Original Article

Structured Fresh Apple Consumption for Birch Pollen Food Allergy Syndrome in an Uncontrolled Phase II/III Trial

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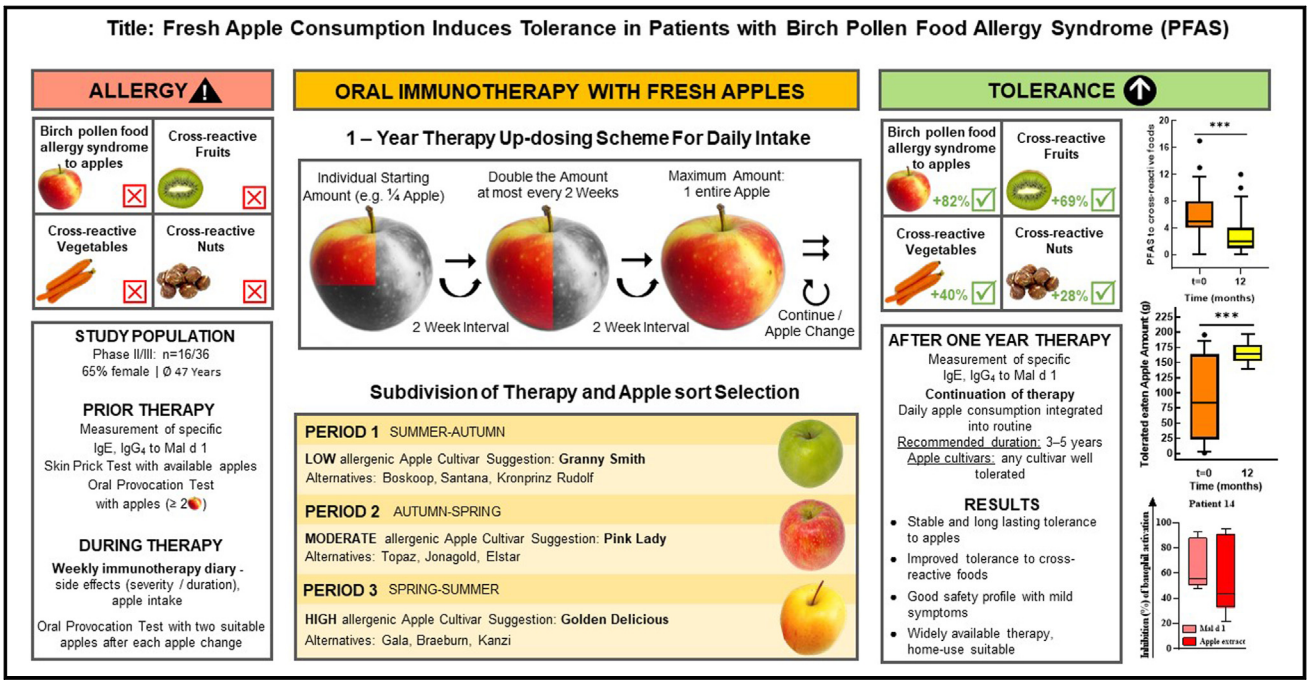
What is already known about this topic? Approximately 70% of patients with birch pollen allergy develop a pollen food allergy syndrome, particularly to apples. To date, there is no standardized therapy available for treating this cross-reactive allergy.

What does this article add to our knowledge? Consumption of increasing doses of fresh apples induces a stable and robust tolerance in birch pollen–allergic patients with pollen food allergy syndrome.

How does this study impact current management guidelines? This study introduces a potential treatment option, promoting tolerance development through regular apple consumption, by offering a globally applicable therapeutic protocol for clinical practice.

VISUAL SUMMARY



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Abbreviations used
AITA- Allergen-specific immunotherapy with apples
DSST- Difficulty swallowing and swollen throat
INEE- Itching nose, eyes, or ears
ISS- Itching or scratching of the mouth and throat
OIT- Oral immunotherapy
OPT- Oral provocation test
PFAS- Pollen food allergy syndrome
SB- Shortness of breath
SCIT- Subcutaneous immunotherapy
SLIT- Sublingual immunotherapy
SPT- Skin prick test
VAS- Visual analog score

BACKGROUND: Birch pollen food allergy syndrome to apples is prevalent, but no approved or standardized treatment exists so far.
OBJECTIVE: To investigate the effect of oral therapy with fresh apples on birch pollen food allergy syndrome to apples using a feasible treatment protocol with different widely available apple cultivars.
METHODS: In this uncontrolled phase II/III study, 42 apple cultivars were tested for their allergen content *in vivo* by skin prick tests and oral provocations. Afterward, 36 patients consumed apples of increasing dose and allergenicity over a period of 12 months. Side effects were documented weekly in a clinical diary. Efficacy was tested before and after therapy by oral provocation and a skin prick test with the Golden Delicious apple. Total IgE, specific IgE, and IgG_4 for Mal d 1 and inhibition of basophil activation were analyzed before and after treatment. Other cross-reactive foods were determined by a questionnaire before and after therapy.
RESULTS: Oral immunotherapy with apples resulted in a consistent and durable tolerance of apples and a significant augmented tolerance to other Bet v 1 cross-reactive foods. After therapy, specific IgG_4 antibodies to Mal d 1 increased significantly; simultaneously specific IgE to Mal d 1 and skin prick test reactivity to apples decreased significantly. Moreover, sera of treated patients displayed blocking activity to Mal d 1.
CONCLUSION: Oral allergy-specific immunotherapy with fresh apples is a promising treatment for birch pollen food allergy syndrome to apples and other Bet v 1 cross-reactive foods. © 2025 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2025;■:■-■)

Key words: *Birch pollen allergy; Birch pollen food allergy syndrome; Immunotherapy; Apple; Bet v 1; Mal d 1*

Up to 70% of European birch pollen—allergic individuals are affected by pollen food allergy syndrome (PFAS).[1,2] PFAS is characterized by mild-to-moderate symptoms of oral pruritus, oropharyngeal pain, itchy ears, or swollen oral mucosa after ingestion of raw plant foods.[1,3,4] It is mediated by IgE antibodies specific for the major birch pollen allergen Bet v 1 and their cross-reactivity with homologous proteins in the plant foods.[1,3,5-8]

To date, no standardized therapy has been established to treat PFAS. Treatment approaches with birch pollen sublingual immunotherapy (SLIT) or subcutaneous immunotherapy (SCIT) have shown controversial results and limited efficacy.[9-19] Kallen et al[20] also recently described the lack of enough evidence to draw firm conclusions about the effect of allergen immune therapy on PFAS. Furthermore, Sánchez Acosta et al[21] demonstrated that SLIT with rBet v 1 induced Bet v 1—specific IgG antibodies lacking sufficient affinity to induce cross-protection to other Bet v 1—homologous proteins. However, treatment of patients with the Bet v 1 homolog in apple, Mal d 1, showed promising results regarding apple-induced PFAS.[21,22] Kopac et al[23] treated PFAS to apple by daily administration of increasing doses of Golden Delicious over 20 weeks, which resulted in transient tolerance, but Mal d 1—specific IgE/IgG_4 and basophil inhibition tests showed no significant improvement after the treatment period. Bouvier et al[24] similarly showed the induction of tolerance to raw apples by continuously consuming raw Golden Delicious. Also, Dijkema et al[25] demonstrated increased apple tolerance by regularly consuming Golden Delicious in yogurt. Moreover, Bergmann et al[26] showed that tolerance to highly allergenic apples can also be achieved by consuming low-allergen apple varieties.

In a previous uncontrolled phase I[27] and phase II[28] studies, our group tested the concept of tolerance induction by oral immunotherapy (OIT) through apple consumption, and a gradually updosing regimen like conventional immunotherapy, as SCIT for respiratory or OIT for peanut allergy, was developed.[29-31] Selected apple varieties were consumed continuously, starting with small amounts of a hypoallergenic variety and ending with a daily intake of 1 to 2 mg/d Mal d 1 contained in highly allergenic cultivars. The results of phase II indicated an improved allergy to apples and also to other cross-reactive plant foods, for example, cherries and carrots. Hay fever symptoms during spring were also decreased.[28] The aim of this open-label, uncontrolled phase III trial was to develop a broadly applicable treatment protocol using commercially available apple cultivars (see Table E1 in this article's Online Repository at www.jaci-inpractice.org) and to further evaluate the efficacy, safety, and side effects of OIT on PFAS.

METHODS

Trial design

Daily apple consumption therapy in the uncontrolled phase III lasted 52 weeks. Weekly immunotherapy diary entries were reviewed. Detailed information on the study design can be found in the papers by Nothegger et al.[27,28] In phase III, the selection of apples was changed to commercially available varieties, and the

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TABLE I. Demographic characteristics and immunologic data before and after therapy

Demographic characteristics	Phase III	Phase II + III	
Sex, n (female/male)	36 (22/14)	52 (34/18)	
Asthma, n (%)	8 (22)	10 (19)	
Polysensitized*	36	52	
Age (y), median (range)	46 (21-68)	47 (21-68)	
Years with pollen food syndrome, median (range)	16 (1-40)	16 (1-40)	
Immunologic characteristics	**Before OIT**	**After OIT**	***P* value* | *r***
Phase III, median (range)			
IgE total (kU/L)	76.50 (13.10-414.00)	53.25 (8.30-362.00)	<.001 | 0.609
Mal d 1−specific IgE (kU/L)	4.61 (0.81-43.00)	2.25 (0.36-23.50)	<.001 | 0.830
Mal d 1−specific IgG_4 (mg/L)	0.08 (0.00-0.94)	0.20 (0.00-3.74)	<.001 | 0.672
Mal d 1 IgG_4/IgE ratio	7.09 (0.00-58.73)	33.50 (0.00-873.02)	<.001 | 0.839
Phase II + III, median (range)			
IgE total (kU/L)	110.50 (13.10-652.00)	88.85 (8.30-670.00)	.027 | 0.369
Mal d 1−specific IgE (kU/L)	4.77 (0.81-43.00)	2.64 (0.36-23.50)	<.001 | 0.500
Mal d 1−specific IgG_4 (mg/L)	0.08 (0.00-2.39)	0.20 (0.00-3.74)	<.001 | 0.678
Mal d 1 IgG_4/IgE ratio	8.10 (0.00-179.43)	32.76 (0.00-873.02)	<.001 | 0.701

Results from phase III (36 patients) and pooled results of phase II (n = 16) and phase III (n = 36) patients (n = 52) are given. Pooled results confirmed results from the phase II trail with all results remaining statistically significant.

OIT, Oral immunotherapy.

*Sensitized to further inhalation allergens (birch, alder, hazel, ash, ragweed, ribwort, mugwort, goosefoot, grass, cat, dog, and house dust mite).

updosing process was changed from 6 weeks to a maximum of 5 months. Two different apple varieties were used for each dose instead of one, that is, 2 low-, 2 medium-, and 2 high-allergen apple varieties, of which 1 main apple variety was eaten more than 70% of the time. This ensured that an appropriate apple variety for treatment was always available and could be changed if the main variety was unavailable or patients grew tired of one. Suitable apple varieties were individually selected based on a skin prick test (SPT). Tolerance to at least 2 varieties was further tested in an oral provocation test (OPT) to determine the starting dose, which was chosen to induce no more than mild PFAS symptoms (visual analog score [VAS] <3 with a range of 0-10, where 0 equals no symptoms and 10 severe symptoms). For therapy initiation, this means, for example, a daily starting dose of 13.6 g, if moderate symptoms occur at 23.6 g during OPT, with only mild or no symptoms at lower doses. A practical guide to therapy implementation is shown in Figure E1, available in this article's Online Repository at www.jaci-inpractice.org. The therapy started in mid-October of 2020, within a 2-week window. The daily amount to be eaten was increased (maximum doubled) every 2 weeks as best as possible until the maximum dose of 1 entire apple daily was reached (maintenance dose). The apple was eaten pure, immediately after cutting it into small pieces. The therapy apples were purchased fresh weekly by the patients themselves and stored in cold storage (2°C-8°C) until use. Each day, a fresh apple was cut and consumed. After 3 months of allergen-specific immunotherapy with apples (AITA), the apple variety was changed to a moderate allergenic and storable apple in January. After the birch pollen season had ended in May, the patient switched to a highly allergenic, stored apple cultivar until the end of the study (1 year). All patients underwent a cessation of 12 weeks after therapy and were re-exposed to the Golden Delicious apple by OPT.

Trial population

The uncontrolled phase III comprised 36 adult patients (aged 18-70 years) with birch pollen allergy and associated moderate-to-severe allergic symptoms to raw apple and other cross-reactive foods, for example, cherries, carrots, or hazelnuts. As results from the phase II trials were confirmed, the 16 phase II patients who had already received 8 months of therapy[28] were included to improve the assessment of therapy and side effects in 52 patients (Table I). The study was conducted at the Medical University of Innsbruck, Austria, and approved by the local ethics committee (EK 1181/2020); it was conducted with the written consent of the participants. Further inclusion and exclusion criteria are described by Nothegger et al.[27]

Efficacy of AITA

The following tests were conducted before and after therapy to analyze treatment efficacy with apples: OPT with Golden Delicious, SPT, and a questionnaire according to Nothegger et al[28] was used to evaluate the cross-reactivity to 28 plant foods.

Safety

Side effects with the severity and duration of symptoms and the eaten amount of apple and its cultivar were documented weekly by the patients in a clinical diary. All reported side effects were considered separately and further grouped into 4 classes and given as percentage of all patients: itching or scratching of the mouth and throat (ISS); itching nose, eyes, or ears (INEE); difficulty swallowing and swollen throat (DSST), and shortness of breath (SB). The severity of symptoms is shown as a VAS of 1 (mild) to 10 (very severe). The duration of symptoms and the consumed amount of apple are given as median in minutes and grams, respectively. The data of the 16 patients of phase II are shown until week 34.

In vivo tests with fresh apples

In addition to the 23 apple cultivars from phases I and II,[28] further 20 apple cultivars were tested by SPT and OPT. This included especially commercially available cultivars commonly found in tyrolean supermarkets as Arlet, Berner Rosenapfel, Boskoop, Braeburn, Delbarestivale, Delprivale, Gloster, Granny Smith, Gravensteiner, Idared, Jonagold, Kanzi, Klarapfel, Kronprinz-Rudolf, Opal, Pinova, Piros, Red Jonaprince, Rubinette, and Summerred. The apples were purchased from regional supermarkets and

farmers from July to October and immediately sliced and frozen at −80°C for prick-testing or stored at cold storage (≤2 days at 2°C-8°C) until use for OPT. Only the peel was tested for all 42 cultivars in prick-to-prick tests before and after therapy because the peel contains the highest amount of Mal d 1. In addition, a standard prick test panel was performed to determine a potential polysensitization. Details are shown in the papers by Nothegger et al.[27,28] OPTs before and after therapy were performed under medical supervision. OPTs during therapy were conducted from home with proper guidelines.

Serum antibody assays

Serum concentrations of total IgE, specific IgE, and IgG_4 for Mal d 1 were quantified before and after AITA by ImmunoCAP (Phadia 250; Thermo Fisher Scientific, Waltham, Massachusetts) according to the manufacturer's specifications.

Basophil inhibition test

Heparinized blood was collected from 5 different untreated individuals with birch pollen−related apple allergy after informed consent and ethical clearance by the local ethics committee (EK1344/2018). The samples from all individuals were incubated with rMal d 1 and apple extract (Golden Delicious) preincubated with the sera collected before and after treatment as described previously.[21] Activated basophils were detected as $CD63^+$ $CD123^+$ $CCR3^+$ cells by flow cytometry. The percentage of inhibition was calculated with the formula (1 − (POST/PRE)) × 100.

Statistical analysis

All statistical calculations were performed using SPSS 29 and GraphPad Prism 9 software. Demographic data, the eaten amount of apple (g), and duration of side effects or allergen-specific immune responses (IgE and IgG) are expressed as median, including ranges. The IgG_4/IgE ratio was calculated by converting IgE kU/L into ng/mL by multiplication with 2.4.[32] The SPT results are shown as histamine-equivalent prick-Index diameter (allergen average wheal diameter divided by the positive control average diameter). The Wilcoxon matched-pairs signed-rank test was used to analyze differences before and after therapy; a P value of $<.05$ was considered significant. The Spearman rank test was used to determine correlations.

RESULTS

Study population

In total, 90 patients were screened for suspected PFAS, and 48 patients could be included in the study. The COVID-19 pandemic within the study period led to 24 consent withdrawals during the screening (n = 18) and treatment (n = 6) of the phase III trial (fear of clinic visits and infection) (Figure 1). The study was completed by 36 patients, and their demographic characteristics are shown in Table I. Results before and after phase III therapy are shown and combined with phase II patient results to better assess efficacy and tolerability in a total of 52 patients. Besides PFAS to apples, 98% reported to have also an allergy to at least 8 other cross-reactive foods (eg, cherry, hazelnut, and carrot; see Figure 2).

Implementation of the phase III study

On average, each patient tested 5 different apple cultivars in oral provocations before therapy. Particularly old or less common market cultivars and summer apples were more suitable for the beginning of AITA. Some typical market cultivars such as Granny Smith or Elstar also showed an acceptable tolerance in OPT. Oral pruritus was the first and most frequent symptom while testing, which increased in intensity after doubling the respective eaten amount of apple, and additional symptoms (itching ear and oral paresthesia) appeared simultaneously. After testing, the therapy started primarily with Boskoop (47%) or Granny Smith (17%). The rest of the patients consumed Pink Lady, Jonagold, Elstar, Kronprinz Rudolf, Lederapfel, or Topaz. After switching to a new apple cultivar, Pink Lady (28%) and Gala (28%) were used most frequently, followed by Braeburn, Elstar, Topaz, and Kanzi. During the last therapy phase, Golden Delicious (42%) and Gala (42%) were mostly eaten besides Braeburn, Pink Lady, Elstar, and Kanzi. All participants independently increased their apple dose at home without the need for clinic visits or emergency medication. A suggested treatment sequence for therapy with broadly available apple cultivars could be Granny Smith (alternatives: Boskoop, Santana, and Kronprinz Rudolf) for low allergen potency, Pink Lady (alternatives: Topaz, Jonagold, and Elstar) for middle allergen potency, and Golden Delicious (alternatives: Gala, Breabum, and Kanzi) for high potency.

Efficacy

OPT with the highly allergenic Golden Delicious apple showed a significant increase in tolerance after treatment in all 52 patients ($P < .001$, $r = 0.847$). At baseline, the onset of symptoms appeared already after consuming 3.6 g of peeled apple. After therapy, tolerance increased to 159.5 g of an unpeeled apple (+82%). Initially, 73% of patients reacted to less than 13 g of Golden Delicious. After therapy, 40% tolerated a whole apple without symptoms and 21% tolerated 125 g to 1 full apple. A nonspecific desensitization effect was excluded by stopping therapy for 3 weeks after treatment (52 weeks), followed by a further 3-month break. The tolerated amount after AITA remained increased and unchanged between the breaks ($P = .759$, $r = 0.051$), indicating a true and long-term tolerance induction (Figure 3). In addition, cross-reactivity was also observed for several plant foods, which also improved after therapy ($P < .001$, $r = -0.820$) for fruits (69%), vegetables (40%), and nuts (28%) (Figure 2). A detailed overview of the 10 most common cross-reactive fruits, vegetables, and nuts, along with changes in tolerance after therapy, is provided in Figure E2 (available in this article's Online Repository at www.jaci-inpractice.org). Wheal size in SPT with 42 apples decreased by an average of 18% ($P < .001$, $r = -0.725$). The most commonly used apple varieties during therapy (Pink Lady, Gala, and Golden Delicious) showed reductions of 24% to 38% in SPT wheal size (Figure 4). In addition to these clinical parameters, the concentration of Mal d 1−specific IgG_4 antibodies increased, while the levels of Mal d 1−specific IgE decreased significantly. This resulted in a significantly increased IgG_4/IgE ratio ($P < .05$ in all, see Table I). Moreover, sera after AITA from 4 of 5 studied individuals (80%) displayed IgE-blocking activity in basophil inhibition tests (Figure 5). IgE-blocking to recombinant Mal d 1 and apple extract was comparable in all individuals, indicating that the majority of blocking antibodies were specific for the major apple allergen.

Safety

The specific immunotherapy with fresh apples was completed by 52 patients and analyzed for side effects for 34

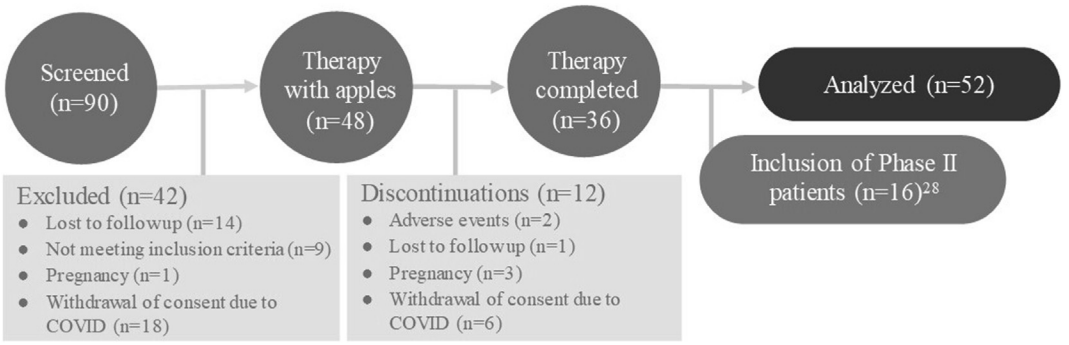

FIGURE 1. Flow diagram showing details of all participants involved for final study analysis. Details of phase II are shown in the paper by Nothegger et al.[28]

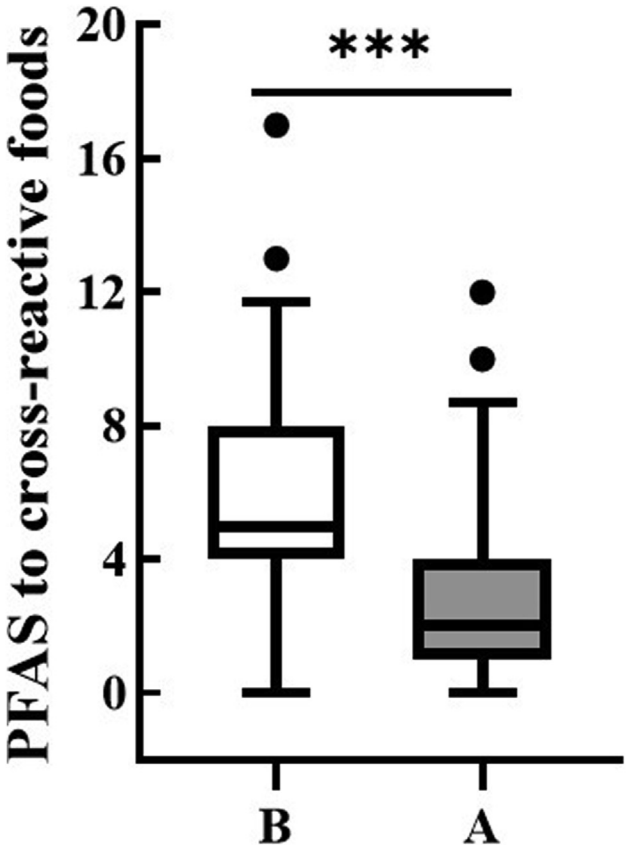

FIGURE 2. Pollen food allergy syndrome (PFAS) to different cross-reactive foods before and after therapy. The boxplots show the number of reactive foods inducing PFAS per patient (**B**: before, **A**: after). Tested foods included 28 different fruits (eg, cherry and peach), vegetables (eg, carrot and celery), and nuts (eg, hazelnut and walnut). ***$P < .001$, Wilcoxon signed-rank test (n = 52).

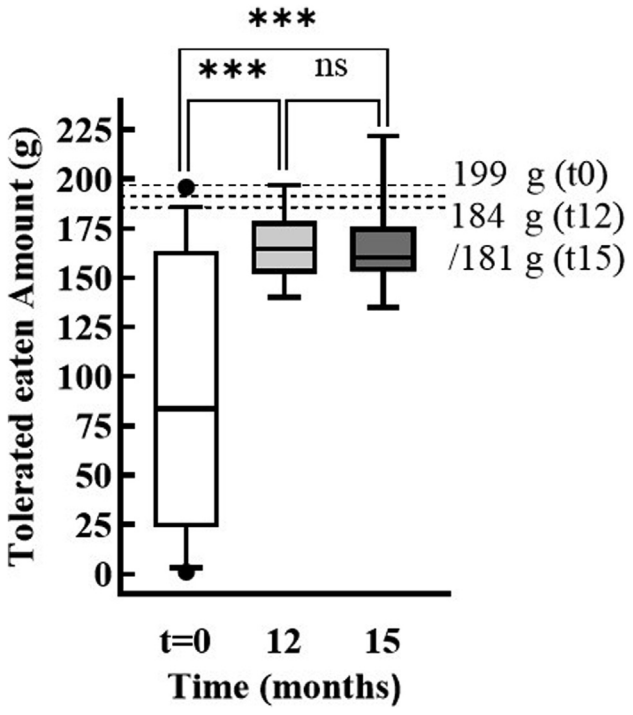

FIGURE 3. Desensitization effect after cessation of treatment. Shown is the maximum tolerated apple amount before ($t = 0$) and after therapy, following a treatment interruption for 3 weeks ($t = 12$) or 3 months ($t = 15$), respectively. Therapy was discontinued between months 12 and 15. The dashed lines from top to bottom show the median weight of apples tested in each provocation; ***$P < .001$, (ns) not significant, Wilcoxon signed-rank test (n = 36).

weeks (n = 16, phase II) or 52 weeks (n = 36, phase III) (Figure 6). The most common adverse event was itching or ISS. Specifically, 85% of phase II and 78% of phase III patients reported having these symptoms, particularly at the start of treatment. The most reported symptom within this group was oral pruritus (67%), followed by palatal pruritus (35%), pharyngeal pruritus (27%), and oral paresthesia (23%) (Figure 7). Toward the end of the therapy, only 56% of phase II and 25% of phase III patients were still affected by ISS. Side effects from the group INEE occurred less frequently, with 38% of patients in phase II and 11% in phase III at baseline and 6% or 0% (phase III) toward the end of therapy. Within this group, ocular pruritus (35%), running nose (29%), nasal pruritus (27%), or ear pruritus (23%) occurred most frequently. The last 2 groups, DSST or SB, appeared in 13% and 6% (phase III) of the patients. The most common adverse

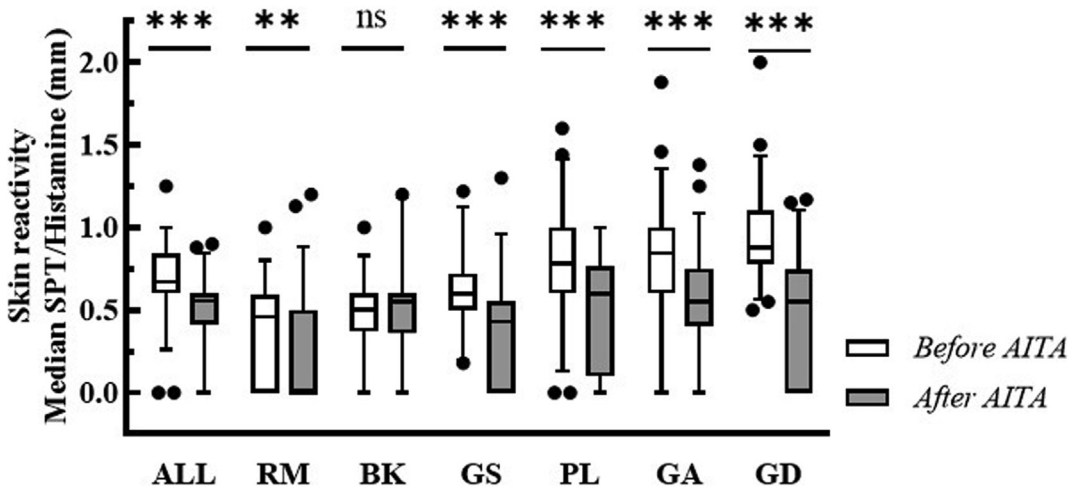

FIGURE 4. SPT with apples before and after therapy. Shown is the skin reactivity to 42 apple cultivars and key therapy cultivars (median SPT/histamine, mm); ***$P < .001$, ** $P < .01$, (ns) not significant, Wilcoxon signed-rank test. n = 52 for ALL, GD, GA, PL, and RM; and n = 36 for GS and BK. *AITA*, Allergen-specific immunotherapy with apples; *ALL*, 42 apple cultivars; *BK*, Boskoop; *GA*, Gala; *GD*, Golden Delicious; *GS*, Granny Smith; *PL*, Pink Lady, *RM*, Red Moon; *SPT*, skin prick test.

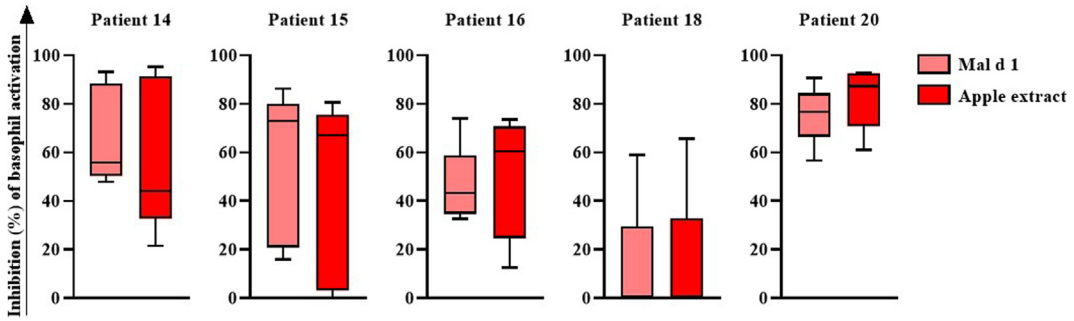

FIGURE 5. IgE-blocking activity to rMal d 1 and apple extract after allergen-specific immunotherapy with apples (AITA). Allergen-activated $CD63^+CD123^+CCR3^+$ basophils were measured in the presence of AITA sera before and after treatment. The percentage of inhibition was calculated using the formula (1 − (POST/PRE)) × 100. Boxplots (5-95 percentile) summarize the mean values of 5 basophil donors for each treated patient and allergen, respectively.

reactions were oropharyngeal pain (12%), lip swelling (10%), dysphagia (10%), and pharyngeal edema (8%). Other less common adverse reactions (10% or less) were rash or pruritus, nausea, abdominal pain, chest pain, diarrhea, or fatigue. Two patients in phase III and 1 patient in phase II discontinued treatment after 16 and 28 weeks of therapy, respectively, due to adverse events (eg, abdominal pain and diarrhea, persistent itching in the mouth and ears, itching and swollen eyes, oral mucositis, and oropharyngeal pain) with a VAS of 5 to 10. Patients were treated with antihistamines, which resulted in rapid improvement. No anaphylactic reactions occurred.

As expected, all side effects were most frequent at the beginning of therapy or after a change to a new apple variety (W7, W13 [phase II], W11-14, W32-35 [phase III]). Similarly, we observed that complaints increased significantly or persisted during high pollen concentrations in the spring and summer seasons (W23-30, birch pollen; W36-40, grass pollen, W45-46, mugwort pollen), $P < .05$ (see Figure 6). The VAS, a parameter for the severity of symptoms during AITA, was entirely mild in phase III and mild to moderate in phase II, showing an average of 1 to 2 (Figure 6). Apples were best tolerated when eaten in the morning after breakfast. At the end of treatment, all but 6 patients in phase II were able to eat a whole apple daily. In phase III, the duration of symptoms was also measured, with 10 minutes at the start of AITA and an average of 5 minutes during the subsequent 16 weeks of therapy, immediately after or during the consumption of an apple. Subsequently, the duration of symptoms decreased to a few seconds, except after a change in apple variety or when pollen levels were high; then it increased to <5 minutes.

Treatment satisfaction and tolerability

The treatment was well tolerated; only 3 of 70 (4.3%) discontinued therapy because of side effects (Figure 1). All patients expressed appreciation for AITA in a questionnaire and would recommend it to others (100%). The most common reason stated was the availability of a fresh, healthy, and natural alternative therapy option (47%). Patients also stated that the therapy

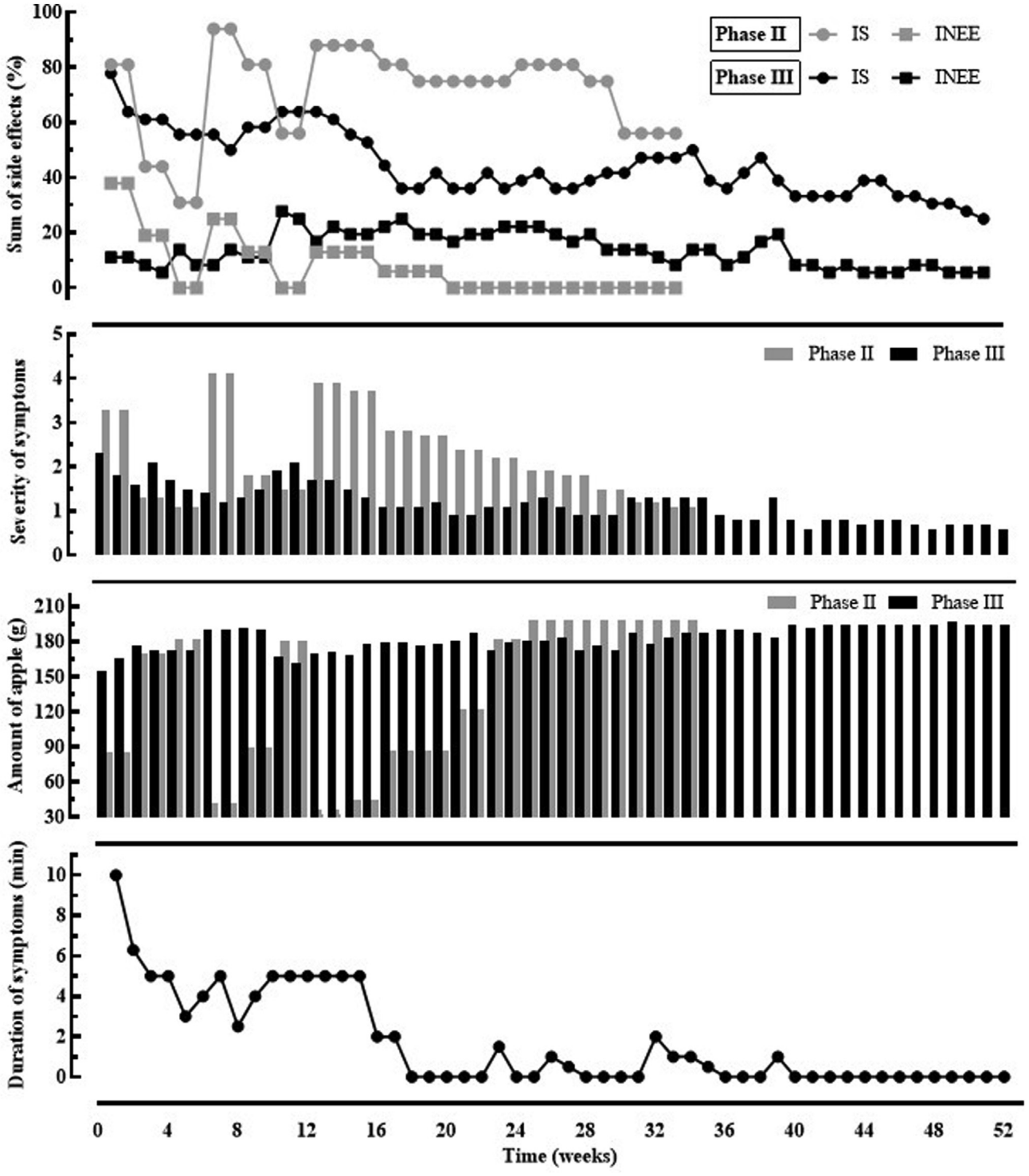

FIGURE 6. Allergen-specific immunotherapy with apples over 1 year. Side effects, severity, duration of symptoms, and the weekly consumed amount of apple over 52 weeks are shown. Side effects are grouped into 2 classes ● itching or scratching of the mouth and throat (IS)/■ itching nose, eyes, or ears (INEE). Symptoms <10% are not shown. Severity of symptoms is shown as visual analog score 1 (mild) to 10 (severe symptoms). Duration of symptoms/consumed amount of apple is given as median in minutes/gram; data of the 16 patients of phase II are shown until week 34 (n = 52).

was time-saving and easy to implement with minimal effort (25%) in their daily routine. Some appreciated the ability to consume other cross-reactive foods (22%) again and that the AITA positively influenced their digestion and quality of life (19%). A few patients also highly appreciated the low number of side effects and the low costs during therapy (8%).

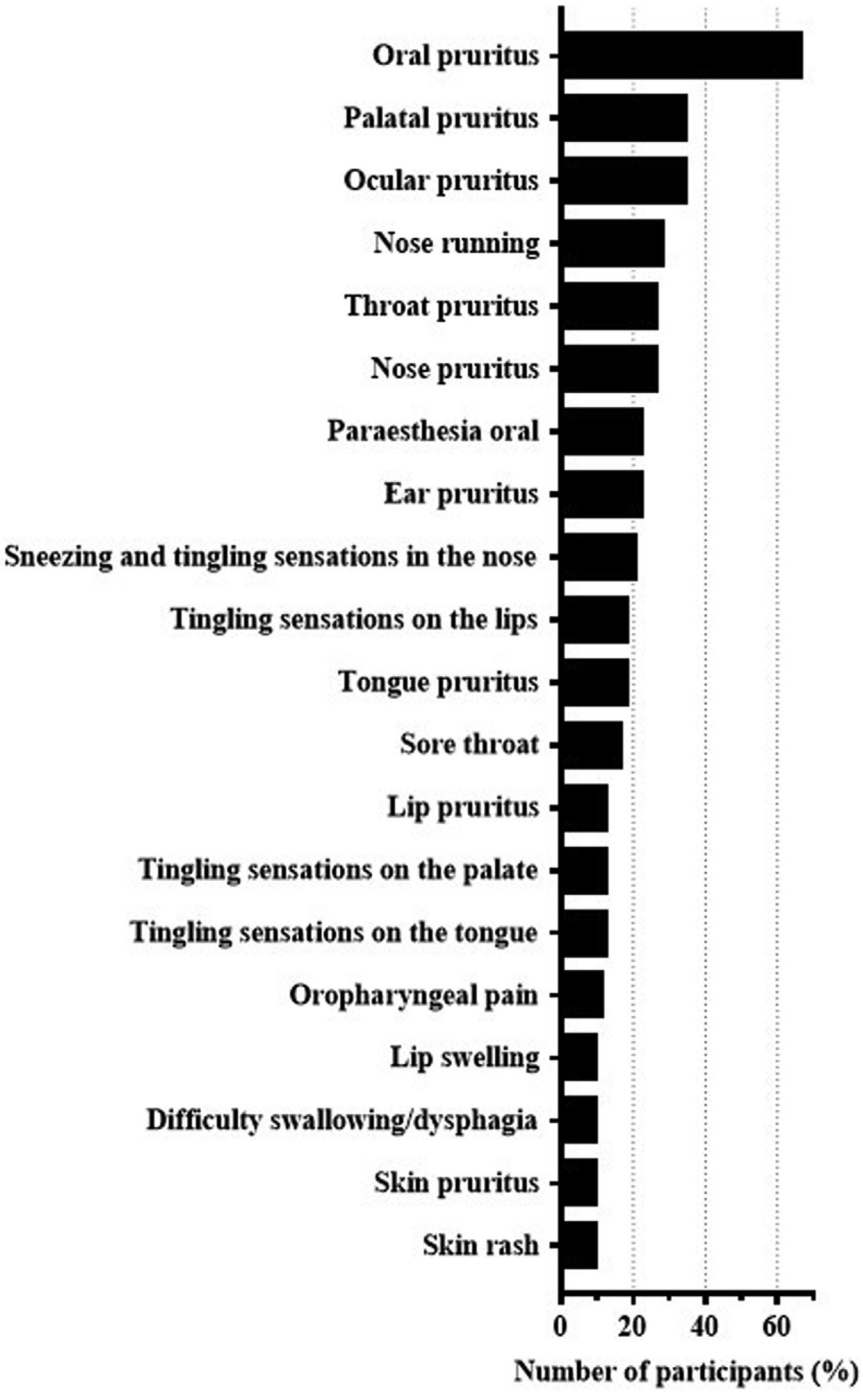

FIGURE 7. Side effects during sublingual immunotherapy with apples. Shown are side effects affecting ≥10% of patients in phase II-III therapy. Symptoms <10% were skin irritation, burning lips, throat mucous, pharyngeal edema, nausea, nasal congestion, throat tingling, burning mouth, hoarseness, coated tongue, abdominal pain, and burning eyes. Symptoms <5% were lip eczema and blisters, burning-swollen tongue, oral swelling, throat tickle, dyspnea, abdominal fullness, flatulence, fatigue, intestinal complaints, diarrhea, chest pain, heartburn, asthma, wheezing, swollen lymph nodes in the neck, chin tingling, anxiety, running-, dry-, red-, or swelling eyes, hypoesthesia oral, periodontitis, and migraine (n = 52).

DISCUSSION

To date, no study has investigated the efficacy of developing tolerance to PFAS through regular apple consumption to this extent. Continuous consumption of different types of apples induced increased tolerance to apples, which was observed after repeated oral provocation at the end of therapy. Before treatment, most patients had symptoms after consumption of a sugar cube-sized piece of Golden Delicious. After treatment, however,

the intake amount of this highly allergenic cultivar increased to three-quarters of an apple on average, even after a 12-week treatment break, indicative of sustained tolerability. In total, 85% of patients were able to eat a whole Golden Delicious apple. Although this study lacks a placebo group, the substantial increase in tolerance in patients with yearlong (median 16 years) symptoms strongly suggests a true therapeutic effect that exceeds. The SPT also showed a significant improvement in wheal size for most apples with a reduction in the wheal size of up to 38% (Figure 4). A significant increase in Mal d 1−specific IgG_4/IgE ratio was noted after treatment. Furthermore, the induced IgG antibodies displayed IgE-blocking activity, which has been associated with clinical improvement of apple allergy[21] and indicates a positive effect of AITA on the allergen-specific response. Previous SLIT studies with peach and peanut protein showed similar results.[21,22,33,34] IgG_4 to Mal d 1 might also have a cross-blocking activity against related allergens in fruits, nuts, and vegetables, especially of the *Rosacea* family, similar to IgG1 antibodies induced by SLIT with recombinant Mal d 1.[35] For instance, PFAS in this phase III study improved for cherry-, peach-, pear-, and apricot-allergic subjects by 64%, indicating a cross-desensitization. This effect was also observed in an OIT study treating cashew allergy with a positive coeffect on pistachio[36] or in a SLIT where peach protein Pru p 3 positively influenced peanut allergy.[34] We did not observe any loss of tolerance to apples after a 3-month therapy break, indicating sustained tolerance induction. Recent OIT studies have shown that the therapy should be continued to support the therapeutic effect.[31] Sicherer et al,[37] for example, advised patients with pollen-related food allergy to include small amounts of allergenic food into their diet and Du Toit et al[38] demonstrated that regular consumption of peanuts lowers the risk of developing or exacerbating peanut allergy and triggers a modulated immune response to peanuts. We therefore recommend continuing the therapy for longer than 1 year, as in conventional immunotherapies, and integrating apples and small amounts of other cross-reactive foods into daily life as routine to maintain or even enhance tolerance, like 2 to 3 apples per week, combined with other seasonally available cross-reactive food sources (eg, cherry and peach).

Apple treatment was safe and well tolerated by most patients, with 3 of 70 (4.3%) dropping out due to side effects. Most symptoms, especially ISS, occurred mainly at the beginning of therapy, after changing apple varieties or during high pollen counts. The individual adaptation of the apple varieties to the patients in phase III resulted in a better overall tolerability of the therapy with only mild symptoms during the whole treatment compared with phase II ($P < .001$, $r = 0.585$). No signs of eosinophilic esophagitis were observed, and AITA could also be used during the pollen season. As therapy is administered at home, it is easily integrated into daily life and does not lead to missed school or work time. In addition, treatment costs are low with a wholesale price for apples of 0.8 to 1.0 €/kg in Austria,[39] and the fruits are available throughout the year. This was highly appreciated by patients, who also had a high treatment satisfaction.

Here we show that also commonly available apple cultivars at the supermarket are well suited for OIT when therapy starts at the right time with freshly harvested apples in late summer to fall. Especially fresh apples are better tolerated than stored ones and should be kept at cool temperatures (eg, in the fridge at 4°C-8°C) after purchase for therapy usage.[40-43] Apples also have further health benefits, as they contain vitamins, fiber, carbohydrates, and secondary plant metabolites (eg, polyphenols), which can positively affect digestion and quality of life.[44-46] Apple polyphenols have been shown to partially mask an antibody binding side when binding to Mal d 1, reducing IgE binding reactivity.[47-49] Another advantage could be the different isoforms of Mal d 1 at different frequencies in each variety of apple to which the patient is exposed.[50] This could lead to a broader spectrum of desensitization of the patient's immune system, potentially enhancing the effectiveness of the therapy. Besides, apple cultivars rich in isoforms with cysteine may be better tolerated as they shield the IgE epitopes.[47-49] Apples have at least a 10 to 16 times higher protein content than SLITs with birch pollen extract, for example, standardized quality tree SLIT tablet,[51,52] which may be an important reason why desensitization to PFAS with apples in this study worked better than conventional treatments for birch pollen allergy.[9-19]

This study investigated the efficacy and safety of allergen-specific immunotherapy with fresh apples in birch pollen−allergic patients with PFAS. The therapeutic concept of the phase II study was improved for a better real-life application, and treatment results were confirmed. The therapy's impact on birch pollen allergy was comprehensively evaluated, but due to the scope of the data, results will be presented in a separate publication. AITA is an inexpensive, highly effective treatment for PFAS with minimal side effects that can be conveniently used at home, saves time, has shown high patient satisfaction, has good potential for clinical use in practice, and has other health benefits in addition to oral desensitization. For those reasons, we think the time is ready to recommend this treatment under medical supervision to patients with PFAS who suffer a significant reduction in quality of life.

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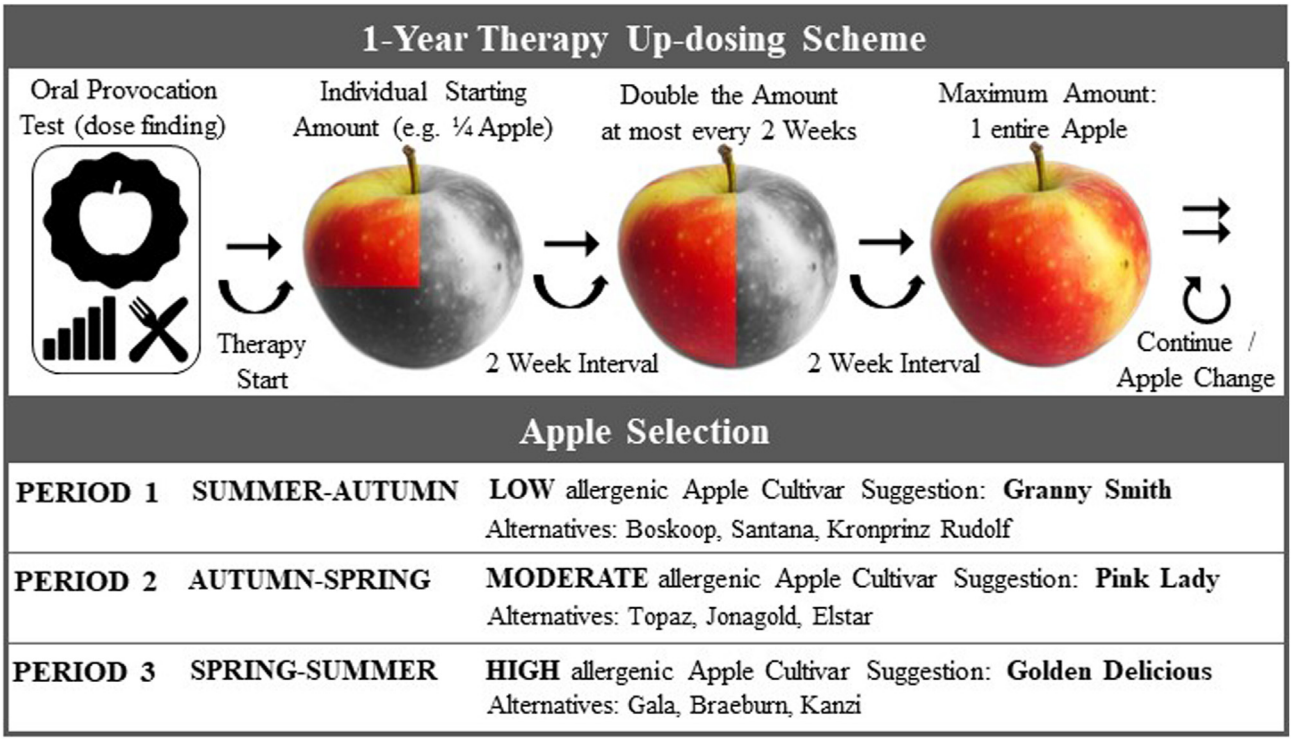

FIGURE E1. Treatment protocol for oral immunotherapy with fresh apples. It is recommended to initiate the oral immunotherapy with fresh apples under medical supervision to check the present status of the allergy and potential exclusion criteria (eg, uncontrolled asthma, sensitization against the heat-stable lipid transfer Mal d 3 protein). The first step is to determine the current threshold of apple tolerance and the individual starting dose (eg, 40 g [equal to approximately a quarter piece of apple]). The dose is determined by an oral provocation test (OPT) before therapy starts with at least 2 suitable low-allergenic apple cultivars (eg, Boskoop or Granny Smith) after being freshly harvested in late summer to autumn (period 1). The respective amount should not induce more than mild symptoms (visual analog score [VAS] <3 with a range of 0-10, where 0 equals no symptoms). The first apple portions in the oral provocation are 0.1 g, 1.0 g, 2.5 g, and 5.0 g of peeled fruit flesh, which are then given in a 5-minute interval. After that if no symptoms occur, the patient starts to eat unpeeled fruit starting with 5 g, 10 g, 20 g, and 40 g in 5-minute intervals. Afterward, the dose is doubled (80 g) in a 30-minute interval and further increased by 100 g until a maximum dose of no more than 1 full apple is reached. The oral provocation is stopped when symptoms reach or surpass VAS 3; the starting dose is the one before reaching VAS 3. The apple portion should be chewed thoroughly for 2 minutes, with some left under the tongue. Food and drink should be avoided for at least 15 minutes. The following 2 OPTs during therapy are conducted by the patient at home before switching to a new apple cultivar. To facilitate the process, the initial portion is increased to 5 g with the peel. The daily eaten therapy starting dose has to be increased (maximum doubled) in 2-week intervals until 1 entire apple per day is reached. The respective apple cultivars are replaced between autumn and spring (at the earliest after 6 weeks and the latest after 5 months) by medium allergenic cultivars, for example, Pink Lady or Topaz (period 2). An OPT predetermines the daily amount to be eaten and is again increased in 2-week intervals. After the birch pollen season ends, the same principle can be used to switch to stored highly allergenic cultivars between spring and summer, for example, Golden Delicious or Gala (period 3). Two different apple cultivars can be used for each dose, of which 1 main apple cultivar should be eaten more than 70% of the time. Therapy apples should be kept in the fridge at 2°C to 8°C until use and prepared fresh every day. The daily dose should best be consumed pure, including the peel, directly after a first meal in the morning. It is recommended to continue treatment after 1 year with a weekly maintenance dose of at least 3 entire, ideally, high-allergenic apples per week (maintenance dose). A clinical checkup is recommended once a year during the first 3 to 5 years of immunization.

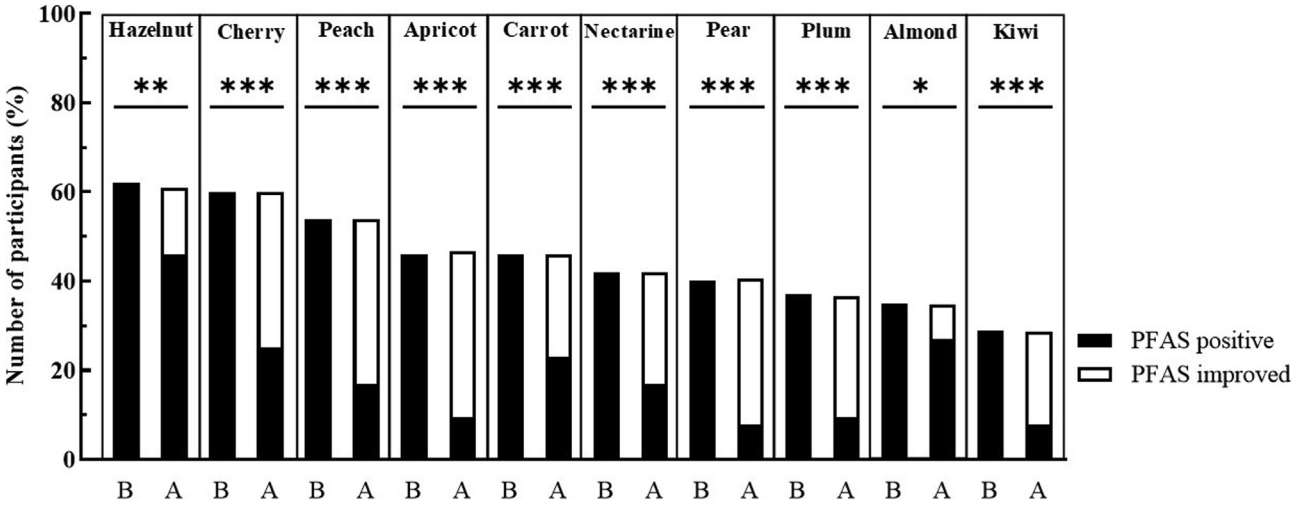

FIGURE E2. Pollen food allergy syndrome (PFAS) to cross-reactive foods before and after therapy. The figure shows the number of patients (%) and the top 10 cross-reactive foods most patients initially reacted to. The bars show the expression of PFAS before (**B**) and after (**A**) therapy; patients who were not tested after therapy are not included in the calculation. $***P < .001$, $**P < .01$, $*P < .05$, Wilcoxon signed-rank test ($n = 52$).

TABLE E1. Apple prick test panel

Cultivar	Positive prick tests, % (n)	Type	Parentage	Discovery/release (breeding year)
Red Love	51 (61)	RC	Scab resistant breed × breeding clone	2010
Summerred	52 (33)	NC	Pollinated seedling	1964
Baya Marisa	53 (64)	RC	Weirouge × breeding clone 166	21st century
R 201 Kissabel	56 (67)	RC	Unknown	21st century
Red Moon	57 (68)	RC	Unknown	21st century
Boskoop	62 (37)	OC	Chance seedling	19th century
Delprivale	62 (42)	NC	Delcorf × Akane	1960s (1990s)
Y 103 Kissabel	74 (89)	RC	Unknown	21st century
White Winter-Callville	78 (93)	OC	Chance seedling	16th-17th century
Delcorf (Delbarestivale)	78 (49)	NC	Jonagrimes × Golden Delicious	1974
Berner Rosenapfel	79 (50)	OC	Chance seedling	19th century
White Transparent (Klarapfel)	81 (55)	OC	Aspa × St Germain	19th century
Gravensteiner	83 (52)	OC	Chance seedling	17th century
Kronprinz Rudolf	83 (48)	OC	Chance seedling	19th century
Santana	83 (98)	NC	Elstar × Priscilla	1996 (1978)
Gloster	84 (101)	NC	Glockenapfel × Richared Delicious	1969 (1951)
Tiroler Spitzlederer	85 (102))	OC	Chance seedling	19-20th century
Piros	85 (58)	NC	Helios × Apollo	1985 (1963)
Kanada Renette	86 (103)	OC	Chance seedling	18th century
Granny Smith	87 (59)	NC	Chance seedling	19th century
Cripps Pink (Pink Lady)	88 (106)	NC	Golden Delicious × Lady Williams	1986 (1973)
Red Jonaprince	90 (60)	NC	Mutant of the cultivar Jonagold	1994
Rubinette (Rafzubin)	90 (52)	NC	Golden Delicious × Cox Orange	1983 (1966)
Fuji Zhen	91 (109)	NC	Ralls Janet × Red Delicious	1960s (1930s)
LB17906	91 (109)	NC	Topaz × Gold Rush	2013 (1999)
CIVG 198 Modi	92 (110)	NC	Gala × Liberty	21st century
Bonita	93 (111)	NC	Topaz × Pink Lady	21st century
Topaz	93 (112)	NC	Rubin × Vanda	1990s (1984)
White Rosemary	93 (112)	OC	Chance seedling	18th-19th century
Elstar	94 (93)	NC	Golden Delicious × Ingrid Marie	1975 (1955)
SQ159 Natyra	94 (112)	NC	scab resistant breed × Elise	2016 (1990s)
Jonagold	96 (65)	NC	Golden Delicious × Jonathan	1968 (1943)
Arlet	96 (65)	NC	Golden Delicious × Idared	1984 (1958)
UEB 32642 (Opal)	96 (65)	NC	Golden Delicious × Topaz	2010 (1991)
Golden Delicious	97 (116)	NC	Chance seedling	19th century
Bay 4210 Sonnenglanz	97 (116)	NC	Pinova × Topaz	21st century
Gala Buckeye	97 (116)	NC	Kidd's Orange Red × Golden Delicious	1965 (1934)
Goldparmäne	97 (96)	OC	Chance seedling	16th century
Idared	97 (66)	NC	Jonathan × Wagenerapfel	1942 (1935)
Nicoter (Kanzi)	97 (66)	NC	Gala × Braeburn Hillwell	2006 (1993)
Braeburn	99 (67)	NC	Chance seedling	1990 (1952)
Pinova	99 (67)	NC	Clivia × Golden Delicious	1986 (1965)

Positive prick reactions (>3 mm) of 42 tested apple cultivars (Baya Marisa, Bonita, Fuji Zhen, Gala Buckeye, Gloster, Golden Delicious, Kanada Renette, LB17906, CIVG 198 Modi, Cripps Pink, R 201 Kissabel, Red Love, Red Moon, Bay 4210 Sonnenglanz, Tiroler Spitzlederer, Topaz, White Rosemary, White Winter-Callville, Y 103 Kissabel [n = 120], SQ159 Natyra [n = 119], Santana [n = 118], Elstar, Goldparmäne [n = 99], Arlet, Braeburn, Delprivale, Granny Smith, Idared, Jonagold, Nicoter, White Transparent, UEB 32642, Pinova, Piros [n = 68], Red Jonaprince [n = 67], Berner Rosenapfel, Delcorf, Gravensteiner, Summerred [n = 63], Boskoop [n = 60], Kronprinz Rudolf, Rubinette [n = 58], 100%) are shown. The apples are grouped into the type new cultivar (NC, apples targeted breeding for marketing), old cultivar (OC, local and traditional apples fallen into oblivion, rare on the market), or red fleshed-cultivar (RC, new bred apples with red flesh). The last 2 columns show the parentage and the time of discovery or release of each cultivar.