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Peanut gastrointestinal delivery oral immunotherapy in adolescents: Results of the build-up phase of a randomized, double-blind, placebo-controlled trial (PITA study)

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Summary

Background: Oral immunotherapy to peanut is effective in desensitizing patients but has significant side effects including anaphylaxis and gastrointestinal symptoms. In most protocols, peanut is administered in a vehicle food.

Objective: In an exclusively adolescent population, we tested a new approach using sealed capsules of peanut (gastrointestinal delivery oral immunotherapy or GIDOIT) to bypass the upper gastrointestinal tract. The primary aim was to assess the efficacy of the oral build-up phase of GIDOIT and the secondary aim to analyse its safety.

Methods: Adolescents with a history of a clinical allergic reaction after peanut ingestion were included in a 2-armed, parallel-design, individually randomized, double-blind, placebo-controlled, multicentre trial after a positive double-blind placebo-controlled oral food challenge (DBPCFC1). A central randomization centre used computer-generated tables to allocate treatments. Peanut (or placebo) capsules were ingested daily over a period of 24 weeks with increments every 2 weeks from 2 to 400 mg of peanut protein (pp). Primary outcome was tolerance of 400 mg of pp at DBPCFC2.

Results: Thirty patients were included between September 2013 and May 2014. At DBPCFC2, unresponsiveness to 400 mg of pp was achieved in 17/21 peanut group patients (2 withdrawn patients) and 1/9 in the placebo group (Intention-to-treat analysis, $P < .001$, absolute difference = 0.7, 95%IC 0.43 0.96). Oropharyngeal symptoms were equally frequent in both groups. No dysphagia or other signs of eosinophilic oesophagitis occurred. Digestive adverse events (AE) were more frequent in the treated group ($P = .02$), but mild and without compliance issues. Only one severe adverse event led to withdrawal in a patient who ingested twice the investigated treatment. Peanut-specific humoral immune responses were modulated.

Conclusion: The GIDOIT protocol demonstrated clinical and immunological efficacy and had an acceptable level of safety with weak oropharyngeal symptoms, no dysphagia, mild digestive events and few severe systemic AE.

Merlin and Evrard equally contributed to this work

^aSee Appendix 1 for PITA group details.

KEYWORDS

adolescent, anaphylaxis, capsules, gastrointestinal delivery, oral immunotherapy, peanut allergy

1 | INTRODUCTION

Peanut allergy is one of the most common forms of food allergy encountered in paediatric practice. It affects about 1%-2% of children in France and Europe.¹ It is widely assumed that in 80% of cases peanut allergy will not be outgrown.²

Peanut oral immunotherapy (OIT) can induce desensitization in peanut-allergic patients. Since the Cochrane review published in 2012, there has been much debate about the modalities of OIT for peanut allergy in children.³⁻¹¹ The treatment is long, compliance is uncertain and adverse events (AE) are seen as a drawback.¹²⁻¹⁷ Digestive symptoms have been commonly reported,^{10,11} in particular abdominal pain, nausea and oropharyngeal symptoms. In addition to these symptoms, the prevalence of eosinophilic esophagitis has been estimated at 2.7% and more with other foodstuffs.¹⁸⁻²⁰ In the many studies published, peanut is usually administered in a vehicle food. We decided to deliver peanut in sealed capsules, incrementally increasing over time the amount of peanut ingested. We postulated that bypassing the upper digestive tract would maintain, or even enhance, the efficacy of the technique by targeting the immune system in the lower digestive tract, which is the main anatomical site of oral tolerance induction.²¹ This technique could reasonably be expected to limit AE particularly those involving the mouth and oesophagus.^{10,11} In addition, reducing the absorption of peanut allergens by the buccal mucosae could limit their diffusion in the blood and hence reduce systemic AE.²² The technique also masks the taste of peanut, thereby avoiding taste aversion. We designated this new modality of immunotherapy GastroIntestinal Delivery Oral Immunotherapy (GIDOIT).

Peanut allergy is a great challenge for physicians because, in the absence of OIT, strict avoidance is the sole management technique. An avoidance diet is demanding for children and their families because of the widespread presence of peanut in food products. In addition, it exposes children to severe reactions in the event of unintended ingestion.²³ Adolescents are particularly at risk because they are inclined to deny the importance of rigorous avoidance and because their behaviour is unpredictable. Most fatalities due to anaphylactic reactions to peanut occur in adolescents or young adults,²⁴ who therefore need strategies to protect them against this risk. Adolescents are able to ingest large-size capsules and therefore to follow a course of OIT. To date, there have been no documented findings on OIT in a population composed solely of adolescents with established peanut allergy and high levels of peanut-specific IgE.

The primary aim of this study was to assess the ability of this new modality of OIT to induce desensitization in peanut-allergic adolescents. The secondary aim was to analyse the AE and particularly those suspected of resulting from the bypassing the upper digestive tract during the build-up phase of the protocol. The desensitized patients were then included in the second phase of the study to evaluate sustained unresponsiveness after maintenance phase. They

subsequently returned to a non-restrictive diet in daily life. We report here only the results of the build-up phase.

2 | METHODS

We conducted a randomized, double-blind, placebo-controlled, multi-centre study to assess the efficacy and safety of GIDOIT in adolescents and to confirm the immunological mechanisms underlying this treatment and their relationship to clinical outcomes.⁴ The study was approved on May 24, 2013 by the Comité de Protection des Personnes Sud-Est VI, France, 2013-A00169-39. All patients gave written informed consent. The protocol was registered in clinicaltrials.gov under the number NCT02046083 and is detailed extensively in supporting information 1 (SI1) and Figure 1 and by Michaud et al.²⁵

Adolescents with peanut allergy aged 12-18 years were recruited in the paediatric allergy units of the university hospitals of Clermont-Ferrand, Saint-Etienne and Lyon. All screened patients had experienced a clinical reaction within 60 minutes after ingestion of peanut and evidenced sensitization as assessed in our previous publications on a local population of children: positive skin prick test (SPT) (3 mm greater than the negative control) and/or a serum-specific IgE level over 12 IU/mL (ImmunoCAP®, Phadia 250®, Thermo Fisher®) for peanut or over 5.8 IU/mL for rAra h 2 fraction of peanut.^{26,27} Exclusion criteria were a history of severe anaphylaxis requiring hospitalization in an intensive care unit, severe persistent asthma or poorly controlled atopic dermatitis, lactose intolerance, allergy to sunflower (contained in the trial capsules), and the inability, for whatever reason, to manage a severe reaction. To avoid misinterpretation, the occurrence of another concomitant severe food allergy (such as to egg, milk or nuts) was also an exclusion criterion. Patients having experienced a severe reaction after food ingestion are at risk of reproducing a severe systemic reaction whose origin would be difficult to differentiate from that of a peanut-induced reaction (Appendix S1; Figure 1).

All patients underwent a double-blinded placebo-controlled oral food challenge (DBPCFC) with sealed capsules (Table S1). A DBPCFC was declared positive when an objective symptom occurred. The positive threshold of DBPCFC was the cumulative amount of ingested peanut resulting in clinical symptoms. Inclusion was confirmed when a convincing objective reaction occurred after a cumulative intake over 20 mg and below or equal to 400 mg of peanut protein (pp) during DBPCFC1. After DBPCFC1, patients were randomly assigned for a build-up phase of 24 weeks to active treatment (peanut) or placebo, in a 2:1 ratio. The random allocation process was performed with a computer software program (Stata) that generates a sequence of blocks of different sizes. Patients ingested a daily treatment dose of 2 to 400 mg of pp (10 mg to 2000 mg of peanut paste) that was increased every 2 weeks (Table S2) while maintaining a peanut-free diet. The first dose of each increment was

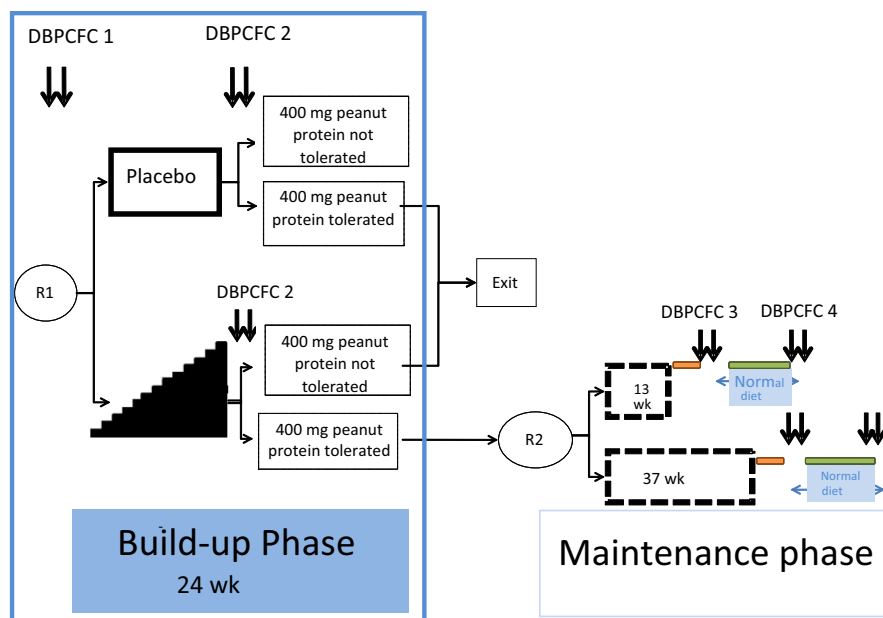


FIGURE 1 Peanut immunotherapy in adolescent protocol. Adolescents aged 12–18 y with peanut allergy proven in a first double-blind placebo-controlled oral food challenge (DBPCFC) will enter a double-blind placebo-controlled build-up phase with a first 2:1 randomization (R1). After a successful build-up phase, duration of the maintenance phase will be determined by a second 1:1 randomization (R2). We report only results of the build-up phase, focused on desensitization (Blue frame).²⁵ Double arrow: DBPCFC. —24-week normal diet; —6-week wash-out [Colour figure can be viewed at wileyonlinelibrary.com]

given in the study centre by a nurse who was blinded. The patients were kept under surveillance for 2 hours before leaving the hospital. On-going treatments were continued, particularly those for asthma management. Daily home intake of the capsules, the times at which they were taken and any AE were recorded in the patient's paper diary. Education sessions were mandatory before and during the protocol. Patients were instructed to remain under supervision for 2 hours after intake. The raw material used to prepare the peanut capsules by the pharmacy department was a paste made with roasted peanut from Argentina with sunflower oil added (MENGUY'S ©). Total protein content of the paste was $\approx 20\%$ (Appendix S2). Thus, we decided to present results in terms of pp to allow comparison with studies performed with defatted peanut powder containing around 50% protein. To avoid mouth and oesophageal contacts, peanut paste was enclosed in sealed gelatin capsules to swallow, allowing allergen gastric/small intestine release. The main objective of the study was to investigate the efficacy and safety of oral escalating immunotherapy using a gastrointestinal delivery system with sealed capsules of peanut up to a maximum daily level of 400 mg of pp. The primary endpoint was assessed at a second DBPCFC performed one day (or 3 days at weekends) after the end of the build-up phase to allow the patient to return to baseline. This waiting period was included in the protocol to avoid cumulating the daily intake of 400 mg of pp and the intake of 2 to 1800 mg of pp during DBPCFC2.

Desensitization was considered to be achieved when no objective symptoms occurred after the ingestion of 400 cumulative mg of pp during DBPCFC2, that is, the reactivity threshold was strictly

over 400 mg. Safety was assessed on the basis of the number and intensity of AE and the number of withdrawn patients (after grade 3b or grade 4 reactions or those whose level of intake remained unchanged for more than 8 weeks).²⁵ The secondary endpoints were the number of patients who increased 4-fold the tolerated dose of cumulative peanut intake during DBPCFC, the percentage of patients affected by AE and immunological profile changes. Adverse events were recorded at each visit to the department and classified as local (rhinitis, conjunctivitis, oropharyngeal symptoms and dysphagia), cutaneous, digestive or general (Table S3), and if appropriate considered as a likely allergic reaction to the treatment. If any reaction occurred during the protocol, symptomatic drugs were prescribed, depending on the severity of the reaction.²⁵ The immunological profile was assessed at each DBPCFC and at mid build-up phase on the basis of SPT and total IgE and IgG4 levels, peanut-specific IgE and IgG4 levels for total peanut and the recombinant fractions rAra h 1, rAra h 2, rAra h 3, rAra h 8 and rAra h 9 (ImmunoCAP®, Phadia 250®, Thermo Fisher®) (Appendix S3).

In all, 60 randomized patients were necessary to detect a 50% difference in the primary outcome with a 2-sided type I error set at $\alpha = .05$ and a statistical power of 90% assuming a 60% rate of desensitization after the build-up phase in the treated group and 10% in the placebo group with a 2:1 ratio.^{5,17,28} Owing to the discrepancy between the number of screened patients and those recruited, an interim analysis was planned after the inclusion of 30 patients to be performed by an independent statistics expert and approved by the ethics committee. For 30 assessable patients, a

difference in the primary outcome between the 2 arms was considered significant for an adjusted α equal to .003 (O'Brien-Fleming, East© software). Thus, as the primary outcome was reached, we stopped randomization after the 30th patient and unblinded the study to record the statistically significant results of the build-up. Statistical analysis was performed with Stata 13 software (StataCorp LP, College Station, TX, USA). Results are presented as mean ($\pm$ standard deviation) or median [interquartile range] according to statistical distribution for continuous data (assumption of normality assessed by the Shapiro-Wilk test) and as the number of patients and associated percentages for categorical parameters. Patient characteristics in the randomization groups were compared by the Chi-square or Fisher's exact tests for categorical variables such as unresponsiveness to 400 cumulative milligrams of pp, and by the Student *t* test or the Mann-Whitney test if the conditions of *t* test were not met (normality and homoscedasticity verified by the Fisher-Snedecor test) for quantitative parameters. Threshold is expressed as median and quartiles [25-75]. Longitudinal analyses were made with mixed models to take into account between- and within-subject variability (random effect) and to study fixed effects such as randomization group, time-point evaluation and their interaction. The residual normality was checked. In accordance with standard practice, we chose to report all individual *P* values for secondary outcomes without any mathematical correction for distinct tests comparing the 2 arms.²⁹ A per-protocol analysis was used to take into account attrition bias because 2 patients did not complete the follow-up stipulated in the study protocol.

3 | RESULTS

3.1 | Study patients

Between July 2013 and December 2015, 51 patients were screened. Among them 30 had a positive DBPCFC1 and entered the study. The remaining 21 patients had an intake threshold higher than 2000 mg of peanut paste (400 mg of pp) and thus were not included (Figure 2). Of the 30 patients included, 21 were allocated to the peanut group and 9 to the placebo group. Baseline characteristics did not differ between the 2 groups except for the prevalence of eczema and the level of specific IgE for rAra h 8 recombinant fraction of peanut (Tables 1 and 2). The baseline immunologic profile of patients is given in Table S4. No patients were suspected of gastro-oesophageal reflux. As required by the protocol, none of the patients included had a peanut threshold over 400 mg of cumulative pp.

3.2 | Primary outcome

Unresponsiveness to 400 cumulative mg of pp (2 cumulative grams of peanut) was achieved in 17 of the 21 patients treated with peanut and 1 of the 9 patients receiving placebo (Intention-to-treat analysis, $P < .001$, Fisher's exact test) (Figure 3). Of these 30 patients, 2 in the treated group withdrew because of side effects

(described below), leaving 28 to undergo DBPCFC2. Two patients both treated with peanut did not increase their intake threshold over 400 mg as targeted, which increased from 30 mg to 222 mg and from 62 mg to 400 mg. Thus, they failed the first outcome but succeeded in the second (increasing 4-fold their unresponsiveness threshold of cumulative intake). For these 2 patients, the number of 2-week step periods without AE was 6 and 11 out of 12. They tolerated the last 2-week steps when ingesting the daily amount of 400 mg of pp without AE. The one patient whose threshold increased under placebo did not complain of any AE during build-up phase but had a grade 3b reaction after ingesting 1800 mg of cumulated pp during DBPCFC2.

3.3 | Secondary outcomes

Between DBPCFC1 and DBPCFC2, the cumulated median pp threshold rose from 122 mg [122-400] to 1800 mg [800-1800] in the peanut group ($P < .001$) and from 122 mg [62-220] to 400 mg [220-400] in the placebo group ($P = .02$), that is, a 15-fold and 3-fold increase, respectively (Table S5). The reactivity threshold was similar in the placebo and peanut groups at baseline ($P = .38$) but differed significantly ($P < .001$) at DBPCFC2. In total, 17 of the 19 patients treated with peanut who finished the build-up protocol increased their reactivity threshold 4-fold between DBPCFC1 and DBPCFC2 as did 2 out of 9 in the placebo group. The statistical significance was high in per-protocol analysis ($P < .001$) and also in intention-to-treat analysis ($P < .001$) (Figure 3).

3.3.1 | Safety

Two patients from the peanut group did not complete the build-up phase, one because of a severe adverse event (SAE) (anaphylactic shock requiring Epinephrine) and the other because of moderate but recurrent respiratory side effects (grade 2) that led us to maintain the same level of peanut intake over 3 additional 2-week periods. Only 3 patients had no adverse reaction at any time during the build-up phase, 2 in the peanut group and one in the placebo group. The number of patients experiencing AE was not different between the 2 groups but the number of AEs per patient was higher in the treated group than in the control group, resulting in a higher number of 2-week steps with AEs in the treated group. The number of doses resulting in any AE was 35 per 1000 doses in the peanut group and 31 per 1000 doses in the placebo group, whereas the number of doses requiring administration of a medication was 91 per 1000 doses in the peanut group and 36 in the placebo group. AEs were more severe in the treated group ($P < .05$) (Tables 3 and 4). Five severe multi-system reactions (grades 3a and 3b) occurred in 4 peanut-treated patients and in none in the control group (ie one vs no anaphylaxis reaction for 1000 doses). One was life-threatening (grade 3b), as noted above. None occurred before 40 minutes after intake. Patients ingesting peanut reported more digestive AE: 68 among the treated patients and 7 among the placebo patients experienced a digestive event at least once during the build-up phase

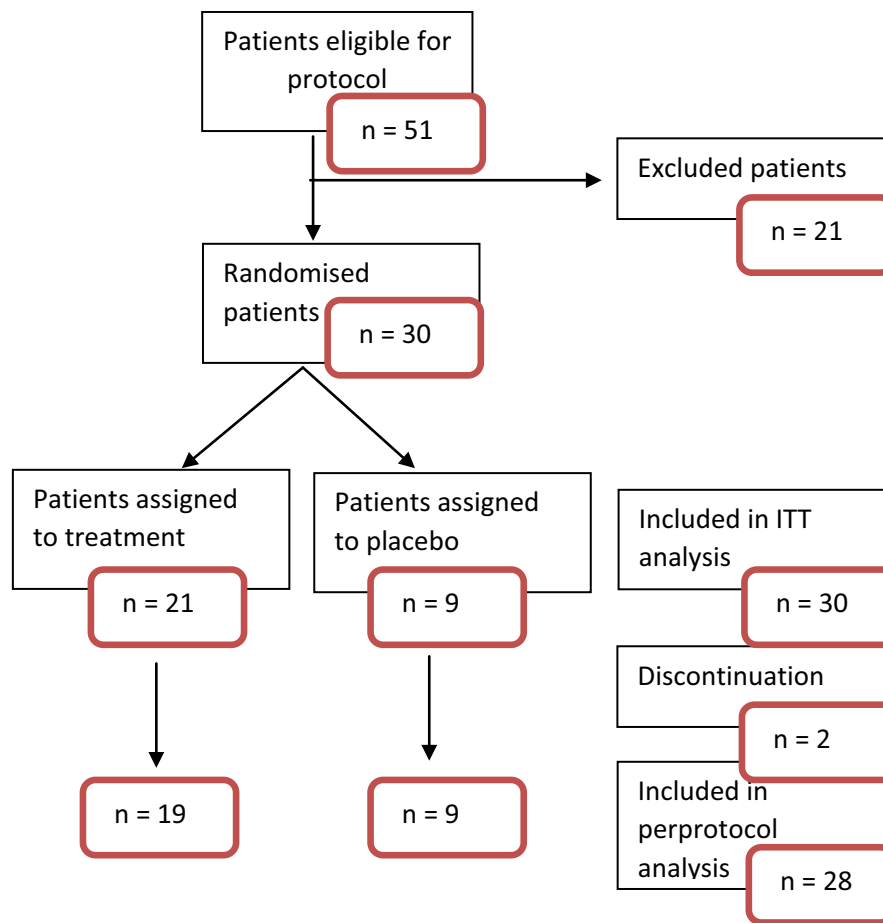


FIGURE 2 Patient inclusion flowchart

(mean per patient = 3.2 events vs 0.8, respectively), mostly abdominal pain and nausea.

The numbers of reported oropharyngeal symptoms (swelling, itching and paraesthesia located in the oral sphere) or dysphagia symptoms were considerably low and not significantly different between peanut and placebo groups (Table 5). No eosinophilic oesophagitis was diagnosed. None of the patients were withdrawn because of gastrointestinal symptoms. Respiratory reactions were also significantly more frequent in the treated patients. General reactions occurred more frequently in the peanut group than in controls but not to a level of significance. The number of rescue medications per patients did not differ between the 2 groups.

3.3.2 | Immunological profile

is shown in Table 2. SPTs decreased from 12.4 ± 6.7 mm to 6.0 ± 4.0 mm between the 2 DBPCFCs ($P < .001$) in the peanut group as against 12.5 ± 6.6 mm to 8 ± 3.8 mm in the placebo group ($P < .05$). Positive histamine controls were unchanged (data not shown). None of the patients acquired sensitization to sunflower seeds during the study. In the peanut group, total IgE but not total IgG4 levels were significantly different after the build-up compared to baseline phase. Specific IgE levels to peanut increased significantly

in the peanut group in comparison to the placebo group at DBPCFC2 ($P = .04$). At mid build-up phase, peanut IgE values had already increased in the peanut group, but not to a level of significance. No significant variations were observed for anti-Ara h 1-, 2- and 3-specific IgE between DBPCFC1 and DBPCFC2. For Ara h 8 IgE assay, the difference evidenced at DBPCFC1 persisted at DBPCFC2. Specific IgG4 levels increased significantly between the beginning and the end of the build-up phase for peanut (0.40 ± 0.45 to 2.98 ± 3.69 mg/mL), and the 3 main allergenic fractions of peanut. Specific Ara h 8 IgG4 varied significantly between the 2 groups owing to a large dispersion of the results. There was no significant variation in specific IgG4 against Ara h 9. No variation in peanut-specific IgE/total IgE ratio was observed during the build-up phase in either group (Figure 4A). In contrast, peanut and Ara h 2-specific IgG4/specific IgE ratios increased in the treated group at DBPCFC2, reaching significance for Ara h 2 ($P = .03$). There was no significant difference between the placebo and treated groups in IgG4/IgE ratios (Figure 4B,C).

4 | DISCUSSION

Our study of GIDOIT with sealed capsules proposes a practical way of inducing desensitization that ensures efficacy and safety in high-

TABLE 1 Baseline characteristics

	Peanut group n = 21 (%)	Placebo group n = 9 (%)
Population		
Male/Female, n (%)	15/6 (71.4)	7/2 (77.8)
Age (y), mean $\pm$ SD	14.5 $\pm$ 1.8	15.0 $\pm$ 1.1
Age at diagnosis mean $\pm$ SD	3.7 $\pm$ 3.6	2.9 $\pm$ 1.5
Body mass index, mean $\pm$ SD	19.6 $\pm$ 3	18.9 $\pm$ 3
Allergy history, n (%)		
Unexpected reaction to peanut occurred after diagnosis ^a	14 (66)	5 (55)
Nut allergy ^b	2 (9)	2 (22)
Other food allergies	6 (28)	3 (33)
Dust Mite allergy	7 (33)	3 (33)
Pollen allergy	11 (52)	4 (44)
Dander allergy	7 (33)	4 (44)
Medical history		
Asthma	9 (43)	5 (55)
Rhino conjunctivitis	3 (14)	1 (11)
Eczema	0	4 (44)
On-going treatment		
Antihistamines	0	2 (22)
Inhaled corticosteroids	10 (48)	2 (22)
Anti-leukotrienes	4 (19)	1 (11)
Sublingual aeroallergen immunotherapy	0	1 (11)
Other treatments	9 (43)	4 (44)

^aClinical reaction occurred between the time of diagnosis and the inclusion in the peanut immunotherapy in adolescent protocol.

^bNon-systemic allergic reaction.

risk adolescents. Although statistically our study population is small, the power of the study is satisfactory and our data are robust because we selected a homogeneous population in terms of body surface. The interim analysis was performed according to usual statistical recommendations particularly with regard to rules about correction of type I error. After unblinding of the study, additional patients were recruited unblinded in the build-up phase so as to be included in the second part of the study devoted to maintenance.

Using peanut in sealed capsules for blinded desensitization is a key point of our protocol. Peanut OIT has been reported to successfully induce desensitization after ingestion of peanut flour or powder.^{4,5,13,14-17,30} These published protocols, in which peanut is administered in a vehicle food, commonly report a wide range of side effects. We chose to use peanut in capsules for theoretical and practical reasons. It could be objected that using capsules results in bypassing the buccal immune cells, especially the dendritic and Langerhans cells. However, from an immunological and pathophysiological perspective, the role of dendritic cells in the small intestine is pivotal in the induction of tolerance.³¹⁻³³ After exposure to dietary antigens, via oral administration, intestinal tolerogenic

CD103+ CD11c+ dendritic cells possess the unique ability to induce Foxp3+ regulatory T cells in the mesenteric lymph node and therefore to mediate T-cell suppression.³⁴ Thus, allergen delivery in the gut is theoretically a suitable route to induce tolerance.³⁵ Consequently, we decided to target directly the antigen towards the immune cells of the gut. This decision was also governed by the observation that carrying allergen directly to the gut of non-sensitized patients can induce greater sensitization than allergens initially digested in the stomach, probably because of a better conservation of the tri-dimensional structure of allergens.³⁶ Conversely, we hypothesized that the same reasoning, based on the use of better preserved allergens, would help in inducing desensitization in highly sensitized patients and probably limit the incidence of AE. In addition, using capsules could reduce the risk of oropharyngeal symptoms and of iatrogenic eosinophilic oesophagitis (see below).¹⁸ The use of capsules rather than flour masks the taste of peanut and allows a true double-blind comparison of the groups, except in the event of a significant gastro-oesophago-pharyngeal reflux. Finally, ingesting capsules also avoids aversion, which is commonly encountered in peanut allergy. To our knowledge, using sealed peanut capsules has been tested in only one study on a small number of patients without placebo controls.³⁷

Efficacy was assessed on the basis of the increase in the reactivity threshold during DBPCFC2 up to at least 400 mg of pp (2 cumulative grams of peanut paste). Peanut allergic patients are potentially at risk of reactions after accidental ingestion of traces. Increasing this threshold of pp up to 400 mg is consistent with protection against accidental ingestion or cross-contamination with peanut and allows protection in daily life, except when fresh peanuts or peanut-based foods are ingested. In 2014, the VITAL Expert Panel calculated various Eliciting Doses (ED) including ED₀₁ (predicted dose to provoke reaction in 1% of the peanut allergic population) at 0.160 mg, and ED₅₀ between 100 and 200 mg of pp.³⁸ Other authors indicated in 2016 that there was no significant difference between quantifying EDs in real life and upon food challenge.³⁹ The build-up phase of the peanut immunotherapy in adolescent (PITA) protocol produced dramatically positive effects consistent with results of other studies in terms of reactivity threshold.³⁻⁵ Among the patients treated with peanut 17, that is, 81% were able to ingest 400 mg of pp in a day without experiencing any objective symptom and achieved our primary endpoint. For most of them, the peanut threshold level was much higher. At DBPCFC2, 42% of the treated patients only reacted when ingesting 1800 mg of pp and 14% even had no reaction to this amount (Figure 3). 19% reacted to the cumulated dose of 800 mg. This threshold exceeded most of the ED given above. Thus, it can be presumed that these patients are protected in daily life. However, at DBPCFC2, 2 patients (9.5%) remained intolerant to 400 mg of pp but had increased their threshold level 6- and 7-fold, respectively. Thus, they achieved the secondary outcome of a 4-fold increase in threshold. In addition, they succeeded in ingesting 400 mg daily of pp at home without any clinical symptoms. Although these 2 patients were not considered desensitized (because the first endpoint was not achieved), the impact of the treatment was

TABLE 2 Immunological profile in the Peanut and Placebo group

		At DBPCFC1	Mid Build-up Phase		At DBPCFC 2		
		Mean $\pm$ SD	mean $\pm$ SD	P*	Mean $\pm$ SD	P**	P***
SPT (mm)	Peanut	12.4 $\pm$ 6.7			6.1 $\pm$ 4.1	<.001	.55
	Placebo	12.5 $\pm$ 6.6			8.0 $\pm$ 3.8	.045	
Total IgE (IU/mL)	Peanut	628 $\pm$ 469	699 $\pm$ 411	.35	840 $\pm$ 573	.004	.31
	Placebo	1403 $\pm$ 1883	1226 $\pm$ 1603	.30	1468 $\pm$ 2403	.744	
Peanut Spe. IgE	Peanut	162 $\pm$ 200	213 $\pm$ 197	.06	195 $\pm$ 173	.102	.04
	Placebo	211 $\pm$ 177	193 $\pm$ 148	.53	163 $\pm$ 128	.090	
Ara h 1 Spec. IgE	Peanut	48 $\pm$ 69	53 $\pm$ 70	.70	65 $\pm$ 97	.1	.27
	Placebo	34 $\pm$ 29	31 $\pm$ 22	.32	34 $\pm$ 29	.944	
Ara h 2 Spec. IgE	Peanut	79 $\pm$ 85	100 $\pm$ 95	.15	97 $\pm$ 87	.251	.10
	Placebo	134 $\pm$ 129	124 $\pm$ 95	.68	105 $\pm$ 72	.238	
Ara h 3 Spec. IgE	Peanut	42 $\pm$ 81	42 $\pm$ 62	.95	43 $\pm$ 66	.438	.34
	Placebo	29 $\pm$ 43	29 $\pm$ 46	.94	23 $\pm$ 30	.235	
Ara h 8 Spec. IgE	Peanut	1.6 $\pm$ 5.2	1.2 $\pm$ 3.5	.47	2.3 $\pm$ 5.3	.270	.03
	Placebo	21 $\pm$ 42	21 $\pm$ 36	.99	44 $\pm$ 97	.137	
Ara h 9 Spec. IgE	Peanut	0.22 $\pm$ 0.74	0.17 $\pm$ 0.47	.71	0.27 $\pm$ 0.74	.676	.42
	Placebo	1.04 $\pm$ 1.65	0.96 $\pm$ 152	.82	1.34 $\pm$ 2.69	.427	
Total IgG4 (mg/mL)	Peanut	0.85 $\pm$ 0.83	0.85 $\pm$ 0.80	.96	0.93 $\pm$ 0.82	.508	.38
	Placebo	0.83 $\pm$ 0.63	0.88 $\pm$ 0.73	.39	0.78 $\pm$ 0.59	.380	
Peanut Spec.IgG4	Peanut	0.40 $\pm$ 0.45	0.99 $\pm$ 0.85	.30	2.98 $\pm$ 3.69	<.001	.005
	Placebo	0.38 $\pm$ 0.37	0.50 $\pm$ 0.53	.08	0.42 $\pm$ 0.44	.233	
Ara h 1 Spec. IgG4	Peanut	0.049 $\pm$ 0.057	0.066 $\pm$ 0.078	.90	0.387 $\pm$ 0.896	.026	.12
	Placebo	0.034 $\pm$ 0.047	0.046 $\pm$ 0.058	.02	0.02 $\pm$ 0.024	.814	
Ara h 2 Spec. IgG4	Peanut	0.092 $\pm$ 0.102	0.312 $\pm$ 0.464	.62	1.467 $\pm$ 2.877	.003	.05
	Placebo	0.076 $\pm$ 0.046	0.107 $\pm$ 0.081	.003	0.077 $\pm$ 0.069	.370	
Ara h3 Spec. IgG4	Peanut	0.228 $\pm$ 0.404	0.247 $\pm$ 0.332	.78	0.555 $\pm$ 0.532	<.0001	.006
	Placebo	0.088 $\pm$ 0.078	0.122 $\pm$ 0.103	.005	0.097 $\pm$ 0.103	.351	
Ara h 8 Spec. IgG4	Peanut	0.016 $\pm$ 0.039	0.015 $\pm$ 0.040	.77	0.018 $\pm$ 0.058	.582	.006
	Placebo	0.106 $\pm$ 0.157	0.114 $\pm$ 0.158	.76	0.173 $\pm$ 0.245	.045	
Ara h 9 Spec. IgG4	Peanut	0.043 $\pm$ 0.132	0.063 $\pm$ 0.217	.84	0.200 $\pm$ 0.771	.146	.36
	Placebo	0.271 $\pm$ 0.575	0.261 $\pm$ 0.558	.56	0.3 $\pm$ 0.579	.997	

*P = mid build-up vs DBPCFC 1; **P = DBPCFC 2 vs DBPCFC 1; ***P = peanut patients vs placebo patients at DBPCFC2.

DBPCFC, double-blind placebo-controlled oral food challenge; ND, not done; Plac., Placebo; SD, standard deviation; Spec., Specific.

manifest. Thus, the threshold progression cannot be considered to be a consequence of intra-individual variability. There are 3 possible explanations of the discrepancy between the day-to-day intake of peanut and the ingestion of peanut during DBPCFC2 in these 2 patients. (i) The modality of intake, which was instantaneous in day-to-day ingestion and cumulative during DBPCFC (Table S2). (ii) In the 24- to 72-hour time gap between the last home intake of peanut and DBPCFC2 patients may have already lost their unresponsiveness. (iii) The context of intake and the season when DBPCFC2 was performed. The lack of reproducibility of the challenge could also account for this slight variation.⁴⁰ The overall rise in threshold in the placebo group (from 122 to 400 mg of pp) could have resulted from day-to-day intra-individual variability. We speculate that this variability could be due to criteria such as seasonal variability, reduced

stress due to habituation to DBPCFC or other factors. While this is an important finding, what should be particularly emphasized is that, despite both groups being affected by seasonal or intra-individual variability, there was a great difference in intensity of the threshold increase between the two. Since biological arguments have been suggested by others for a secondary loss of unresponsiveness, the persistence of tolerance after OIT will be addressed in our protocol in the long term.⁴¹

In the interest of safety, the management of AEs underwent rigorous assessment. The patients taking part had all attended education sessions, and before enrolment and at each visit they were tested on their knowledge of the practical management of their allergy. In both the treated and placebo groups they were able, therefore, to identify a wide range of side effects.⁵ Unlike in other

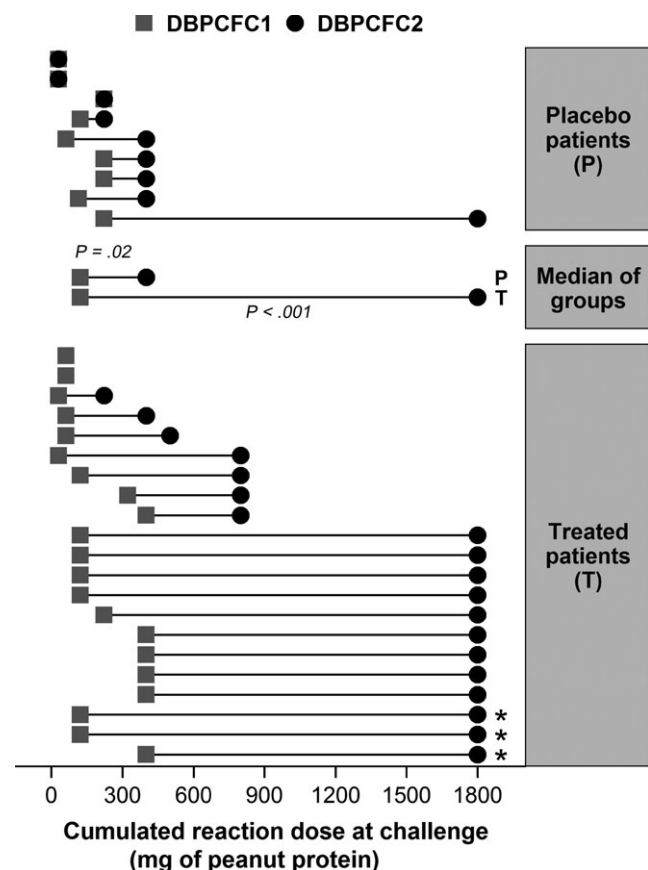


FIGURE 3 Peanut protein intake threshold at DBPCFC1 and DBPCFC2 (intention to treat analysis: $P < .001$): cumulated reaction dose at challenge (mg of peanut protein). In all, 30 adolescents underwent oral immunotherapy either with peanut capsules ($n = 21$) or with placebo capsules ($n = 9$). Two patients were withdrawn from the treated group (no DBPCFC2). DBPCFC2 was negative at 400 mg of pp in 17 of the 19 treated patients and one of the placebo patients ($P < .001$). (DBPCFC = double-blind placebo-controlled oral food challenge)

types of AE, the number of local symptoms was not significantly different between treated and control patients. Interestingly, and in contrast to previous reports, oropharyngeal symptoms (swelling, itching and paraesthesia located in the oral sphere) in our study were not significantly more frequent in treated patients.⁷ Unfortunately, we did not assess quality of life and so are unable to evaluate the daily impact of the clinical effects observed.⁴² Nevertheless, compliance during this escalation phase turned out to be excellent, since no patient was withdrawn because of protocol non-adherence. Thus, good compliance could account for the limited reduction in quality of life. Although oropharyngeal symptoms are not severe they are uncomfortable, and can result in compliance difficulties. A decrease in these symptoms could therefore have enhanced compliance during the escalation phase. No patient in our study experienced dysphagia. Thus, monitoring of AEs evidenced no sign leading to the diagnosis of esophagitis. Conversely, Virkud et al,¹¹ who used peanut powder administered in a food vehicle, confirmed the occurrence of eosinophilic esophagitis by

endoscopy. In a recent publication on a build-up phase with opened capsules of peanut in food, Bird et al³⁰ reported 14% of oesophageal pain in the treated group as against none in the placebo group. As noted above, bypassing the oral and oesophageal mucosa could explain the small number of upper digestive tract AEs in our population and is a strong argument in support of the efficacy of our concept of shunt. The non-exposure of oral immune cells to the antigen could account for the rareness of local reactions in our treated population compared to results of recent published studies.^{10,11}

Gastrointestinal AEs, as in other studies, were frequent in our treated patients.⁷ In all but one patient, repeating the same level of intake during an additional 2-week period, in some cases associated with antihistamine treatment, was sufficient to allow them to carry on with the escalation protocol. The median duration of the build-up phase was therefore 190 days (13.5 2-week periods, range [173; 259] days, [12.36; 18.50] 2-week periods). Finally, no patient was withdrawn because of the recurrence of digestive symptoms, unlike in a recent study using opened peanut capsules during which 21% of the patients were withdrawn because of digestive side effects, mainly vomiting.³⁰ Most of them (4 ie 14%) were withdrawn primarily because they experienced recurrent digestive AEs. Two (7%) were withdrawn because of lack of compliance following digestive (and other) AEs. As we observed no compliance problems due to digestive symptoms, we speculate that adverse reactions were milder in our patients.

Severe AEs grade 3 were infrequent. Three patients experienced a total of 4 non-life-threatening reaction grade 3a. All promptly and fully recovered after symptomatic treatment at home without consultation of a physician or hospitalization. Thus, the AEs did not lead to withdrawal. The only patient who experienced a grade 3b reaction had not rigorously followed the protocol. At mid-day, 2 hours after ingestion and a physical workout, he experienced an anaphylactic reaction that required the intervention of the emergency service for administration of epinephrine. He received salbutamol, oral steroids and antihistamines. He fully recovered promptly, and was released from hospital in the afternoon. After being withdrawn from the study, the patient declared he had taken twice the prescribed dose of 100 mg of pp. Accidental double dosing is a commonly reported AE in other studies. This incident occurred despite patient selection and educational training programmes. Investigators should exercise caution with regard to this issue to avoid SAEs. This single incident (1 patient out of 21 = 4.8%) is to be compared with the 57 widely dispersed (from 0% to 31% of patients) reactions described by Wasserman in 36 of 352 patients recruited at 5 sites and treated with epinephrine during escalation.⁴³ The slow escalation of peanut intake and the education sessions provided for the management of AEs at their very early onset could account for the rareness of severe anaphylaxis in our treated patients. The relatively low rate of protein in our peanut paste could also partly explain the rareness of life-threatening AEs. Dirks et al²² showed that absorption of dietary proteins by the buccal mucosae is very rapid, leading to blood histamine release in 10 minutes. The authors postulated that this

TABLE 3 Safety results during the build-up phase

	Peanut group n = 21			Placebo group n = 9			P
Withdrawals	2			0			
Number (%) of patients with AE							
None	2 (9.5%)			1 (11.1%)			.67
Any (≥ 1)	19 (90.5%)			8 (88.9%)			
Number of reactions/patient $\pm$ SD	6.19 $\pm$ 3.17			3.66 $\pm$ 2.29			.02
Number (%) of 2-week steps resulting in AE	130 (44.5%)			33 (27.3%)			.001
Intensity of the AE ^a							
1	93			31			.02
2	32			2			
3	5 (incl.1 case of 3b)			0			
4	0			0			
Number of AEs	TN	% P	% _{oo} D	TN	% P	% _{oo} D	P (TN)
Local	30	57	7	8	55	5	.88
Cutaneous	48	81	12	16	55	9	.22
Digestive	68	76	17	7	55	4	.001
Respiratory	61	57	15	7	55	4	<.01
General	18	33	4	2	22	1	.22
Number of treatments for AE	88			21			.65
Number of treatments per patient (mean [Q25-Q75])	4 [2-7]			2 [1-3]			.06
Oral antihistamines (mean [Q25-Q75])	3 [1-6]			0 [0-1]			<.01
Oral steroids (mean [Q25-Q75])	1 [0-2]			0 [0-1]			.03
Beta-2-mimetics (mean [Q25-Q75])	1 [0-4]			0 [0-1]			.02

AE, adverse event; TN, total number.

% P: percentage of patients affected by AEs; %_{oo} D: Number of AEs for 1000 doses received.

^aSee Table S3.

TABLE 4 Safety results during the build-up phase: severe multi-system adverse reactions

Patient n°	ReactionDose (mg pp ^a)	Grade	Reaction time	Local	Cutaneous	Digestive	Respiratory	General	Anaphylactic shock	Epinephrine	Withdrawal
8	150	3a	40 min	No	Pruritus Eyelid oedema	Abdominal pain	No	No	No	Yes	No
10	400	3a	120 min	No	Generalized urticaria	No	Asthma attack	No	No	No	No
10	400	3a	90 min	No	Neck and face oedema	No	Asthma attack	No	No	No	No
15	200 (twice 100)	3b	60 min	No	No	No	No	Yes	Yes	Yes	Yes
17	60	3a	45 min	No	Generalized urticaria	Vomiting	No	No	No	No	No

^amg of peanut protein.

absorption could explain the elicitation of food-induced systemic reactions. Moreover, another interesting study has showed that severe reactions occur mainly in the 30 minutes after swallowing the eliciting food.⁴⁴ In our patients, conversely, severe reactions were relatively rare and occurred within 40-120 minutes. The symptoms, such as urticaria, oedema and asthma, were typically IgE-mediated. We speculate therefore that shunting the upper digestive tract is

beneficial since it reduces absorption of allergens by the buccal mucosae and hence their release in the blood, which in turn reduces systemic reactions.⁴⁴

A second patient was excluded because she had moderate side effects (grade 2) that led us to maintain the same level of peanut intake over four 2-week periods. According to the statistical plan, the number of 2 withdrawn patients was compatible with continuing the

TABLE 5 Safety results during the build-up phase. Local symptoms: number of local reactions and in brackets percentage of patients

Local adverse events	Peanut (n = 21)	Placebo (n = 9)	P
Any	30 (57)	8 (55)	.88
Conjunctivitis	10 (24)	2 (22)	.96
Rhinitis	21 (43)	5 (33)	.77
Oropharyngeal symptoms	6 (19)	1 (11)	.71
Dysphagia	0 (0)	0 (0)	ns

protocol, recruiting additional patients and keeping those who had finished the build-up phase ($P < .003$). Hence, our standardized progressive desensitization protocol without an initial 1-day escalating phase but with initial very weak antigen exposure (as low as 10% of the lowest accepted threshold of 10 mg peanut, ie 2 mg of pp) appears relatively safe and well tolerated in high-risk adolescents.

Why should adolescents be specifically targeted for the management of OIT? Peanut allergy significantly affects their quality of life.⁴² No practical solution is available for these patients at an age when they are about to leave their family circle, change their medical environment and possibly interrupt compliance with their avoidance regimen. Most studies on peanut allergy involve children and none have been made specifically of adolescents.^{6,13,14,17,28,30} Although most of our patients experienced a successful 24-week period of daily intake, it was important to confirm a threshold increase in pp intake by comparing the results of DBPCFC1 and DBPCFC2. Finally, results on efficacy and safety during the build-up phase tend to describe peanut allergic adolescents as a subgroup of patients particularly receptive to peanut OIT. In view of the limitations of our study (exclusion of concomitant severe food allergies and population of solely adolescents), these results need to be confirmed in multi-sensitized adolescents and in other populations of children.

Changes in the immunological profile included changes in SPT and in the antibody response to allergens that are characteristic of desensitization. During the build-up phase, we observed an increased level of peanut-specific IgE in the treated group. These results are in line with those of other reports of allergen immunotherapy, in particular to food allergens.^{45,46} In previous studies involving peanut OIT, an increase in specific IgE was observed as of 2 months of treatment and continued for at least 12–18 months.^{5,14,47} This increase was also associated with a significant increase in specific IgG4 measurements. However, although the IgG4 increase was highly significant in our peanut group, the intergroup assessment of variability did not reach significance for IgG4/IgE ratios in our mixed model, possibly owing to a lack of power consecutive to the great dispersion of values.

Analysis of the peanut paste used to prepare the capsules confirmed the presence in significant and stable amounts of 3 major peanut allergen fractions, Ara h 1, Ara h 2 and Ara h 3, in the raw material. This assay had to be performed before clinical challenge against these molecular compounds (Appendix S2).⁴⁸ At baseline, our patients were sensitized against Ara h 2 and to some extent against Ara h 1 and Ara h 3. In our patients, changes in the antibody response to the three allergenic fractions Ara h 1, Ara h 2 and Ara h

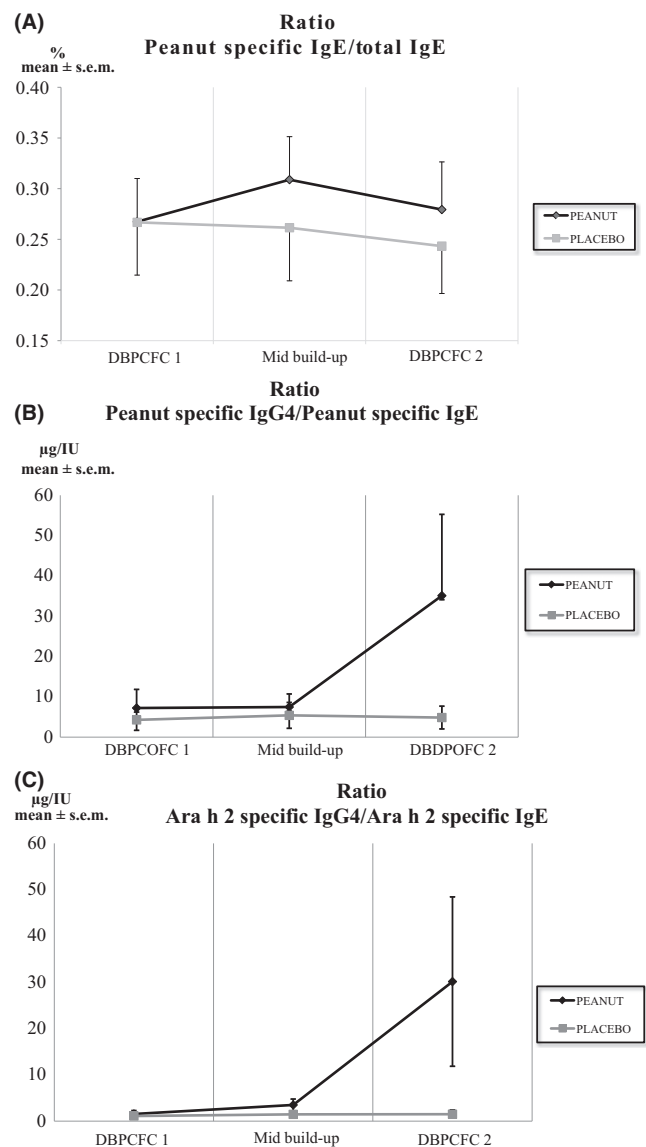


FIGURE 4 Immunological profile: (A) Ratio of peanut-specific IgE (IU/mL)/total IgE (IU/mL). The serum ratio of peanut-specific IgE over total IgE did not change significantly between DBPCFC1 and DBPCFC2 in the peanut group in comparison with the placebo group. (B) Ratio of peanut-specific IgG4 (μ g/mL)/peanut-specific IgE (IU/mL). The ratio of peanut-specific IgG4 over specific IgE increased between DBPCFC1 and DBPCFC2 in the peanut group without reaching significance ($P = .06$). (C) Ratio of Ara h 2-specific IgG4 (μ g/mL)/Ara h 2-specific IgE (IU/mL). The ratio of Ara h 2-specific IgG4 over Ara h 2-specific IgE increased significantly ($P = .03$) in the peanut group between DBPCFC1 and DBPCFC2. Like the ratio of peanut antibodies (B), when compared with the placebo group this increase did not reach statistical significance. DBPCFC = double-blind placebo-controlled oral food challenge

3 were similar to those of the total extract: a simultaneous increase in the specific IgE and specific IgG4 values and their IgG4/IgE ratio. These results are closely similar to those observed in three recent publications.^{49–51} Among the storage pps, Ara h 2 and Ara h 6 are predictive factors for the occurrence of systemic reactions.^{52,53} We

observed dramatic changes in our treated patients in Ara h 2 levels of IgE and IgG4 after the build-up phase. The effects of the increase in the Ara h 2 ratio of IgG4/IgE after the build-up phase on the clinical sensitivity of patients to the intake of peanut could explain the rareness of severe AEs. The follow-up of immune status should help in managing the clinical course of patients and in predicting relapses.⁵⁴⁻⁵⁶ Ara h 8-specific IgE levels cause a biological cross-reactivity with respiratory allergens of the PR10 family.⁵⁵ The differences observed at baseline between the peanut and placebo groups in the levels of anti-Ara h 8 IgE and IgG4 persisted at DBPCFC2. These differences were a consequence of high levels of anti-Ara h 8 IgE detected in 2 placebo patients. As expected, the anti-Ara h 9-specific IgE level did not change significantly between DBPCFC1 and DBPCFC2 in the peanut group, which is consistent with the rareness of the lipid transfer protein syndrome in our region (only 2 patients had specific anti-Ara h 9 IgE at baseline greater than 0.35 IU/mL). The peanut-specific IgE/total IgE ratio did not change significantly, a stability that could be explained by the increase in total IgE levels, which counterbalance the increase in peanut-specific IgE levels. Hence, the changes in the immune profile may not be restricted to immunity directed against peanut. We performed an ancillary study, therefore, using the immuno solid-phase allergen chip (ISAC) technique, to evaluate the impact of peanut GID0IT on associated sensitizations (data not shown).

In conclusion, our study provides an effective standardized way to induce peanut desensitization in high-risk adolescents, offering them protection against severe anaphylactic reactions after unintended intake. The PITA trial showed a low incidence of oropharyngeal symptoms and no signs suggestive of eosinophilic oesophagitis. In light of these findings, safety regarding local AEs is therefore enhanced by the administration of peanut paste in sealed capsules. Owing to the low incidence of life-threatening AE, the trial have an acceptable safety profile. In addition masking the taste of peanut, using sealed capsules probably had a positive impact on compliance. Further studies of larger populations are needed to confirm these results and to assess the long-term persistence of food tolerance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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APPENDIX 1

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Supporting Information (SI)

Peanut Gastro-Intestinal Delivery Oral Immunotherapy in Adolescents: results of the build-up phase of a randomized, double-blind, placebo-controlled trial (PITA study)

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Appendix 1 Study design

Educational sessions were organized collectively and individually with monitoring of the level of knowledge regarding food diet, behavior in the event of an adverse reaction according to the severity of the event. The first dose of each increment was taken in the study centre after recording of any side effects experienced and treatments followed during the previous 2-week period, and a full clinical examination. Patients were observed for 2 hours in the research unit, which was equipped with emergency facilities, and then released. On this occasion, the patients were recommended not to do any sport in the 2 hours after daily intake nor to expose themselves to any condition of stress likely to be induced either by effort or sun exposure. It was ensured that rescue medications were available. If a reaction occurred during the build-up phase, the level of peanut intake could be maintained for two additional periods of 2 weeks, i.e. for a maximum duration of 6 weeks. The protocol could be discontinued at any time by the investigator or the patient. Discontinuation was mandatory when ordered by the independent supervisory board in the event of a serious adverse reaction or inability or unwillingness to increase the intake of peanut after more than four 2-week periods. A wash-out period of 24 to 72 hours was observed before DBPCFC2.

At the end of the build-up phase, patients who tolerated a 2-gram cumulative intake of peanut (400 mg of peanut protein) during a second DBPCFC were randomized (R2) for the maintenance phase. The protocol of this second phase has been detailed in clinical trials¹⁸. This second phase was not assessed in the statistics.

Appendix S2. Assays of peanut proteins and allergenic fractions in peanut paste (Rougé personal communication)

Storage proteins are sequestered in dehydrated vacuoles and so are poorly accessible to extraction buffer (Maleki SJ, Chung SY, Champagne, & al. The effects of roasting on the allergenic properties of peanut proteins. *J. Allergy Clin. Immunol.* 2000;106:763-768. doi:10.1067/mai.2000.109620). The effects of roasting on the allergenic properties of peanut proteins prompted an evaluation of the protein and allergen contents of the peanut paste in crude seeds. Extraction procedures included preliminary homogenization at high dynamic pressure (500 bar) with unbuffered deionized water. Peanut protein content was measured in two samples of crude peanut by a bi-synchronic method (Pierce®). SDS-PAGE was performed in 12.5% acrylamide gel. Protein fractions were then stained and finally estimated from capsules prepared with 95.7 % of peanut extract in triplicate by the ELISA method using polyclonal specific IgG. Protein content was 21 mg/100 mg of peanut extract. From ELISA quantification measurements, the amounts of the major peanut allergens Ara h 1, Ara h 2, and Ara h 3 in protein extracts from samples XCRDARG 14-0003 and XCRDARG 14-0009 was estimated to account for $\approx 12\%$, $\approx 6\%$ and $\approx 15\%$ of the peanut protein content, respectively. In the peanut extract prepared from the XCRDARG 14-0003, protein content was estimated to be 20 mg of protein for 100 mg of capsule. Allergenic fractions were 2.4 mg for Ara h 1, 1.2 mg for Ara h 2, and 3.0 mg for Ara h 3.

Appendix S3. Methods (completed)

Biological markers Complementary tests were performed before each OFC: the results of skin prick tests to peanut were compared with those of the local reaction to histamine hydrochloride (10 mg/ml) and a negative saline solution control. IgE assays were expressed in IU/mL and IgG4 in mg/mL (ImmunoCAP®, Phadia 250®, Thermo Fisher®). In addition to assays performed at the beginning and end of the build-up phase, specific peanut IgE and IgG4 assays were done midway through the build-up phase. Cross-reactivities were evaluated during the initial assessment. Any adverse event was investigated according to its clinical aspect, including complementary assays included specific IgE levels for lupine, walnut, almond, hazelnut, pistachio, sunflower, and cross carbohydrate determinants (horseradish peroxidase and bromelain).

Assignments were centrally prepared, stratified by site in a 2:1 a ratio in each unit, with equilibration every 6 subjects, according to the principles of the Children Clinical Research Center, INSERM CIC 1405. Blinding and anonymization of data were performed in accordance with the declaration of Helsinki.

Safety was assured by recording any adverse event at any time during the protocol. The data monitoring safety committee could recommend interruption of the trial if a patient's safety was compromised or in the event of severe or repeated allergic reactions leading to more than three times stagnation at a given step.

Table S1. Double blinded placebo-controlled oral food challenge to peanut: increments of intake

Time	Increments in mg of peanut paste	Increments in mg of peanut proteins	Cumulative doses in mg of peanut paste	Cumulative doses in mg of peanut proteins
30 min	10	2	10	2
1 h	20	4	30	6
1h30	40	8	70	14
2h	80	16	150	30
2h30	160	32	310	62
3h	300	60	610	122
3h30	500	100	1110	222
4h	890	178	2000	400
4h30 *	2000	400	4000	800
5 h *	5000	1000	9000	1800

* An additional 30 minutes can be added at each level when needed, at the physician's discretion.

Table S2. Build-up phase: increments of intake (2-week periods)

Week	Doses in mg of peanut paste	Doses in mg of peanut proteins
1-2	10	2
3-4	20	4
5-6	40	8
7-8	80	16
9-10	160	32
11-12	300	60
13-14	500	100
15-16	750	150
17-18	1000	200
19-20	1250	250
21-22	1500	300
23-24	2000	400

Table S3. Scoring of AE.

Anaphylaxis was defined by Sampson in 2006 as the rapid appearance (minutes to hours) after exposure to a probable allergen **of symptoms indicative of an adverse effect on two organs.** (Sampson (HA & coll. Second symposium on the definition and management of anaphylaxis. J Allergy Clin Immunol 2006;117:391–7).

Skin: Pruritus or general rash, swollen lips, tongue or glottis

Respiratory: Dyspnoea, wheezing, laryngeal cough, diminished peak expiratory flow, hypoxemia

Digestive: Abdominal cramps, vomiting, diarrhoea

General: Blood pressure drop or association of symptoms of organ failure: hypotonia, fainting, incontinence.

The symptoms occurring during DBPCFC and during the build-up phases will be classified by the severity grades of Ring and Messmer (modified). (Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977; 1: 466–9).

Grade 1: Conjunctivitis, rhinitis, oral allergy syndrome, simple general urticaria, swollen lips and/or face with no symptoms of asphyxia (laboured breathing), cough or isolated wheezing, nausea or abdominal pain.

Grade 2: Moderate multi-system effects with signs on the skin and mucosae (angioedema) ± digestive signs (vomiting, diarrhea) ± asthma (acute bronchospasm): cough, dyspnoea, wheezing, drop in peak expiratory flow (15% or more of expected or known values) ± tiredness and tachycardia.

Grade 3a: Severe multi-system effects requiring specific therapy: bronchospasm or signs of laryngeal oedema with signs of asphyxia, anaphylaxis (symptoms of adverse effects on several organs, including respiratory symptoms).

Grade 3b: Anaphylactic shock (faintness, agitation, loss of consciousness, collapse), circulatory collapse, tachycardia or bradycardia, cardiac arrhythmia.

Grade 4: Cardiorespiratory arrest.

Severe adverse events (SAE) were defined as all life-threatening events i.e. grade 3b and grade 4 events, leading to patient withdrawal.

Table S4. Data at baseline

	Patient	SPT (mm)	Total IgE	Peanut Spe IgE	ara_h1 spe IgE	ara_h2 Spe IgE	ara_h3 spe IgE	ara_h8 spe IgE	ara_h9 spe IgE
Peanut	1	12	1253	335	78,8	148	11,8	6,65	0,11
	2	22	947	373	160	163	104	0,1	0,12
	3	35	1071	13,9	0,12	11,5	0,1	0,03	0,1
	4	10	416	99,7	28,8	162	1,11	0,1	0,1
	5	13	107	34,2	7,96	21	0,89	0,1	0,1
	6	17	427	65,8	12,5	53,7	1,3	0,1	0,1
	7	15	509	162	23,8	49,3	39,7	0,1	0,1
	8	8	100	47,4	19,1	21,4	7,8	0,1	0,1
	9	12	1101	205	86,3	72,1	23,9	0,1	0,13
	10	8	108	57,1	9,12	29,8	15,7	0,1	0,1
	11	5	225	33	6,02	37,4	0,86	0,1	0,1
	12	7	505	58,7	22,2	17,9	5,17	23,4	3,44
	13	12	903	473	52,7	280	159	0,1	0,1
	14	11	691	8,72	0,1	3,01	1,31	0,1	0,1
	15	15	1911	646	236	203	212	0,14	0,13
	16	14	75	30	6,15	20,7	1,59	0,1	0,41
	17	10	523	52,1	8,05	41,8	0,72	1,2	0,1
	18	10	841	595	205	250	292	0,1	0,1
	19	5	462	1,97	0,1	2,28	0,1	0,53	0,25
	20	8	389	40	7,42	37,2	0,12	0,83	0,1
Placebo	21	12	272	62,2	28,4	29,6	6,43	0,1	0,1
	22	11	2046	489	47,5	208	133	0,13	0,1
	23	26	83	36,5	1,49	29,2	0,54	0,1	0,1
	24	4	6121	301	23,1	215	26,6	128	3,78
	25	15	315	95,5	22,7	65,1	2,18	0,1	0,1
	26	5	216	50,8	30,5	28,8	4,15	1,27	0,1
	27	16	534	260	78,9	127	46,8	11,5	3,96
	28	15	636	181	25,5	90	8,73	14,2	1,28
	29	11	1336	29	0,53	25,5	0,15	31,2	0,24
	30	10	1340	457	77,6	417	35,4	0,16	0,1

Table S5. Results on efficacy: reactivity threshold at DBDPFC1 and DBDPFC2

		Peanut patients (n = 21)	Placebo patients (n = 9)	p
DBPCFC2 *	Negative	17 (81%)	1 (11%)	<0.001
	Positive	2 (9.5%)	8 (89%)	
DBPCFC1 median [Q25 Q75]		122 [122-400]	122 [62-222]	0.38
DBPCFC2 median; [Q25 Q75]		1800 [800-1800]	400 [222-400]	<0.001
<i>p (DBPCFC2/DBPCFC1)</i>		<i>p <0.001</i>	<i>p =0.02</i>	
4 fold increase		17	2	< 0.001

* = patients tolerating ingestion of 400 mg of peanut proteins