list of author information is available at the end of the article

http://doi.org/10.1016/j.waojou.2020.100458
Received 5 May 2020; Received in revised from 3 August 2020; Accepted 13 August 2020
Online publication date xxx
1939-4551/© 2020 The Author(s). Published by Elsevier Inc. on behalf of World Allergy Organization. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Conclusion: This study indicates that SLIT with *Artemisia annua* drops is an effective and safe treatment option in Chinese patients with *Artemisia Annua*-induced AR.

Keywords: Sublingual immunotherapy, Allergic rhinitis, *Artemisia annua*, Efficacy, Safety

INTRODUCTION

Allergic rhinitis (AR) is a global allergic disease, which has a pronounced negative impact on quality of life and leads to an increased economic and health-related burden on both the affected individual and society.[1] In the recent decade, the prevalence of self-reported AR in China has increased markedly from 11.1% to 17.6%.[2] A recent study has reported that the incidence of physician-diagnosed AR is as high as 32.4% in Northern China,[3] with the majority of patients having seasonal allergic rhinitis (SAR) due to outdoor aeroallergens.

Artemisia species, or mugwort, is the most important outdoor aeroallergen throughout China.[4,5] It has been reported that about 11.3% of the Chinese patients with respiratory allergies are sensitized to *Artemisia* pollen, with the number of patients sensitized to this allergen being over 50% in Northern China.[5] The commonly occurring species of *Artemisia* in China is *Artemisia annua*, or sweet sage wort, which is mainly distributed in the area around the northern part of Yangtze River.[4] This plant produces a large quantity of small pollen grains and causes widespread SAR over a long period from the beginning of July until the end of September each year.

Medications generally used to relieve AR symptoms have been shown to be partially effective and without long-term benefits in 40% of patients with hay fever in a general practice setting.[6] In contrast, allergen specific immunotherapy (AIT) is the only treatment modality able to induce prolonged remission and alter disease progression.[7] Although subcutaneous immunotherapy (SCIT) has been introduced into clinical practice for over 100 years and confirmed to be efficacious,[8,9] considering the inconvenience of injection and potential risks of adverse events (AEs) in SCIT, sublingual immunotherapy (SLIT) has been developed as an alternative to facilitate access to AIT and to provide a very favorable safety profile. By enhancing the benefit-risk ratio in favor of superior convenience of self-administration without supervision of medical personnel, SLIT has been shown to be preferred as an effective and safer alternative to SCIT in recent years.[10] Moreover, most clinical trials and meta-analysis confirm a significantly more reliable efficacy and better safety profile of SLIT compared with placebo for management of pollen-induced AR or allergic rhinoconjunctivitis.[11-17]

Compared to pollen specific SLIT widely performed in AR patients in Europe[18] and North America,[19-21] little research has focused on the efficacy and safety profile of AIT with *Artemisia annua*. Recently, *Artemisia annua* drops have been developed as the first registered, standardized pollen allergen extracts for SLIT in patients with *Artemisia* pollen-induced SAR; with phase 1 and phase 2 clinical trials in a small group of Chinese patients with *Artemisia annua*-sensitized SAR. These trials indicated that this preparation did not result in any serious adverse events and also determined the effective safe dose for *Artemisia annua* SLIT (unpublished data). The aim of the present study was therefore to evaluate the efficacy and safety of SLIT with this new preparation in a large cohort of Chinese SAR patients sensitized to *Artemisia annua*, using a placebo-controlled, double-blind, randomized, multicentre clinical trial design.

METHODS

Study design

This phase 3, randomized, double-blind, placebo-controlled study (registered at clinicaltrial.gov as NCT03990272) was conducted from March of 2017 (approximate 4 months before the local natural *Artemisia* pollen season) to October

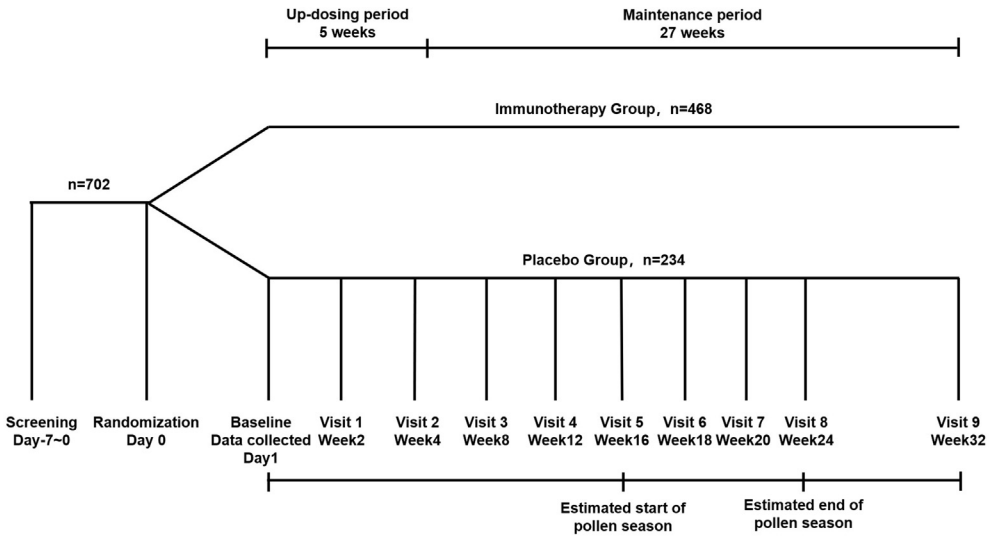


Fig. 1 Study design. The treatment began approximately 4 months before start of the *Artemisia* pollen season and continued throughout a single pollen season

of 2017, and involved patients from 13 centers across Northern China.

A total of 702 eligible patients were recruited and randomized into SLIT group and placebo group at the ratio of 2:1 according to a computer-generated block-randomization scheme. All patients and investigators were blinded to the study treatments by use of a placebo, which was identical to *Artemisia* drops adjunct in taste and appearance, and blinding was maintained through the trial until the database was locked.

All patients received treatment for 32 weeks involving a "5-week" up-dosing phase (1.25 biological unit to 2400 biological unit) and a "27-week" maintenance phase (2400 biological unit) with daily sublingual drops (Fig. 1), which were provided by Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd., China. The allergen extract used for immunotherapy was glycerinated allergen extract of *Artemisia annua*, with the main allergenic components Art an1 and Art an3, while the placebo drops were physically identical to active medication, but without allergenic components. The drops were administered sublingually for 1 min before swallowing and nothing was allowed by mouth for 5 min after the administration of the drops. The participants were provided with rescue medications to use in a stepwise manner during the treatment, to alleviate AR symptoms as needed. Treatment compliance was evaluated with daily diary card, in which antiallergy medications, symptoms and adverse events were required to be recorded. The investigator inspected the vials and residue medications to confirm adherence to the regimen.

Treatment began approximate 4 months before the expected start of the pollen season according to the peak period of outpatients with SAR in the previous two autumns.[3,4] The start of the natural peak pollen period[22] in 2017 was defined as the first of 3 consecutive days with a pollen count of 50 pollen/m^3 or greater each day. The end of peak pollen period was defined as the last occurrence of 3 consecutive days with $\geq$50 pollen/m^3 each day.

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the corresponding review board in each center, and written informed consent was obtained from each subject prior to entry into the study.

Subjects

Patients with allergic rhinitis due to *Artemisia* pollens were recruited into the study. All patients had experienced *Artemisia*-related SAR for over 2 years; with symptom scores for 2 or more nasal symptoms $\geq$2 points during July to October in 2016. *Artemisia annua* allergy was further verified by the presence of specific immunoglobulin E (sIgE) $\geq$ 3.5 kU/L, using UniCAP system (Phadia, Uppsala, Sweden). Polysensitization was defined

as *Humulus*- or *Artemisiifolia*-sIgE levels (ImmunoCAP) ≥0.7 kU/L (ImmunolCAP), but < *Artemisia*-sIgE level.

Patients were excluded from the study if they had previously received AIT, suffered from persistent or unstable asthma, perennial allergic rhinitis (eg, house dust mite sensitized patients with perennial allergic symptoms), cancer, or other serious diseases that could render them unfit to receive allergen immunotherapy, or any nasal condition that could confound the results of the study (chronic rhinitis, chronic rhinosinusitis with/without polyps). Similarly, patients who were non-compliant with the study protocol and treatment, pregnant and sexually active women of child-bearing potential, patients taking β-antagonists or those who had taken systemic corticosteroids in last 4 weeks, and patients whose *Humulus*- or *Artemisiifolia*-sIgE levels (ImmunoCAP) were ≥ *Artemisia*-sIgE level, were also excluded.

Demographic and clinical data including age, sex, atopic status, comorbid allergic diseases, sIgE, and nasal/ocular symptom scores at the previous pollen season were collected before initiation of treatment.

Efficacy and safety assessments

The primary efficacy endpoint was set as the daily combined scores of medication and rhinoconjunctivitis symptoms (CSMRS) (ranging from 0 to 6); which was calculated as the combined score of daily average scores of 6 rhinoconjunctivitis symptoms (rhinorrhea, nasal congestion, nasal itching, sneezing, ocular pruritus, and watery eyes) and the daily rescue medication score (RMS).[23]

Nasal and ocular symptoms were rated on a 4-point scale of 0–3; with 0 = no symptoms, 1 = mild symptoms (sign/symptom clearly present, but minimal awareness; easily tolerated), 2 = moderate symptoms (definite awareness of sign/symptom that is bothersome but tolerable), and 3 = severe symptoms (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping). Relief medication was scored for daily use as follows: 0 = no use of rescue medication, 1 = use of oral and/or topical non-sedative H1 antihistamines (Clarityne or Patanol), 2 = use of intranasal corticosteroids (Rhinocort) with/without H1 antihistamines, and 3 = use of oral corticosteroids with/without intranasal corticosteroids, with/without H1 antihistamines.

Two secondary endpoints were investigated in the current study. The daily total nasal symptom score (dTNSS) was the sum of four nasal symptom scores for nasal congestion, discharge, itching and sneezing (ranging from 0 to 12). The daily RMS (dRMS) was assessed as the rescue medication score (ranging from 0 to 3).

Safety was evaluated by the occurrence and severity of adverse events (AEs) and the casual relationship between AEs and the experimental drug. All AEs were recorded in dairy card and categorized as mild (no impact on the activities of daily living), moderate (decreased or affected performance of the activities of daily living) or severe (an inability to perform the activities of daily living or death).

Statistical analyses

The full analysis set (FAS) population, same as the intent-to-treat population, included all randomized subjects who had received at least 1 dose of study medication during the peak pollen season and who had at least 1 post-dose efficacy assessment and rescue medication score. The per protocol set (PPS) population included all subjects in FAS population who completed the study with no serious violation of the protocol. The safety analysis was performed with the safety set (SS) population, which included all randomized subjects who had received at least 1 dose of the study medication in the study.

The sample size was estimated by power statistics using the CSMRS as the primary outcome measure. We estimated that using a 2:1 group design, at least 582 subjects would be required between the two treatment groups to detect a mean difference of 0.32 (combined SD, 1.12) (according to previous data in phase 2 clinical trials in a small group of Chinese patients with *Artemisia annua*-sensitized SAR), with 90% power and a 2-tailed α value of 0.05. Considering a drop-out rate of 20% and randomization block size as 6, we recruited 702 participants, with 468 subjects in the SLIT group and 234 patients in the placebo group. The last observation carry forward was used for missing values of the primary outcome.

SPSS statistical software, Version 22.0 (IBM Corp., Armonk, NY, USA) was used for data analysis, and two-side tests were performed with a significance level of 0.05. Descriptive statistics were used for baseline and post-treatment variables. Efficacy endpoints were assessed in the FAS and PPS population, respectively. Analysis of covariance (ANCOVA) was performed to compare differences in efficacy endpoints. Mean and median differences between groups were estimated. ANCOVAs were performed on scores adjusted for baseline, treatment center, age and gender. An interaction term (treatment group * sensitization status) was included in the regression model. The minimum clinically significant difference (MCSD), the important threshold of change in the outcomes between SLIT and Placebo groups in both the FAS and PPS populations, was estimated as one half of the SD (baseline or control) according to Norman and colleagues.[24] If the left limit of the covariance-based 95% confidence interval (CI) surrounding the between-group difference was greater than MCSD, the clinical significance was certain.

RESULTS

Demographic characteristics

A total of 966 subjects with allergic rhinitis symptoms were screened for eligibility and 702 subjects were randomized at a ratio of 2:1 into the SLIT group (n = 468) and placebo group (n = 234) as shown in Fig. 2. Data for 30 participants (20 participants in SLIT group and 10 participants in placebo group) from one center (Center 13) were excluded from the SS population, as the sealed envelope containing the blinding information on treatment was lost. Furthermore, 35 patients withdrew their consent before the pollen season and did not accept the treatment, whereas 5 patients were lost to follow-up. Thus, the FAS population included 632 subjects. A total of 42 patients were excluded from PPS, primarily as a result of change of residence over 7 days during the pollen season.

Both groups were comparable in age, gender, atopic status and co-morbidity of allergic asthma, allergic conjunctivitis and atopic dermatitis

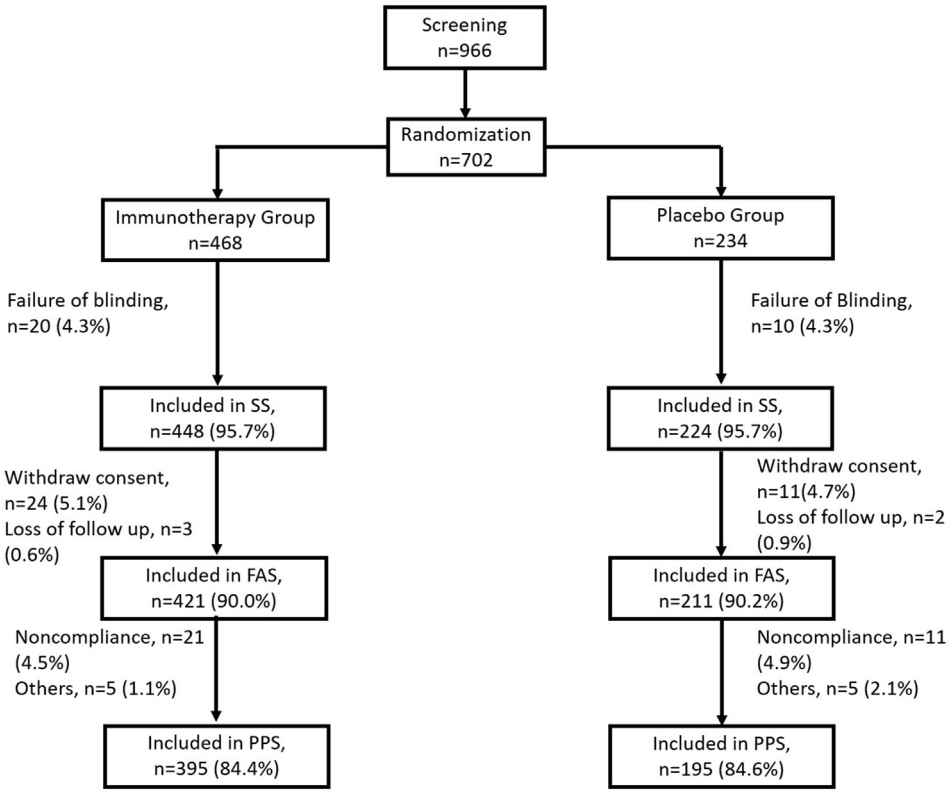


Fig. 2 Subjects disposition. A total of 702 subjects were recruited and randomized into the two treatment groups to receive either *Artemisia annua* sublingual immunotherapy (SLIT) or placebo. Overall, 672, 632 and 590 subjects were included in the safety set (SS), full analysis set (FAS) and per protocol set (PPS) population, respectively

(Table 1). Similarly, nasal and ocular symptoms scores at pollen season of 2016 were generally comparable between both groups (Table 1).

Primary endpoint of clinical efficacy

CSMRS in peak pollen period was significantly improved in *Artemisia annua*-SLIT group compared to placebo group. There was a 22.3% reduction in the mean CSMRS (1.46 ± 0.47 vs. 1.88 ± 0.42, $P < 0.0001$) in the active treatment group relative to the placebo treatment group in the FAS population (Table 2). The median values were found to be 1.44 vs. 1.87 (a 23.0% reduction) in *Artemisia annua* SLIT group and placebo group, respectively (Supplementary Table E1). Similarly, there was a 23.6% reduction in the mean CSMRS in the active treatment group relative to placebo treatment group in the PPS population (1.49 ± 0.52 vs. 1.95 ± 0.46, $P < 0.0001$) (Table 2). The median values in PPS were found to be 1.49 vs. 1.93 (a 22.8% reduction) in *Artemisia annua* SLIT and placebo groups, respectively (Supplementary Table E1). The MCSDs in CSMRS between SLIT and placebo groups in FAS and PPS populations were estimated to be 0.21 and 0.23, respectively. As the left limit of 95% CI of adjusted mean difference in improvement of CSMRS was 0.289 and 0.321, respectively, which was greater than

Variables	SLIT (n = 421)	Placebo (n = 211)
Age (year)	37.5 ± 8.73	38.7 ± 9.43
Sex, n (%)		
Male	192 (45.6%)	103 (48.8%)
Female	229 (54.4%)	108 (51.2%)
Height (m)	1.68 ± 0.079	1.678 ± 0.077
Weight (kg)	66.58 ± 12.30	66.91 ± 11.36
BMI (kg/m^2)	23.58 ± 3.19	23.64 ± 2.791
Nasal symptom scores at the previous pollen season	9.4 ± 1.83	9.3 ± 1.90
Nasal inching	2.2 ± 0.79	2.1 ± 0.85
Sneezing	2.5 ± 0.58	2.5 ± 0.59
Rhinorrhea	2.5 ± 0.65	2.4 ± 0.71
Nasal obstruction	2.2 ± 0.85	2.2 ± 0.87
Ocular symptom scores at the previous pollen season	3.7 ± 1.30	3.8 ± 1.42
Ocular pruritus	2.4 ± 0.68	2.5 ± 0.72
Watery eyes	1.3 ± 0.95	1.3 ± 1.03
Specific IgE (kU/L)	4.1 ± 0.79	4.2 ± 0.78
Allergic Asthma, n (%)	9 (2.1%)	4 (1.9%)
Allergic conjunctivitis, n (%)	388 (92.2%)	198 (93.8%)
Atopic dermatitis, n (%)	28 (6.7%)	10 (4.7%)
Food Allergy, n (%)	6 2 (14.7%)	29 (13.7%)
Drug Allergy, n (%)	44 (10.5%)	32 (15.2%)
Other allergic disease, n (%)	10 (2.4%)	4 (1.9%)
Polysensitization, n (%)	75 (17.8%)	38 (18.0%)

Table 1. Demographic characteristics of the subjects in full analysis set population. *BMI, body mass index; SLIT, sublingual immunotherapy*

	FAS Population		PPS Population	
	SLIT	Placebo	SLIT	Placebo
N	421	211	395	195
Mean ± SD	1.46 ± 0.47	1.88 ± 0.42	1.49 ± 0.52	1.95 ± 0.46
P value	<0.0001		<0.0001	
Improvement	22.3%		23.6%	
Adjusted mean difference (95% CI)	0.371 (0.289–0.453)		0.420 (0.321–0.518)	

Table 2. Daily combined scores of medication and rhinoconjunctivitis symptoms during the peak pollen period. *FAS, full analysis set; PPS, per protocol set; SLIT, sublingual immunotherapy; SAR, seasonal allergic rhinitis; CI, confidence interval*

the MCSD, this indicated that the improvement of CSMRS in SLIT group was clinically significant.

Analysis of the improvements in CSMRS using a regression model, including the interaction term between treatment variable (SLIT or placebo) and sensitization status (monosensitization or polysensitization), indicated evidence of difference based on sensitization status ($P < 0.001$ in both FAS and PPS populations). In specifically *Artemisia annua* monosensitized patients, *Artemisia annua* SLIT significantly reduced mean daily CSMRS by 24.1% (1.48 ± 0.54 vs. 1.95 ± 0.44) and 27.0% (1.43 ± 0.55 vs 1.96 ± 0.44) in the FAS and PPS populations, respectively. However, the reduction in mean daily CSMRS in polysensitized patients was not significantly different between SLIT and placebo groups in the FAS (1.39 ± 1.01 vs. 1.38 ± 0.94) and PPS (1.39 ± 1.00 vs. 1.45 ± 0.94) populations.

As far as each center was concerned, the mean daily CSMRS in peak pollen period for active treatment in nine centers indicated a significant advantage over the placebo in FAS population (Fig. 3A); whereas in PPS population, only in one center (Center 10), the mean daily CSMRS for active treatment demonstrated no significant difference from that for placebo (Fig. 3B).

Secondary endpoints of clinical efficacy

The secondary efficacy endpoints of mean daily TNSS and mean daily RMS were also significantly improved with *Artemisia annua* SLIT compared to placebo (Table 3). Patients receiving active treatment reported a 19.0% reduction in mean dTNSS during the peak pollen period relative to placebo recipients (3.84 ± 1.06 vs. 4.74 ± 0.98, $P < 0.0001$) in FAS population. In PPS population, the mean values were found to be 3.79 ± 1.08 vs. 4.88 ± 1.01 in *Artemisia annua* SLIT and placebo groups ($P < 0.0001$), respectively, which reported a 22.3% reduction. The MCSDs in dTNSS PPS population was estimated to be 0.505, less than the left limit of 95% CI of adjusted mean difference (0.672); indicating that the difference in improvement of dTNSS between the *Artemisia annua* SLIT group and placebo group was also clinically meaningful.

Subjects who received active *Artemisia annua* SLIT treatment reported a 22.0% reduction in mean dRMS throughout the peak pollen period relative to placebo recipients (0.56 ± 0.27 vs. 0.71 ± 0.26, $P < 0.0001$) in FAS and a 26.0% reduction (0.54 ± 0.29 vs. 0.73 ± 0.27, $P < 0.0001$) in PPS. The estimated MCSDs of dRMS in FAS population and PPS population was 0.13 and 0.135, respectively. The 95% CIs surrounding the adjusted between-group difference (0.101–0.206 in FAS and 0.088–0.175 in PPS) included values less than the estimated MCSDs, therefore, the clinical significance of rescue medication use reduction in the *Artemisia annua* SLIT group is uncertain. When each center was considered separately, the mean daily RMS in the *Artemisia annua* SLIT group during peak pollen period was found to be significantly reduced in seven centers (Center 2, 4, 5, 6, 7, 11 and 12) compared to the placebo SLIT group in statistics (Supplementary Figure E1 A and B).

Safety

Artemisia annua SLIT was well tolerated, with no deaths, drug-related serious allergic reactions,

http://doi.org/10.1016/j.waojou.2020.100458

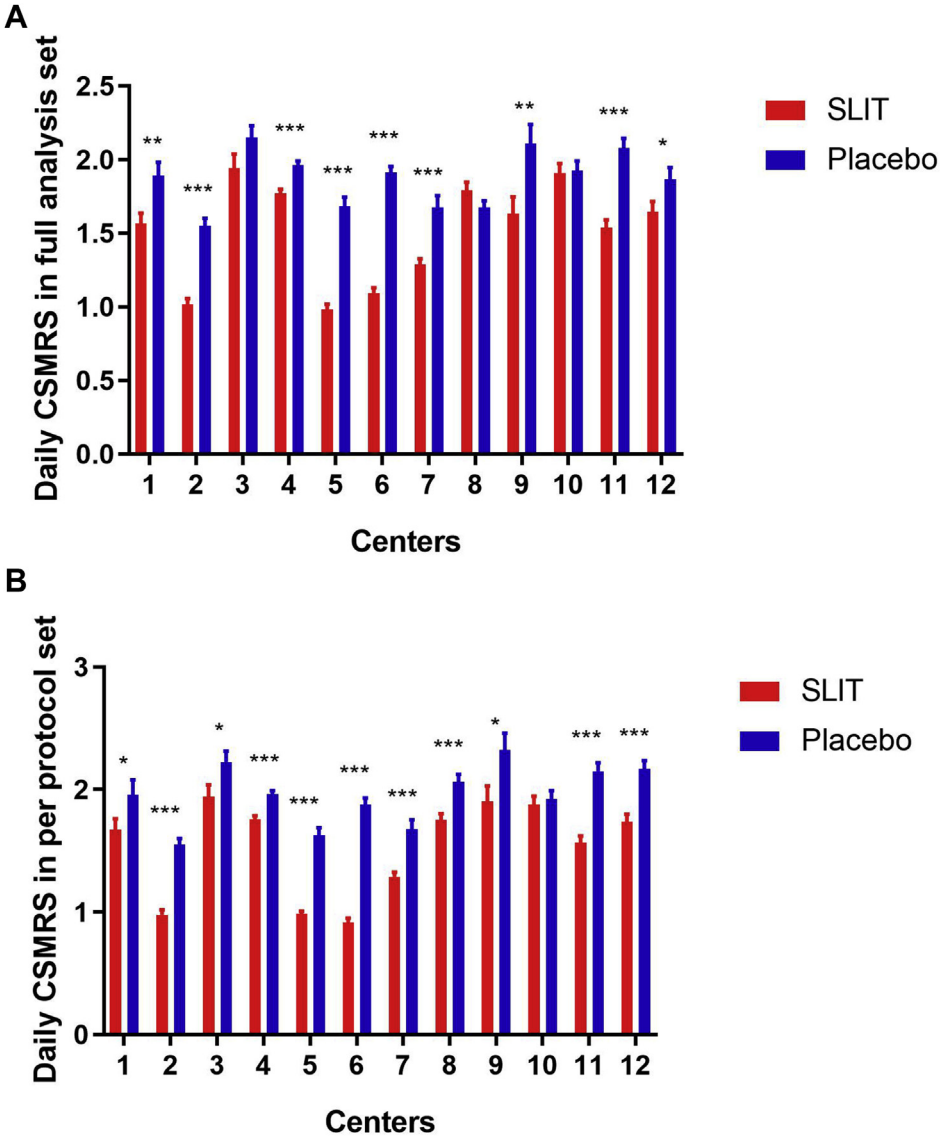


Fig. 3 Effect of *Artemisia annua* sublingual immunotherapy on daily combined scores of medication and rhinoconjunctivitis symptoms (CSMRS) in separate centers. (A) daily CSMRS during peak pollen period in full analysis set population; (B) daily CSMRS during peak pollen period in per protocol set population. FAS, full analysis set; PPS, per protocol set. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$

anaphylaxis, or life-threatening events during the whole treatment. Among the 6245 AEs reported in the current study, 2344 events were considered as drug-related and occurred in 425/672 patients (63.2%) included in the SS population. The percentage of participants reporting AEs was 91.5% (410/448 in *Artemisia annua* SLIT group and 205/224 in the control group). Similarly, the incidence of AEs per subject was also comparable in both groups during the entire 32 weeks of treatment (10.11 vs 10.24, respectively). Although the percentage of subjects who reported drug-related AEs was higher in *Artemisia annua* SLIT group (295/448, 65.8%) compared with control group (130/224, 58.0%), the incidence of drug-related AEs per subject were similar in both groups (5.51 vs. 5.53). Most AEs were early onset, transient, self-limiting reactions; with the most common drug-related AEs in the *Artemisia annua* SLIT group reported to oral paresthesia (36.8%). Table 4 lists the AEs with an incidence of 5% or greater. Overall, four patients in the active group and five patients in the placebo group each reported a severe AE (Table 5), none of which was considered to be associated with the study drug.

DISCUSSION

Artemisia annua is generally considered as the main aeroallergen responsible for SAR during late

	FAS population		PPS population	
	SLIT	Placebo	SLIT	Placebo
N	421	211	395	195
Daily total nasal symptom score				
Mean ± SD	3.84 ± 1.06	4.74 ± 0.98	3.79 ± 1.08	4.88 ± 1.01
P value	<0.0001		<0.001	
Improvement	19.0%		22.3%	
Adjusted mean difference (95% CI)	0.663 (0.447–0.879)		0.883 (0.672–1.094)	
Daily rescue medication scores				
Mean ± SD	0.56 ± 0.27	0.71 ± 0.26	0.54 ± 0.29	0.73 ± 0.27
P value	<0.0001		<0.0001	
Improvement	22.0%		26.0%	
Adjusted mean difference (95% CI)	0.154 (0.101–0.206)		0.131 (0.088–0.175)	

Table 3. Summary of average daily total nasal symptom score and daily rescue medication scores during peak pollen period. *FAS, full analysis set; PPS, per protocol set; SLIT, sublingual immunotherapy; CI, confidence intervals*

summer and autumn in Northern China. Rigorous clinical trials have confirmed that AIT can significantly relieve AR symptoms and reduce the use of medications, not only during but also after discontinuation of AIT.[25-27] Despite the widespread and adverse socioeconomic impact of *Artemisia* allergy in China, not much attention has been paid to date to either research focused on AIT with *Artemisia annua* allergen or the development of commercially available allergen extracts for *Artemisia annua*-AIT. To our knowledge, this is the first confirmatory randomized, double-blinded, placebo-controlled, multicenter, phase 3 clinical trial to investigate the clinical efficacy of SLIT with a liquid preparation of *Artemisia annua* extract in a Chinese adult population with *Artemisia annua*-associated SAR. Our study demonstrated that *Artemisia annua* SLIT was a safe and significantly efficacious therapy; as indicated by elicitation of a significantly greater reduction in combined scores of symptoms and the rescue medication compared to placebo SLIT, in *Artemisia annua*-sensitized individuals during the pollen season. Indeed, our findings has further indicated that *Artemisia annua* SLIT is likely to have more pronounced effects in *Artemisia annua*-monosensitized individuals than in polysensitized individuals. When applied in future clinical practice of SLIT, *Artemisia annua* drops is promising to greatly benefit the most prevalent SAR patients sensitized to *Artemisia annua* in China.

In this regard the present study demonstrated that the mean CSMRS was reduced by 22.3% in the FAS population and by 23.6% in PPS population, after *Artemisia annua* SLIT, relative to placebo SLIT in peak pollen period. These results meet the clinically relevant efficacy criteria established by World Allergy Organization criteria,[28] a difference of at least 20% between active and placebo groups. In this regard, our data for dTNSS in the PPS population have also indicated that patients receiving *Artemisia annua* SLIT had greater and highly statistically significant reductions of greater than 20% compared with placebo during peak pollen period. Furthermore, these results also compared favorably with a recent Cochrane meta-analysis, which compared efficacy of different pharmacologic agents for allergic rhinitis[29] and demonstrated that compared with placebo, the effect sizes for leukotriene pathway modifiers, antihistamines, and intranasal corticosteroids were, respectively, 5%, 7%, and 18%.

Several clinical trials of SLIT have reported significant improvements in the clinical endpoints for

Adverse events No. (%)	Treatment emergent		Treatment related	
	SLIT[a]	Placebo[b]	SLIT[a]	Placebo[b]
Oral paresthesia	169 (37.7)	56 (25.0)	165 (36.8)	55 (24.6)
Nasopharyngitis	126 (28.1)	62 (27.7)	2 (0.4)	2 (0.9)
Sneezing	116 (25.9)	66 (29.5)	46 (10.3)	26 (11.6)
Nasal pruritus	100 (22.3)	47 (21.0)	37 (8.3)	18 (8.0)
Rhinorrhea	91 (20.3)	56 (25.0)	32 (7.1)	19 (8.5)
Eye pruritus	88 (19.6)	59 (26.3)	41 (9.2)	23 (10.3)
Nasal congestion	80 (17.9)	49 (21.9)	25 (5.6)	15 (6.7)
Throat irritation	59 (13.2)	22 (9.8)	40 (8.9)	15 (6.7)
Oropharyngeal pain	51 (11.4)	28 (12.5)	14 (3.1)	9 (4.0)
Cough	41 (9.2)	26 (11.6)	9 (2.0)	8 (3.6)
URTI	41 (9.2)	19 (8.5)	1 (0.2)	0
Ear pruritus	40 (8.9)	11 (4.9)	27 (6.0)	7 (3.1)
Headache	32 (7.1)	14 (6.3)	6 (1.3)	2 (0.9)
Throat-clearing	30 (6.7)	17 (7.6)	5 (1.1)	4 (1.8)
Diarrhea	29 (6.5)	13 (5.8)	5 (1.1)	1 (0.4)
Tongue itching	29 (6.5)	2 (0.9)	29 (6.5)	2 (0.9)
Swollen tongue	24 (5.4)	0	24 (5.4)	0

Table 4. Adverse events experienced by 5% or more of subjects in safety set population. *SLIT, sublingual immunotherapy. URTI, upper respiratory tract infection. a. N = 448 in SLIT group. b. N = 224 in Placebo group*

Case No.	Group	Adverse events	Relation to the treatment
1	Placebo	Tibial fracture because of accident	Not treatment-related
2	SLIT	Eczema requiring hospitalization	Probably not treatment-related[a]
3	SLIT	Bronchopneumonia	Probably not treatment-related[b]
4	SLIT	Forearm fracture because of accident	Not treatment-related
5	Placebo	Incomplete intestinal obstruction	Not treatment-related
6	Placebo	Ureteral calculus	Not treatment-related
7	Placebo	Benign paroxysmal positional vertigo	Not treatment-related
8	Placebo	Lumbar disc herniation	Not treatment-related
9	SLIT	Anterior cruciate ligament rupture	Not treatment-related

Table 5. Severe adverse events and its relation to the treatment. *SLIT, sublingual immunotherapy. a. The patient had a medical history of continuous eczema since October 2016, which is six months before initiation of the treatment. b. Bronchopneumonia was diagnosed on the last day previous to the last visit*

active intervention compared to placebo. Our results are similar to the reduction scores generally observed in SLIT clinical trials of other allergens for treatment of AR.[20,21,30,31] The improvements of CSMRS and TNSS were statistically and clinically significant. The mean values for differences in dRMS in peak pollen period observed in PPS population in the current study were similar to those observed in the North American timothy grass AIT study (26%).[20] However, the improvements of RMS were statistically significant but may not be clinically significant, because 95% CIs of adjusted mean difference included values of uncertain clinical significance. Both rescue medication use and symptoms in the *Artemisia annua* SLIT group were significantly reduced in seven centers in statistics as indicated by CSMRS and RMS decrease. In other centers, although there was similar use of rescue medication between the *Artemisia annua* SLIT group and placebo SLIT group, significant improvement of CSMRS was probably achieved because of remarkable alleviation of symptoms in the *Artemisia annua* SLIT group. Similarly, Blaiss and colleagues[21] have reported a 22% improvement in the median daily symptom score in grass pollen-induced ARC patients following timothy grass AIT versus placebo AIT, which is comparable to a 22.3% improvement in the mean dTNSS observed in the present study. Moreover, our finding that *Artemisia annua* SLIT was more effective in *Artemisia* annua-monosensitized patients than in polysensitized patients is also in accordance with the recommendations of the World Allergy Organization (WAO) taskforce, which has been suggested that exposure to confounding allergen/s during the relevant allergen season is likely to be a critical factor that reduces the statistical power to detect meaningful differences in clinical AIT trials.[32]

The optimal stage to begin AIT treatment is of paramount importance for clinical outcome. In accordance with other RCT clinical trials,[18,20,21,31,33,34] AIT treatment in the present study was also started approximate 4 months in advance of the estimated pollen season. Although both Durham and colleagues[30] and Dahl and colleagues[34] have demonstrated efficacy of SLIT with a particular preparation of grass allergen tablets in two separate randomized multicentre trials in SAR patients, these studies demonstrated that starting SLIT only 8 weeks before the PS, resulted in a much smaller treatment effect over placebo (16% reduction in symptoms),[30] compared to starting SLIT 16 weeks before the pollen season (30% reduction in symptoms).[34]

Apart from satisfactory clinical efficacy, our results also revealed a favorable safety profile for *Artemisia annua* SLIT. Although 65.8% patients in the SLIT group experienced drug-related AEs, these AEs were early onset, transient, and self-limiting; with no serious AEs. The rate and severity of AEs observed in this study in this study are in accordance with those reported in other SLIT studies. One recent study investigating the long-term efficacy of Japanese cedar pollen SLIT in AR patients sensitized to Japanese cedar pollen has demonstrated a cumulative AEs incidence rate of 93.0% within 8 weeks of treatment,[35] which was comparable to the 91.5% AEs rate observed in the present clinical trial. The percentage of subjects who reported drug-related AEs in the present was 65.8% in *Artemisia annua* SLIT group, which was lower than the 84.9% of subjects experiencing AEs in the 5-grass-pollen SLIT study in Europe,[18] but higher than the 12% of subjects experiencing AEs to ragweed sublingual-liquid immunotherapy trial in North America.[19] It is possible that the differences in drug-related AEs in these studies may be a consequence of differences in both the types and potencies of the allergens employed for SLIT.

The current study, however, is limited in some ways. Firstly, the duration of treatment and the follow-up period were relatively short compared to many other studies investigating the long-term effects of AIT in patients with allergic disease. Although Lou and colleagues[36] have reported that *Artemisia annua*-SLIT significantly reduced nasal symptoms in *Artemisia annua*-sensitized patients and that the improvement in symptoms was sustained throughout to the next year, this study was conducted in a relatively small number of patients from a single center. Thus, further long-term immunotherapy studies are needed to determine the optimal duration of treatment regimens and evaluate the carry-over effect. Secondly, immunological biomarkers of immunotherapy, such as specific allergen antibody levels (IgE and

immunoglobulin G4), were not supervised throughout the treatment. Thus, further investigations to determine the effect of *Artemisia*-specific SLIT on the immune system are also warranted.

CONCLUSION

In conclusion, this multicentre, randomized, double-blind, placebo-controlled phase 3 clinical trial demonstrated that the standardized liquid drop preparation containing *Artemisia annua* allergen extracts employed for SLIT was well tolerated and effective in reducing the symptoms of allergic rhinitis caused by *Artemisia annua* pollen in a Chinese adult population. When applied in future SLIT, the registered *Artemisia annua* drop will provide great potential to benefit the huge amount of SAR patients sensitized to *Artemisia annua* in China. Although this preparation indicated first-season efficacy when administered as pre-seasonal and co-seasonal specific immunotherapy, these findings need to be confirmed over the long-term. Further studies are also needed to identify the immunologic mechanisms involved, which might point the way forward in better management of AR, as well prevention of its progression to asthma in susceptible individuals.

Abbreviations

AIT; allergen specific immunotherapy, ANCOVA; Analysis of covariance, AR; allergic rhinitis, AE; adverse event, CSMRS; combined scores of medication and rhinoconjunctivitis symptoms, CI; confidence interval, FAS; full analysis set, sIgE; specific immunoglobulin E, PPS; per protocol Set, (d)RMS; (daily) rescue medication score, SS; safety set, SAR; seasonal allergic rhinitis, SCIT; subcutaneous immunotherapy, SLIT; sublingual immunotherapy, (d)TNSS; (daily) total nasal symptom score.

Consent for publication

All authors agreed to the publication of this work.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the corresponding review board in each center, and written informed consent was obtained from each subject prior to entry into the study.

Funding

This work was supported by grants from the national natural science foundation of China (81630023, 81420108009, 81970850, 81870698, 81400444 and 81470678), China, National Key R & D Program of China (2018YFC0116800), China, Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2019-I2M-5-022), China, the Program for Changjiang Scholars and Innovative Research Team (IRT13082), China, Beijing Municipal Administration of Hospitals' Mission Plan (SML20150203), China, Beijing Municipal Administration of Hospitals' Youth Program (QML20150202), China, Beijing Natural Science Foundation (Z141107002514122), China, Capital Health Development Foundation (2016-1-2052), China, and Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University (BTBU) (20181045), China, the Public Welfare Development and Reform Pilot Project (2019-10), China.

Authors' contributions

All the authors contributed significantly to the study: HL, XW, QW, CZ, ZX, QZ, JM, SZ, HZ, RM, HZ, HL, WX collected the data. HL and XW performed statistical analyses. HL, CW and LZ wrote the manuscript. CW and LZ designed and supervised the study.

Declaration of competing interest

All authors declare no financial or commercial conflicts of interest.

Acknowledgments

We thank Dr. Hui Chen for her assistance in statistical analysis.

Appendix A.Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2020.100458.

Author details

[a]Department of Otolaryngology Head and Neck Surgery, Beijing TongRen Hospital, Capital Medical University, Beijing, 100730, China. [b]Beijing Key Laboratory of Nasal Diseases, Beijing Institute of Otolaryngology, Beijing, 100005, China. [c]Department of Allergy, Beijing Shijitan Hospital, Capital Medical University, Beijing, 100038, China. [d]Department of Allergy, No. 202 Hospital of PLA (General Hospital of Northern Theater Command), Shenyang 110003, China. [e]Department of Otorhinolaryngology, Second Hospital of Shanxi Medical University, Taiyuan, 030001, China. [f]Department of

Otorhinolaryngology, Peking University People's Hospital, Beijing, 100044, China. [g]Department of Otolaryngology Head and Neck Surgery, First Hospital of Shanxi Medical University, Taiyuan, 030001, China. [h]Department of Otolaryngology, West China Hospital, Sichuan University, Chengdu, 610041, China. [i]Department of Otorhinolaryngology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, China. [j]Department of Otorhinolaryngology, Tianjin Medical University General Hospital, Tianjin, 300052, China. [k]Department of Otolaryngology Head and Neck Surgery, General Hospital of Ningxia Medical University, Yinchuan 750004, China. [l]Department of Otorhinolaryngology, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830011, China. [m]Department of Otorhinolaryngology, Shanxi Provincial People's Hospital, Xi'an, 710068, China. [n]Department of Otorhinolaryngology, Qingdao Municipal Hospital, Qingdao, 266011, China. [o]Department of Allergy, Beijing TongRen Hospital, Capital Medical University, Beijing, 100730, China.

REFERENCES

1. Brozek JL, Bousquet J, Agache I, et al. Allergic rhinitis and its impact on asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017;140:950–958.
2. Wang XD, Zheng M, Lou HF, et al. An increased prevalence of self-reported allergic rhinitis in major Chinese cities from 2005 to 2011. *Allergy*. 2016;71:1170–1180.
3. Wang XY, Ma TT, Wang XY, et al. Prevalence of pollen-induced allergic rhinitis with high pollen exposure in grasslands of northern China. *Allergy*. 2018;73:1232–1243.
4. Tang R, Sun JL, Yin J, Li Z. Artemisia allergy research in China. *BioMed Res Int*. 2015;2015:179426.
5. Gao Z, Fu WY, Sun Y, et al. Artemisia pollen allergy in China: component-resolved diagnosis reveals allergic asthma patients have significant multiple allergen sensitization. *Allergy*. 2019;74:284–293.
6. Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop G, World Health O. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108:S147–S334.
7. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol*. 1998;102:558–562.
8. Noon L, Cantar BO. Prophylactic inoculations against hay fever. *Lancet*. 1911;1:1572–1573.
9. Freeman J. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Historical document. *Ann Allergy*. 1960;18:427–434.
10. Canonica GW, Bachert C, Hellings P, et al. Allergen immunotherapy (AIT): a prototype of precision medicine. *World Allergy Organ J*. 2015;8:31.
11. Nelson H, Cartier S, Allen-Ramey F, Lawton S, Calderon MA. Network meta-analysis shows commercialized subcutaneous and sublingual grass products have comparable efficacy. *J Allergy Clin Immunol Pract*. 2015;3: 256–266 e253.
12. Durham SR, Penagos M. Sublingual or subcutaneous immunotherapy for allergic rhinitis? *J Allergy Clin Immunol*. 2016;137:339–349 e310.
13. Moingeon P, Cox L. Relevance of a 5-grass sublingual tablet for immunotherapy of patients with grass pollen allergy in North America. *Expet Rev Clin Immunol*. 2016;12:617–623.
14. Maloney J, Durham S, Skoner D, et al. Safety of sublingual immunotherapy Timothy grass tablet in subjects with allergic rhinitis with or without conjunctivitis and history of asthma. *Allergy*. 2015;70:302–309.
15. Yang Y, Zhou W, Chen A. Efficacy of sublingual immunotherapy for cedar pollinosis: a systematic review and meta-analysis. *Ann Allergy Asthma Immunol*. 2016;117:348–353.
16. Feng B, Wu J, Chen B, et al. Efficacy and safety of sublingual immunotherapy for allergic rhinitis in pediatric patients: a meta-analysis of randomized controlled trials. *Am J Rhinol Allergy*. 2017;31:27–35.
17. Nurmatov U, Dhami S, Arasi S, et al. Allergen immunotherapy for allergic rhinoconjunctivitis: a systematic overview of systematic reviews. *Clin Transl Allergy*. 2017;7:24.
18. Wahn U, Tabar A, Kuna P, et al. Efficacy and safety of 5-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2009;123:160–166 e163.
19. Creticos PS, Esch RE, Couroux P, et al. Randomized, double-blind, placebo-controlled trial of standardized ragweed sublingual-liquid immunotherapy for allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2014;133:751–758.
20. Nelson HS, Nolte H, Creticos P, Maloney J, Wu J, Bernstein DI. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol*. 2011;127:72–80, 80 e71-72.
21. Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. *J Allergy Clin Immunol*. 2011;127:64–71, 71 e61-64.
22. Pfaar O, Bastl K, Berger U, et al. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. *Allergy*. 2017;72:713–722.
23. Pfaar O, Demoly P, Gerth van Wijk R, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy*. 2014;69:854–867.
24. Norman GR, Sloan JA, Wyrwich KW. Interpretation of changes in health - related quality of life: the remarkable universality of half a standard deviation. *Med Care*. 2003;41(5):582–592.
25. Bergmann KC, Demoly P, Worm M, et al. Efficacy and safety of sublingual tablets of house dust mite allergen extracts in adults with allergic rhinitis. *J Allergy Clin Immunol*. 2014;133:1608–1614 e1606.
26. Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. *Allergy*. 2006;61:198–201.
27. Jacobsen L, Niggemann B, Dreborg S, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy*. 2007;62:943–948.

28. Canonica GW, Bousquet J, Casale T, et al. Sub-lingual immunotherapy: world allergy organization position paper 2009. *World Allergy Organ J*. 2009;2:233–281.

29. Wilson AM, O'Byrne PM, Parameswaran K. Leukotriene receptor antagonists for allergic rhinitis: a systematic review and meta-analysis. *Am J Med*. 2004;116:338–344.

30. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2006;117:802–809.

31. Okamoto Y, Okubo K, Yonekura S, et al. Efficacy and safety of sublingual immunotherapy for two seasons in patients with Japanese cedar pollinosis. *Int Arch Allergy Immunol*. 2015;166:177–188.

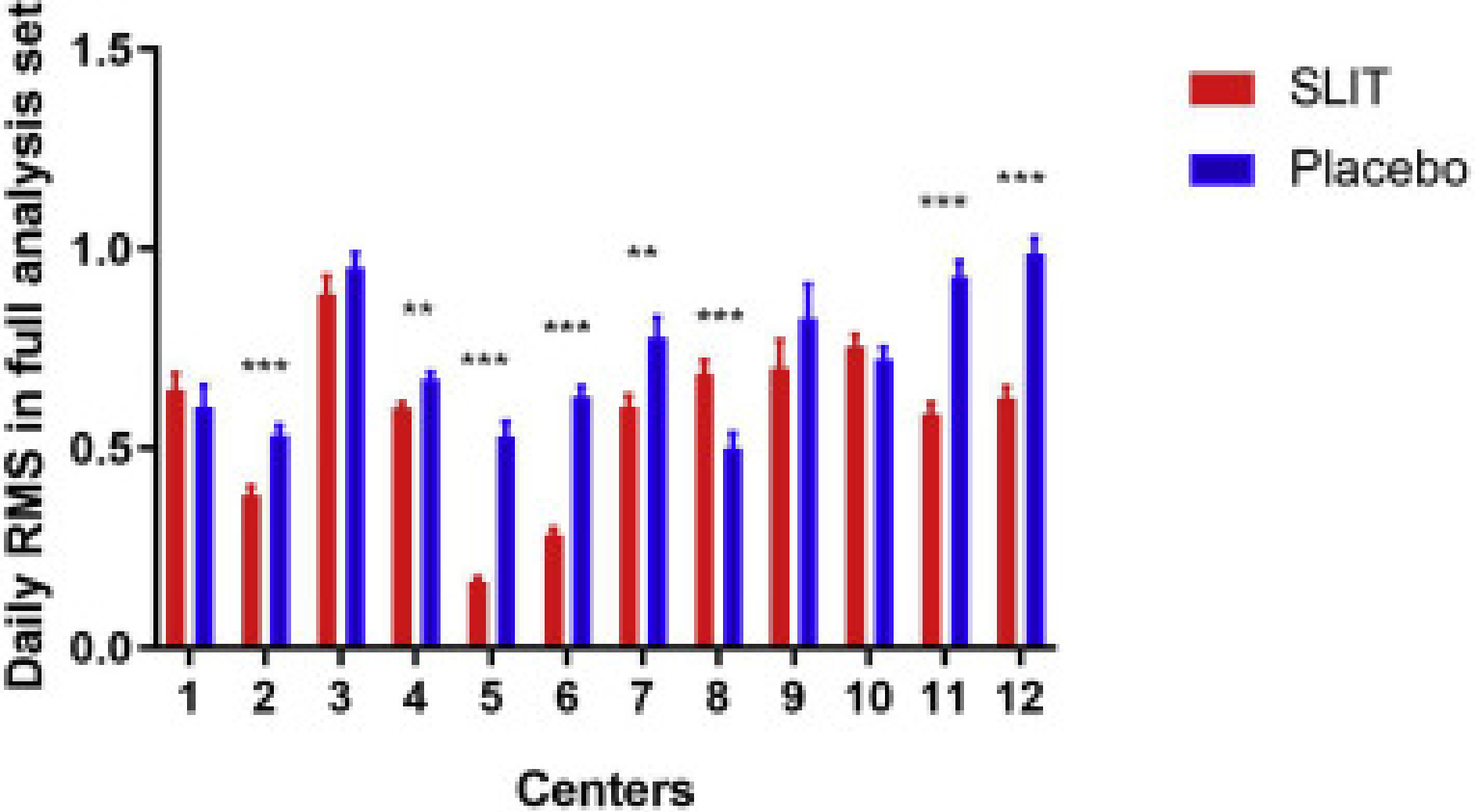
32. Canonica GW, Baena-Cagnani CE, Bousquet J, et al. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. *Allergy*. 2007;62:317–324.

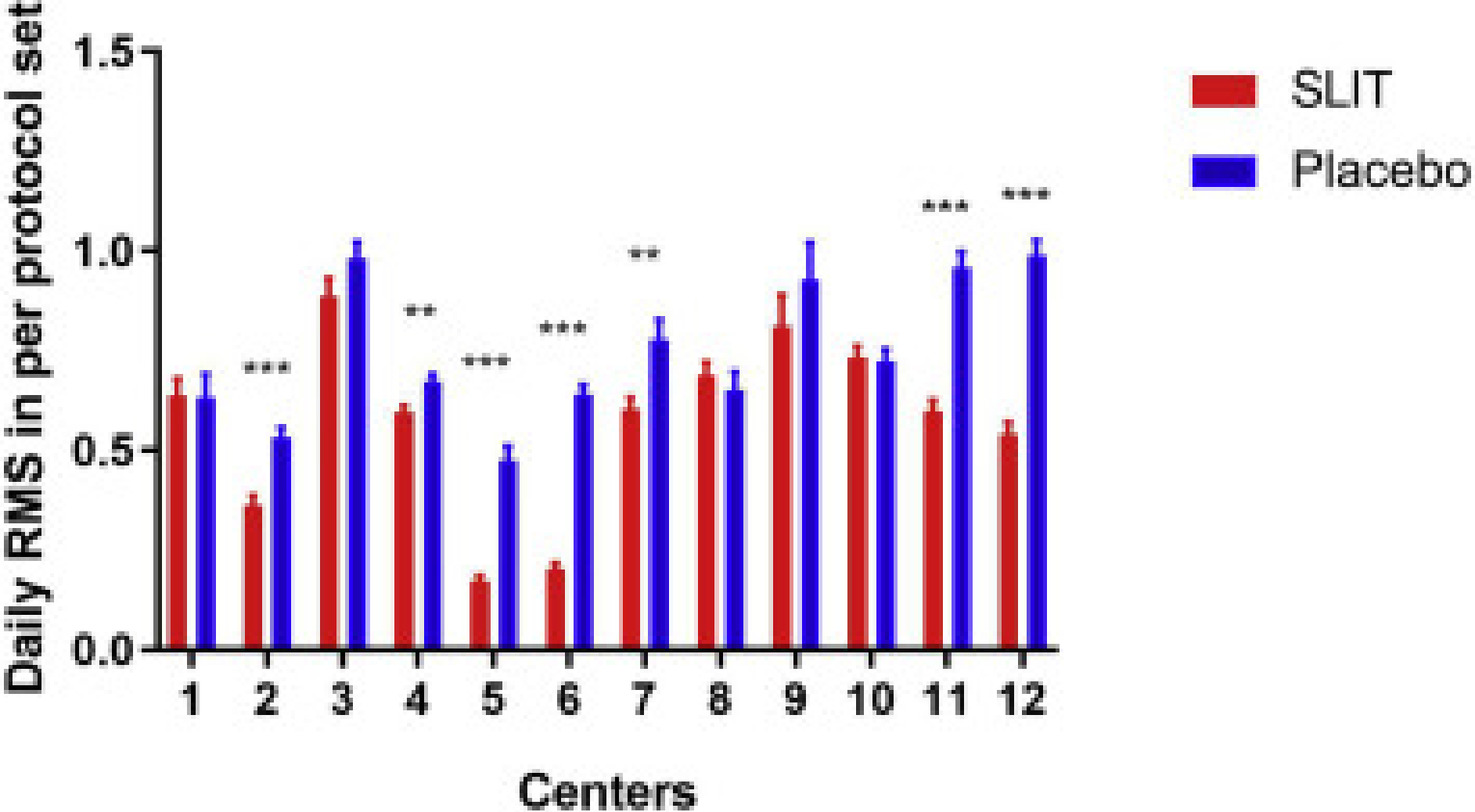
33. Didier A, Malling HJ, Worm M, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol*. 2007;120:1338–1345.

34. Dahl R, Kapp A, Colombo G, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2006;118:434–440.

35. Gotoh M, Yonekura S, Imai T, et al. Long-term efficacy and dose-finding trial of Japanese cedar pollen sublingual immunotherapy tablet. *J Allergy Clin Immunol Pract*. 2019;7:1287–1297 e1288.

36. Lou H, Huang Y, Ouyang Y, et al. Artemisia annua-sublingual immunotherapy for seasonal allergic rhinitis: a randomized controlled trial. *Allergy*. 2020;75:2026–2036. https://doi.org/10.1111/all.14218.


Daily RMS in full analysis set
1.5
1.0
0.5
0.0
SLIT
Placebo
***
**
***
***
**
***
***
***
1
2
3
4
5
6
7
8
9
10
11
12
Centers


Daily RMS in per protocol set
1.5
1.0
0.5
0.0
SLIT
Placebo
***
**
***
***
**
***
***
1
2
3
4
5
6
7
8
9
10
11
12
Centers

Supplementary Table E1. The median of daily combined scores of medication and rhinoconjunctivitis symptoms during the peak pollen period.

	FAS Population		PPS Population	
	SLIT	Placebo	SLIT	Placebo
N	421	211	395	195
Median	1.44	1.87	1.49	1.93
(min-max)	(0.46-3.10)	(0.81-3.11)	(0.37-3.10)	(0.68-3.32)
Improvement	23.0%		22.8%	

FAS, full analysis set; PPS, per protocol set; SLIT, sublingual immunotherapy; SAR, seasonal allergic rhinitis.