# Efficacy, safety and quality of life in a multi-center, randomized, placebo-controlled trial on low-dose peanut oral immunotherapy in peanut allergic children

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| **Abstract:** | Background: Only two, small placebo-controlled trials on peanut- oral immunotherapy (OIT) have been published. We examined the efficacy, safety, immunological parameters, quality of life (QoL) and burden of treatment (BoT) of low-dose peanut-OIT in a multicenter, double-blind, randomized placebo-controlled trial.<br>Methods: 62 children aged 3-17 years with IgE-mediated, challenge-proven peanut allergy were randomized (1:1) to receive peanut-OIT with a maintenance dose of 125-250 mg peanut protein or placebo. The primary outcome was the proportion of children tolerating ≥300 mg peanut protein at oral food challenge (OFC) after 16 months of OIT. We measured occurrence of adverse events (AEs), immunological changes, QoL prior and post OIT and BoT during OIT.<br>Results: 23/31 (74.2%) children of the active group tolerated at least 300 mg peanut protein at final OFC compared to 5 /31 (16.1%) in the placebo group (p<.001). 13/31 (41.9%) children of the active versus 1/31 (3.2%) of the placebo group tolerated the highest dose of 4.5 g peanut protein at final OFC (p<.001). There was no significant difference between the groups in the occurrence of AE-related drop-outs or in the number, severity and treatment of objective AEs. In the peanut-OIT group, we noted a significant reduction in peanut specific IL-4, IL-5, IL10 and IL-2 production by PBMCs compared to the placebo group, as well as a significant increase in peanut specific-IgG4 levels and a significant improvement of QoL. 86% of children evaluated the BoT positively.<br>Conclusion: Low-dose OIT is a promising, effective and safe treatment option for |

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| | peanut allergic children, leading to improvement of QoL, a low BoT and immunological changes showing tolerance development. |

# Efficacy, safety and quality of life in a multi-center, randomized, placebo-controlled trial on low-dose peanut oral immunotherapy in peanut allergic children

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## Abstract

**Background**: Only two, small placebo-controlled trials on peanut- oral immunotherapy (OIT) have been published. We examined the efficacy, safety, immunological parameters, quality of life (QoL) and burden of treatment (BoT) of low-dose peanut- OIT in a multicenter, double-blind, randomized placebo-controlled trial.

**Methods**: 62 children aged 3-17 years with IgE-mediated, challenge-proven peanut allergy were randomized (1:1) to receive peanut-OIT with a maintenance dose of 125-250 mg peanut protein or placebo. The primary outcome was the proportion of children tolerating ≥300 mg peanut protein at oral food challenge (OFC) after 16 months of OIT. We measured occurrence of adverse events (AEs), immunological changes, QoL prior and post OIT and BoT during OIT.

**Results**: 23/31 (74.2%) children of the active group tolerated at least 300 mg peanut protein at final OFC compared to 5 /31 (16.1%) in the placebo group ($p<.001$). 13/31 (41.9%) children of the active versus 1/31 (3.2%) of the placebo group tolerated the highest dose of 4.5 g peanut protein at final OFC ($p<.001$). There was no significant difference between the groups in the occurrence of AE-related drop-outs or in the number, severity and treatment of objective AEs. In the peanut-OIT group, we noted a significant reduction in peanut specific IL-4, IL-5, IL10 and IL-2 production by PBMCs compared to the placebo group, as well as a significant increase in peanut specific-IgG4 levels and a significant improvement of QoL. 86% of children evaluated the BoT positively.

**Conclusion**: Low-dose OIT is a promising, effective and safe treatment option for peanut allergic children, leading to improvement of QoL, a low BoT and immunological changes showing tolerance development.

## What is known already about the topic?

Only two, small placebo-controlled trials on peanut- oral immunotherapy using a relatively high maintenance dose of peanut protein have been published so far showing good efficacy. But safety concerns have been raised.

## What does this article add to our knowledge?

With this placebo controlled trial we could show that low-dose oral immunotherapy in peanut allergic children is effective, has an excellent safety profile, leads to improvement of quality of life, a low burden of treatment and immunological changes showing tolerance development.

## How does this study impact current management guideline?

Low-dose oral immunotherapy is effective and safe and thus might be a promising treatment option for peanut allergic children.

## Short title

Efficacy, safety and quality of life of low-dose peanut oral immunotherapy in a placebo-controlled trial

## Abbreviations

| | |
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| **AE** | Adverse event |
| **BoT** | Burden of treatment |
| **FAQLQ-PF/CF/TF** | Food Allergy Quality of life Questionnaire-Parent/Child/Teenage form |
| **GI** | Gastrointestinal |
| **HRQL** | Health-related quality of life |
| **IgE** | Immunoglobulin E |
| **IL-4** | Interleukin-4 |

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| **IQR** | Interquartile range |
| **IT** | Immunotherapy |
| **ITT** | Intention to treat |
| **MCID** | Minimum clinical important difference |
| **OAS** | Oral allergy syndrome |
| **OFC** | Oral food challenge |
| **OIT** | Oral immunotherapy |
| **PBMCs** | Peripheral blood mononuclear cells |
| **PP** | Per protocol |
| **QoL** | Quality of life |
| **SAE** | Severe adverse event |
| **SCORAD** | Scoring Atopic Dermatitis |
| **SPT** | Skin prick test |
| **Th2** | T helper 2 |
| **URI** | Upper respiratory infection |

## Keywords

Oral immunotherapy, tolerance, induction, children, peanut allergy, desensitization

## Introduction

Peanut allergy is a common disease in childhood with estimated prevalence rates ranging from 0.4% in Europe[1] to 3% in Australia[2]. Ingestion of only small quantities of the allergen may lead to potentially life-threatening allergic reactions[3]. Thus peanut is the most common allergen to induce food-induced anaphylaxis in childhood[4]. Patients are advised to strictly avoid peanut but accidental reactions are common due to widespread use of peanut in the food industry[5]. Thus, patients are also advised to carry self-administered epinephrine at all times. Overall, quality of life (QoL) in patients with peanut allergy is reduced[6, 7]. Therefore there is a need for an allergen-specific therapy in this group of patients.

Recent research has focused on the therapeutic option of oral allergen-specific immunotherapy. Published trials on peanut oral immunotherapy (OIT) have demonstrated clinical desensitization of most of the patients, although different doses for maintenance were used[8-18]. However, all trials were small and only two were placebo-controlled. Mild to moderate adverse reactions were reported in the majority of the patients. Some patients even suffered from anaphylactic reactions associated to OIT dosing. Although OIT seems an effective treatment option for peanut allergic patients, safety has to be evaluated more carefully.

Hypothetically, using a low maintenance dose and a long up-dosing period in peanut-OIT might lead to the same efficacy but better safety profile than using a higher maintenance dose for a shorter up-dosing period. The aim of this double-blind, placebo-controlled study was to assess efficacy for clinical desensitization and safety of OIT as well as possible changes in immunological parameters, in quality of life after OIT, and the burden of treatment in peanut allergic children using the lowest

161 maintenance dose so far reported. It is one of the first placebo-controlled peanut-OIT
162 trials where oral food challenges (OFC) were conducted prior and post OIT, where a
163 high enough top dose of peanut protein was included into the final OFC to define a
164 proper threshold after OIT in individual patients, where safety was assessed
165 thoroughly and the first where changes in quality of life and burden of treatment
166 (BoT) were investigated in a placebo-controlled way.
167

# Methods

## Study overview

This investigator-initiated, multicenter, double-blind, randomized, placebo-controlled, parallel-group trial was conducted at seven German sites (see online repository, **1.1**). We recruited patients consecutively in the outpatient clinics or from a list of peanut sensitized children followed within these tertiary care clinics. The study protocol and consent forms were approved by all ethics committees. All caregivers of the study participants gave written informed consent prior to the start of the study. The study was registered with the German Clinical Trials Register (DRKS00004553).

## Study population

Eligible patients were 3 to 17 years of age with a serum peanut-specific IgE >0.35 kU/l and challenge-proven clinically relevant peanut allergy. Parents of the patients had to be capable of understanding the proposed intervention of the study as well as being able to follow written emergency instructions. Patients were excluded if they had participated in another trial, if they were receiving any other form of immunotherapy including IT using inhalant allergens or if they suffered from a severe disease (e.g. uncontrolled asthma despite proper treatment). Children with controlled asthma or a history of severe allergic reaction (severity Grade V [19] like respiratory arrest, bradycardia, arterial hypotension, cardiac arrest or loss of consciousness) after peanut consumption were not excluded.

## Study endpoints

This study compared active peanut-OIT with placebo-OIT in children with peanut allergy. The primary endpoint was defined as the proportion of children tolerating a

single dose of ≥300 mg peanut protein at final OFC after a maximum of 14 months of up-dosing and two months of a maintenance phase of OIT in both groups. Secondary outcomes for efficacy were full clinical desensitization defined as the proportion of children tolerating the top, single dose of 4.5 g peanut protein at final OFC, median changes of the maximum tolerated single dose at initial and final OFC and comparison of the severity of reaction between initial and final OFC. Other secondary outcomes included safety measurements like severity and number of adverse events, number of accidental allergic reactions to peanut, change in the severity of other atopic diseases as well as changes in immunological parameters, quality of life and the burden of treatment.

**Randomization**

After initial OFC, study participants were randomly assigned (1:1) to the active or placebo group via block randomization with a size of 4 using Dat Inf, Rand List, version 1.2. A stratification for age (≤ or >6 years) and peanut-specific IgE (≤ or >50 kU/l) was performed by an independent statistician.

**Study design**

During the screening visit, the patient's history was obtained (doctor's diagnosed asthma, allergic rhinitis, atopic dermatitis and other primary food allergies), a physical examination and screening for peanut sensitization was conducted. After approximately eight weeks, children were admitted to our ward for an open oral peanut challenge (=initial OFC). After this OFC, patients were eligible to be randomized. OIT was started the next day on the ward. On the day of the initial OFC as well as on the day of final OFC - "post OIT" (after the maintenance phase of OIT) - patients received a physical examination including a SCORAD, a spirometry if

compliance allowed, a skin prick test (SPT) performed as a prick-to-prick test with the natural, roasted whole peanut, and blood samples for analysis of B-cell markers (peanut-, Ara h 2-, timothy-, birch-, mugwort-, dermatophagoides pteronyssinus-, cladosporium herbarum-, dog- and cat-specific IgE and peanut-specific IgG4 (CAP-System FEIA®, Thermo Fisher)) and T-cell cytokine production in cell culture supernatants (described in Blumchen et al[19]).

**Open oral peanut challenges (OFC)**

Prior to the start of OIT - at initial OFC - as well as after the maintenance phase at final OFC children received an open oral peanut challenge using a modified PRACTALL protocol[20] with 2-hour time intervals between dose steps as previously described[19]. In summary, patients received whole crushed roasted peanuts in boiled apple sauce as a matrix in increasing titration steps for a maximum of three days (first day: 3 mg - 10 mg - 30 mg - 100 mg, second day: 100 mg - 300 mg – 1,000 mg – 3,000 mg, third day: 4,500 mg peanut protein). The procedure was stopped if objective clinical symptoms were observed. This dose was considered to be the eliciting dose. The last single dose the patient tolerated just before the eliciting dose was defined as the maximum tolerated single dose.

**Procedures for OIT:**

Peanut flour (light roasted, 12% fat, 50% protein) from the Byrd Mill Company, Virginia, USA was used as the peanut protein source for OIT mixed in a vehicle of chocolate pudding for masking (see online repository, **1.2**). The placebo group received the vehicle without peanut flour. Patients received the first dose of peanut-/placebo-OIT on the ward. The starting dose of peanut- /placebo-OIT varied depending on the eliciting dose patients reacted to at initial OFC. If patients had an

eliciting dose of 3 mg, 10 mg, 30 mg, 100 mg or ≥300 mg peanut protein at initial OFC they started OIT on a dose of 0.5 mg, 1 mg, 3 mg, 10 mg or 30 mg peanut protein, respectively. The same OIT dose was administered again the next day. After two hours of monitoring, patients were instructed to take this dose daily, approximately at the same time. Up-dosings were planned every two weeks under medical monitoring in the outpatient clinics of the study centers (see online repository **TABLE E1)**. The up-dosing phase lasted a maximum of 14 months or shorter if the patients reached their individual planned maintenance dose. The planned final maintenance dose was determined by the eliciting dose patients had reacted to at initial OFC: Patients with an eliciting dose of 3 mg to 100 mg peanut protein at initial OFC were gradually increased to 125 mg whereas patients with an eliciting dose of 300 mg to 4,500 mg peanut protein were dosed up to 250 mg peanut protein as an OIT-maintenance dose. The maintenance phase lasted for 8 weeks (+/-2 weeks).

**Safety outcomes**

Adverse events (AE) were recorded daily by parents in a diary and were assessed every one to two weeks by the blinded study physician either during up-dosing visits or a telephone interview. AEs were recorded as possibly related or related to peanut-/ placebo-OIT if symptoms occurred within two hours after ingestion. AEs were also categorized as being either objective (e.g. hives, flush, angioedema, vomiting, diarrhea, conjunctivitis, rhinitis, sneezing, coughing, wheezing, shortness of breath) or subjective symptoms (e.g. pruritus, abdominal pain, nausea, oral itching, hawking, globus sensation or diverse symptoms (joint-, ear- and throat pain, headache, fever). Severity of possible allergic reactions was determined by the investigator using a modified grading system for food-induced anaphylaxis[19, 21]. As judged by the study physician, AEs were also categorized as being a possible allergic reaction after

accidental peanut exposure. By assessing the parents' diary, patient's spirometry, peak flow and SCORAD, the study physician determined whether an atopic comorbidity as asthma, allergic rhinitis and atopic dermatitis improved, worsened or remained stable during the study on the day of final OFC.

**Health-related quality of life (HRQL) and Burden of treatment (BoT)**

To measure changes in health-related quality of life (HRQL), the German translation of the Food Allergy Quality of Life Questionnaire was sent out to mothers (FAQLQ-PF[22], parental form, proxy measurement), children (FAQLQ-CF[23], child form) and teenagers (FAQLQ-TF[24], teenage form) 4 weeks before initial OFC and 4 weeks after final OFC (online repository **1.6**). For comparison of changes in HRQL before and after OIT in both study groups only complete data sets were considered for analysis (PP analysis). Results represent the median change in total score and each domain score for each study group prior and post OIT. The greater the negative change in score the better was the improvement of HRQL.

The BoT questionnaire was sent out to the families three to four months after starting OIT. Mothers of children (3-12 years), children (8-12 years) and teenagers (13-17 years) were asked to rate the advantages and disadvantages of OIT-treatment on a 7 point-scale ranging from 1 (=extremely positive) through 4 (=neutral) to 7 (=extremely negative)[25, 26]. Mothers and patients were also asked if they would perform OIT again. Results are presented for each treatment group as numbers of mothers or children who reported on a positive (score 1-3) or a negative BoT (score 4-7) and who would and would not perform OIT once more. HRQL- and BoT data of teenagers were not included in data analysis due to the small number of teenagers within the study.

298

299 **Statistical Analysis**

300 Values are expressed as median and interquartile ranges [IQR] unless otherwise
301 indicated, or counts and percentages as appropriate. For primary and secondary
302 endpoints, data are presented as either proportions or as the median change
303 between pre- and post-OIT values (median of post-OIT minus pre-OIT values). All
304 patients randomized were included for the analysis of the primary endpoint as the
305 intention to treat (ITT) population. For the robustness of the statistical analysis of the
306 primary endpoint a worst case analysis was also conducted where all drop outs of the
307 placebo group were considered to reach the primary endpoint and all drop outs of
308 active group were considered to fail the primary endpoint. Data of the primary
309 endpoint as well as all other secondary endpoints were also analyzed per protocol
310 (PP) including all patients who received the intervention and completed the final
311 OFC. Safety outcomes were analyzed from all patients within the ITT population
312 receiving at least one dose of placebo-/peanut-OIT, also including all drop out-
313 patients until the time they discontinued the study. Group comparisons between
314 randomization arms of continuous variables were performed using the Kruskal-Wallis
315 test. The primary endpoint and other categorical variables were compared between
316 randomization arms using the chi-squared test for contingency tables (Fisher exact
317 test). All statistical tests were 2-tailed, and a two-sided p-value of .05 was considered
318 for significance. The statistical analyses were performed using R version 2.5.1
319 (http://www.r-project.org, library Design, Hmisc, ROCR) and Statistical Package for
320 the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

321

# Results

## Study population

Of 186 children with suspected peanut allergy approached for the study, 119 refused to participate, four were tolerant to peanut at initial OFC and the youngest patient vigorously refused to eat the vehicle (chocolate pudding) (see **FIG 1**, CONSORT flow diagram). Thus sixty-two participants with a median age of 6.8 years (range: 3.2 to 17.8 years), median peanut-specific IgE of 81.5 kU/l (range: 0.57-624 kU/l), median Ara h 2-specific IgE of 44.7 kU/l (range: 0.04- 256kU/l) and median maximum tolerated single dose at initial OFC of 30 mg peanut protein (range: 1-3,000 mg) were randomized to receive either active, peanut-OIT (n=31) or placebo-OIT (n=31). Ten of 62 patients discontinued during the study (see CONSORT diagram): One patient of the peanut-OIT group withdrew consent after randomization but before receiving the allocated intervention. This patient was still included in the ITT- analysis (n=62 in ITT). See **FIGURE 1** and the online repository (2.1) for further explanations of all drop-outs. There were no significant differences between the peanut-OIT and the placebo group in demographical and immunological baseline characteristics (**TABLE I**).

## Efficacy

After a median of 13 months [10-14 months] of the up-dosing and 9.5 weeks [8.5-11.4 weeks] of the maintenance phase, 24 patients of the placebo-OIT and 28 patients of the peanut-OIT group finished the study with a final OFC. 50% in each randomization group reached their planned maintenance dose. The median maintenance dose was 125mg peanut protein [50-250mg] in the peanut-OIT and “125mg placebo” [31.3-225mg] in the placebo-OIT group. Within the ITT population 23 of 31 patients (74.2%) of the peanut-OIT group tolerated ≥300 mg peanut protein

whereas only 5 of 31 patients (16.1%) within the placebo-OIT group tolerated this dose at final OFC (p<.001) (**TABLE II, FIG 2**). Also in the worst-case analysis (p=0.01) as well as in the per protocol analysis (p<.001) the primary endpoint was met (**TABLE II**). As a secondary endpoint, 13 patients of the peanut-OIT group (41.9% of the ITT population) tolerated the maximum dose of 4.5 g peanut protein at final OFC compared to only one patient (3.2%) within the placebo-OIT group (p<.001). With a median of 20 mg [10-100] peanut protein, the maximum tolerated single dose at final OFC remained unchanged (fold change=1, [0.33-4.3]) when compared to the median maximum tolerated single dose at initial OFC (30 mg [8.3-100] peanut protein) within the placebo group. In comparison, the maximum tolerated single dose increased by a factor of 12.1 [4.3-97] from a median of 30 mg [10-300] to a median of 1,000 mg [825-4,500] peanut protein at final OFC within the peanut-OIT group.

Within the first six of eight dose steps of the final OFC the patients of the placebo group experienced more and more severe reactions than the peanut-OIT group (**FIG3**). However, comparing the number of grade IV reactions during all dose steps (3 mg to 4.5 g peanut protein) at final OFC, there was no difference between the peanut-OIT (n=7) and placebo group (n=7) (**FIG 3**).

**Safety**

Two patients in each group discontinued due to adverse events (6.5% of the total ITT population), one of these patients in each group due to a severe adverse event (SAE) being judged to be related to the OIT dose (**FIG 1**, for details see online repository **2.1** and **TABLE E2**).

All patients suffered from adverse events (AEs). But only a small number of all placebo-OIT doses (1.2%) and 4.3% of all peanut-OIT doses were associated with AEs (=AEs related to OIT = occurring within two hours after OIT-ingestion, **TABLE E2**). There was a significantly higher proportion of OIT doses associated with AEs in the peanut-OIT group than in the placebo-OIT group (p= .001) mainly due to a significantly higher number of mild, subjective AEs related to peanut-OIT (**TABLE E2**). Thus, significantly more patients of the peanut-OIT group (83%) suffered from subjective AEs related to OIT than patients of the placebo-group (45%) (p= .002). Especially subjective symptoms like tingling in the mouth, globus sensation, hawking, and abdominal pain were reported in a significantly higher number in patients receiving peanut-OIT than in those receiving placebo (**TABLE III** and **E2**). None of the subjective symptoms related to OIT had to be treated.

More than half of the patients in both groups suffered from objective symptoms within two hours after ingestion of OIT (**TABLE III**). However, less than 1% of all OIT doses were associated with objective symptoms related to OIT (**TABLE E2**), mainly skin symptoms (hives and angioedema), vomiting, diarrhea and coughing. There was no significant difference in the number of OIT doses associated with objective AEs, the number of patients suffering from objective AEs related to OIT, the severity or the treatment of these symptoms between randomization groups (**TABLE III and E2**).

Regarding individual objective symptoms, wheezing was the only symptom related to OIT reported significantly more often in the peanut-OIT group (in all eight times by 6 patients) than in the placebo-OIT group (once by one patient, p=0.045) (**TABLE III and E2**). However, there was no difference between groups concerning all other individual symptoms, e.g. coughing or shortness of breath (**TABLE III and E2**). There

were more OIT doses associated with objective AEs during the up-dosing phase than during maintenance, but to a similar extent in both groups (**TABLE E2**). Dose reductions due to AEs could be a sign of more severe AEs during OIT. However, 12/30 patients within the placebo-OIT group (40%) and 14/31 patients within the peanut-OIT group (45%) needed at least one dose reduction due to AEs during the course of OIT.

Five patients within the placebo-OIT and three patients within the peanut-OIT group experienced an SAE (**TABLE III and E3**). In each group, one patient suffered from an SAE related to OIT and leading to study discontinuation as mentioned in more detail in the online repository.

Within the placebo group, 14 patients experienced 24 allergic reactions which were considered to be caused by an accidental ingestion of peanut. In contrast, only five patients of the peanut-OIT group experienced eight accidental reactions ($p<.001$, **TABLE III**).

After the course of OIT, no difference was found concerning the number of patients in the two groups with newly diagnosed atopic diseases (bronchial asthma, atopic dermatitis, allergic rhinoconjunctivitis), with new sensitization to one of the inhalant allergens tested or with worsening of established atopic diseases at baseline (**TABLE III**).

**Immunological parameters**

There were no significant differences between the peanut and placebo OIT groups concerning the baseline levels of immunological parameters (**TABLE I, FIG 4**).

Comparing immunological markers within one randomization arm before and after OIT, a significant reduction in the wheal size of the peanut SPT, peanut specific IL-4, IL-5, IL10, IL-2, IFN-γ and TNF-α production by PBMCs and a significant increase in peanut specific-IgG4 levels and decrease of the ratio of peanut specific-IgE to IgG4 could be noted for the peanut-OIT group but not for the placebo-OIT group (**FIG 4**). There was no significant change pre/post OIT within one randomization arm for peanut - and Ara h 2-specific IgE. When comparing the median changes from baseline between randomization arms, a significant increase in peanut specific-IgG4 and decrease of the ratio of peanut specific-IgE/IgG4 as well as a significant reduction in IL-4-, IL-5-, IL-10- and IL-2- production could be demonstrated for the peanut-OIT group in comparison to the placebo-OIT group (**FIG 4**). There were no significant differences between groups for the changes of wheal size of peanut SPT, peanut specific- IgE, Ara h 2-IgE, IFN-γ- and TNF α-production (**FIG 4**).

**Health-related quality of life (HRQL) and Burden of treatment (BoT)**

Before start of OIT, baseline HRQL did not differ between the placebo and active group in all domains except for the domain of “risk of accidental exposure” in children (online repository **TABLE E4**). After final OFC, mothers of both study groups (n=38) filled out the FAQLQ-PF after a median of 9.5 weeks (IQR [5-15.3]), children (n=17) filled out the FAQLQ-CF after a median of 11 weeks (IQR [7-16]) after final OFC. Taking a minimum clinically important difference (MCID) of 0.5 for a significant clinical improvement of HRQL after an intervention[27] mothers of the peanut-OIT but not the placebo OIT group reported a median improvement of HRQL of greater than 0.5 in score within the domain of “social and dietary limitations” post OIT (**FIG 5**). There was no meaningful median change in HRQL reported by mothers within the total score and the domains of emotional impact and food-related anxiety in the

placebo group or the peanut-OIT group (**FIG 5**). However, comparing the placebo and active group there was no significant group difference in median change in HRQL for all domains of the FA-QLQ-PF reported by mothers post OIT.

When children reported on a possible change in HRQL pre and post OIT (**FIG 5**) the median improvement for the total score as well as for each domain of the FAQLQ-CF exceeded the MCID of 0.5 in the peanut-OIT group. Within the placebo group, median changes ranged between 0 and 0.25. By group comparison, children of the peanut-OIT group reported a statistically significant improvement in HRQL within the two domains of “risk of accidental exposure” and “emotional impact” when compared to the placebo group.

BoT measures during OIT could be analyzed from 50 of 56 mothers and 21 of 23 children, which were answered a median of 20.5 weeks (IQR [19-23]) after starting OIT. 22/27 mothers (82%) of the peanut-OIT group and all mothers of the placebo group (n= 23) reported a positive BoT (=BoT score 1-3). Only one mother from the peanut-OIT group (3.7%) and two from the placebo group (8.7%) would not perform OIT again. Nine of eleven children of the active group (82%) and 9/10 children of the placebo group (90%) were positive about their treatment. One child of each group spoke against performing OIT again.

# Discussion

## Efficacy

This study is the first study to target highly sensitive peanut-allergic patients with a low-dose peanut OIT in a randomized, placebo-controlled fashion showing a good efficacy for clinical desensitization, an excellent safety profile, a prevention of accidental reactions, immunomodulatory capacity, improvement of HRQL and a low BoT. Efficacy was highly significant with 74% of the active group meeting the primary endpoint in tolerating a dose of at least 300 mg peanut protein at final challenge in contrast to only 16% of the placebo group. For the first time ever, we report also on a significant reduction in the number of accidental reactions during OIT within the active group (n=8) vs the placebo group (n=24).

Even with a slow, long-term up-dosing period (median 13 months) and a low maintenance dose (median 125 mg peanut protein), efficacy in this placebo-controlled trial on peanut-OIT is similar to other studies on OIT using higher maintenance doses and a shorter up-dosing period, challenging the hypothesis that a higher maintenance dose may lead to better efficacy. Comparing efficacy for desensitization in studies on peanut-OIT is difficult because of the variations in recruited study populations, maintenance doses, duration of up-dosing and maintenance, and the definition of the endpoint for desensitization.

However, our result of the primary endpoint is almost equal to two recently published trials on peanut OIT which recruited a similar risk group of highly peanut allergic children of a similar age and degree of sensitization, also including children with a history of anaphylaxis[15, 18]. Using a higher maintenance dose of 300 mg peanut protein than in our study, Bird et al reported on 79% of patients within the active

group tolerating at least 300 mg peanut protein at final challenge in comparison to 19% of the placebo group[18]. Choosing an even higher maintenance dose of 800 mg peanut protein, Kukkonen et al were able to demonstrate in a non-controlled trial that 67% of the children in their peanut-OIT group tolerated a maximum cumulative dose of 1,255 mg peanut protein at final challenge[15] which might be comparable to our results with 68% of the active group tolerating a cumulative dose of 1,443 mg peanut protein (data not shown) at final OFC. Recently published in a direct comparison, Vickery et al also demonstrated that using a very high maintenance dose (e.g. 3,000 mg peanut protein) does not lead to a better efficacy than using a lower maintenance of 300 mg[16].

Interestingly, in this study we could also show that even a lower maintenance dose than the planned one of 125mg/250mg peanut protein lead to a reasonable efficacy: Fourteen of the active patients did not reach their planned maintenance dose but had a median maintenance dose of 50mg peanut protein (range: 2.5- 225mg peanut protein). Nine of these fourteen (64%) tolerated at least 300mg peanut protein at final OFC. In contrast, only one of twelve patients of the placebo group who did not reach their planned maintenance dose reached the primary endpoint with a median maintenance dose of “32.5mg peanut protein” (**FIG E2**).

In choosing the dose of at least 300 mg peanut protein to be tolerated at final OFC as the primary endpoint we aimed for the protection from severe allergic reactions to accidental ingestion to peanut in most of the patients within the active group, post OIT. Recently, Baumert et al demonstrated in a model for quantitative risk assessment that an increase in the eliciting dose to ≥300 mg peanut protein post OIT or even more - as in our case to ≥1,000mg as the eliciting dose - would lead to a

significantly higher and clinically meaningful reduction in the risk of experiencing an accidental allergic reaction after eating snack chips mixes, cookies, doughnuts or ice cream in peanut allergic patients[28]. Our results strengthen this risk assessment. This is the first report directly demonstrating a protection from accidental reaction by OIT with a significant reduction in number of accidental reactions within the peanut-OIT group in comparison to the placebo group (**TABLE III**). Thus we could demonstrate that low-dose OIT clinically desensitizes most of the peanut allergic patients to an extent that they are protected from severe allergic reaction after unintended exposure.

This study included fourteen patients who tolerated ≥300mg peanut protein at initial OFC (**TABLE E5**). Although receiving a low maintenance dose of only 225- 250mg peanut protein, this group of patients also seemed to profit from OIT. Eighty percent of the patients of the active group with a maximum tolerated dose of 300mg peanut protein and 100% of the active patients tolerating 1,000mg or 3,000mg peanut protein at initial OFC passed the final OFC with a maximum dose of 4,500mg peanut protein. Immunological modulation and a reduction of accidental reactions seemed to occur in the active treated patients. More moderate AEs related to OIT like wheezing seemed to be a rare event. These results generate the hypothesis that this group of patients might be a good target population for peanut OIT outside of specialized OIT centers. But further studies with a larger population with this kind of patients have to confirm this hypothesis.

**Safety**

Similar to other published studies on peanut-OIT[8, 10, 11, 29] including the two placebo-controlled trials[17, 18], 90% of the patients of the active group suffered from AEs related to OIT, mainly mild to moderate in severity (**Table III**). Also, the majority of these symptoms were of subjective nature. About two thirds of patients in the peanut-OIT group suffered at least once from symptoms of the oral cavity and/or abdominal pain. However, more than three-quarters of the placebo-group (77%) also experienced treatment related AEs.

Comparing the active and placebo groups, there was no difference in the number of drop outs due to AEs, occurrence of SAEs and occurrence of objective, OIT-related AEs, in severity of symptoms, treatment of symptoms, or worsening of preexisting atopic diseases. The only, highly significant difference between the groups could be demonstrated for the two subjective symptoms of OAS and abdominal pain. “Wheeze” was the only objective, OIT-related symptom which occurred significantly more often in the active group versus the placebo group in this study. But with only a lower significance (p=.04) the clinical significance is debatable since there was no difference when treatment with salbutamol was analyzed in both groups (**TABLE III and E2**).

Our excellent safety profile might result from the slow up-dosing and the low maintenance dose used in this protocol. Looking at the proportion of drop outs (13-21%) in other studies recruiting a similar study population but using faster up-dosing and a higher maintenance dose[14, 15, 18] the proportion of drop outs due to AEs (6.7%) in this study is much lower. There was no need of epinephrine treatment for AEs related to OIT and absence of development of eosinophilic oesophagitis. Moreover, antihistamine and steroid treatment was lower than previously reported by others[15].

577

578 **Immunological changes**

579 Similar to results published previously, we were able to demonstrate a reduction of
580 peanut SPT and a marked increase in peanut specific IgG4 post OIT in comparison
581 to the placebo group[8, 16-18]. Uniquely, like in our pilot trial[8] but now shown for the first
582 time in comparison to a placebo-group, we again found not only an *in vitro* peanut-
583 specific suppression of Th-2 cytokines such as IL-4 and IL-5 but also a general
584 suppression of cytokine production for IL-2, IL-10, IFN-γ and TNF-α in the peanut-
585 OIT group post OIT. No change was noted in the placebo-OIT group. Similar results
586 for the possible induction of anergy but not for a shift to Th1 cytokine upregulation
587 were also reported by Gorelik et al.[30]. They demonstrated a reduction of IL-5-, IL-13,
588 but also of IFN-γ-, IL-10- and TNF-α-production of CD4+ T cells co-cultured with
589 myeloid dendritic cells after 12 months of a maintenance peanut OIT with 2 g of
590 peanut protein ingested daily.

591

592 **Health-related quality of life (HRQL) and Burden of treatment (BoT)**

593 After peanut-OIT there was a significant improvement in HRQL (decrease in score)
594 for the domain of “risk of accidental exposure” and “emotional impact” in children
595 when compared to the placebo group approximately 11 weeks after final OFC. If one
596 considers an improvement of >0.5 MCID as significant[27], the HRQL even improved
597 for all domains in children and for the domain of “social and dietary limitations” in
598 mothers’ proxy reports of the active group. This is the first trial on OIT showing a
599 significant improvement of HRQL after OIT in a placebo-controlled study design. Two
600 previously published studies on peanut-OIT showed a significant improvement of
601 HRQL post OIT in parents’ proxy reports[13, 31] and in children and teenagers reports[31]
602 using the same questionnaires but not comparing their results to a control group.

To our knowledge, this is the first time that BoT has been analyzed for an OIT study. Although patients and parents had to cope with a daily therapy which might have also elicited disgust, AEs and included also a daily two-hour interval of parental monitoring of their children, the majority of mothers and children reported (after a median of four months on OIT) being positive (=low BoT) about this treatment and would start this kind of therapy again.

**Limitations of this study**

Since an unblinded OFC protocol was used in this study overreporting of allergic reactions during baseline OFCs and underreporting at final OFCs due to change of attitude of the children towards peanut ingestions might have occurred. However, efficacy results are so robust and similar to other efficacy data on peanut-OIT published so far[15, 18] this effect seems marginal. Additionally, the OFC protocol used in this study- with a two-hour interval between dose steps[19]- differs from other OFC protocols used in OIT trials possibly changing the sensitivity of threshold and severity of reactions during OFC which might impact the efficacy data of this trial. However, the eliciting dose for peanut-induced allergic reactions in 5% of this study population ($ED_{05}$)[19] is comparable to the $ED_{05}$ of other published peanut allergic populations being challenged with 15 to 30 minute intervals[32-34]. Therefore the sensitivity for threshold might not be too different to other published studies on peanut OIT. Due to differences in the reporting of the severity of reaction during OFC the data of this current study cannot be compared to others. Therefore it might well be that due to a two hour interval between dose steps more severe reactions could have been avoided.

**Summary**

In conclusion, we have been able to demonstrate for the first time in a placebo-controlled way that using a low maintenance dose in peanut OIT has a very good safety profile with an efficacy similar to that reported by other studies using higher maintenance doses. Treatment with low-dose peanut-OIT leads to immunological changes, pointing to the possible development of immunological anergy due to OIT. Despite daily treatment and daily monitoring for two hours, children showed a significant improvement in HRQL post OIT, which was demonstrated here for the first time in a placebo-controlled manner. Furthermore, overall BoT seems to be very low for this kind of therapy. However, further placebo-controlled, long-term studies with a larger number of patients, especially including more teenagers, are needed to verify the reduction of allergic reaction after accidental exposure due to OIT and to further evaluate safety.

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655

## TABLE I Baseline characteristics of study participants

| | | placebo-OIT (n=31) | peanut-OIT (n=31) |
|---|---|---|---|
| **Age (years), median [IQR]** | | 7.9 [4.6-10.7] | 6.6 [4.8-9.8] |
| **Sex, male, n (%)** | | 19 (61.3) | 19 (61.3) |
| **Weight (kg), median [IQR]** | | 22.4 [18.2-36.4] | 24.0 [18.7-31.8] |
| **Positive family history of atopy, n (%)** | | 29 (93.5) | 26 (83.9) |
| **Asthma/ increased airway reactivity, n (%)** | | 20 (64.5) | 13 (41.9) |
| **Atopic dermatitis, n (%)** | | 22 (71) | 19 (61.3) |
| **Allergic rhinitis, n (%)** | | 18 (58.1) | 14 (45.2) |
| **Further systemic food allergies[+], n (%)** | | 12 (38.7) | 9 (29) |
| **History for accidental allergic reaction to peanut and to unknown cause, n (%)** | | 29 (93.5) | 31 (100) |
| **History of accidental allergic reaction to peanut and to unknown cause with severity grade ≥ IV [++] n (%)** | | 16 (51.6) | 18 (58.1) |
| **Eliciting single dose of peanut protein (mg) at initial OFC, median [IQR]** | | 100 [10-200] | 100 [30-1000] |
| **Cumulative eliciting dose (mg peanut protein) consumed at day of allergic reaction of OFC, median [IQR]** | | 143 [13-272] | 143 [43-1400] |
| **Maximum tolerated single dose of peanut protein (mg) at initial OFC, median [IQR]** | | 30 [3-65] | 30 [10-300] |
| **Patients tolerating ≥ 300 mg peanut protein at initial OFC[+++], n (%)** | | 4 (12.9) | 10 (32.3) |
| **Severity[++] of reaction at OFC, n (%):** | **Grade I** | 1 (3.2) | 1 (3.2) |
| | **Grade II** | 13 (41.9) | 11 (35.5) |
| | **Grade III** | 11 (35.5) | 10 (32.3) |
| | **Grade IV** | 6 (19.4) | 9 (29) |
| **Peanut SPT (mm), median [IQR]** | | 8 [7-9.8] | 8 [6.5-9.5] |
| **Total IgE (kU/l), median [IQR]** | | 434 [267-758] | 347 [193-766.5] |
| **Peanut specific-IgE (kU/l), median [IQR]** | | 73.1 [31.3-197] | 89.5 [6.9-217] |
| **Ara h 2 specific-IgE (kU/l), median [IQR]** | | 48.8 [20.5-85.7] | 44.6 [6.4-99.7] |
| **Peanut specific-IgG4 (mgA/l), median [IQR]** | | 0.38 [0.15-0.97] | 0.63 [0.18-0.89] |

Continuous values are presented as medians with interquartile range. Kruskal-Wallis test was used for statistical analysis. Categorical variables are presented as number of participants and percentage using the chi-squared test for statistical analysis.

[+]defined as either historical or challenge proven systemic reaction to food allergens other than peanut,

[++] for detailed definition of grading the severity of allergic reactions see Blumchen et al.[19],

666 [+++] number of patients with an eliciting single dose of 1 g, 3 g or 4.5 g peanut protein

667 at initial OFC

668

669 **TABLE II: Clinical efficacy endpoints**

| | placebo-OIT | peanut-OIT | p values |
|---|---|---|---|
| **Primary endpoint:** | | | |
| **Primary endpoint: Intention to treat analysis** | **n=31** | **n=31** | |
| **Patients tolerating ≥300 mg peanut protein at final OFC, n (%)** | 5 (16.1) | 23 (74.2) | **<.001** |
| **Patients newly tolerating ≥300 mg peanut protein at final OFC*, n (%)** | 1 (3.2) | 13 (41.9) | **<.001** |
| **Primary endpoint : Worst case analysis** | **n=31** | **n=31** | |
| **Patients tolerating ≥300 mg peanut protein at final OFC, n (%)** | 12 (38.7) | 23 (74.2) | **.01** |
| **Primary endpoint : Per protocol analysis** | **n=24** | **n=28** | |
| **Patients tolerating ≥300 mg peanut protein at final OFC, n (%)** | 5 (20.8) | 23 (82.1) | **<.001** |
| **Secondary endpoints:** | | | |
| **Secondary endpoint: Intention to treat analysis** | **n=31** | **n=31** | |
| **Patients tolerating the maximum dose of 4,500 mg peanut protein at final OFC, n (%)** | 1 (3.2) | 13 (41.9) | **<.001** |
| **Secondary endpoint: Per protocol analysis** | **n=24** | **n=28** | |
| **Patients tolerating the maximum dose of 4,500 mg peanut protein at final OFC, n (%)** | 1 (4.2) | 13 (46.4) | **.002** |
| **Maximum tolerated single dose of peanut protein (mg) at final OFC, median [IQR]** | 20 [10-100] | 1000 [825-4500] | **<.001** |
| **Change in maximum tolerated single dose of peanut protein (mg) at final OFC, median [IQR]** | 0 [-7.5-13.5] | 997 [592.5- 4200] | **<.001** |

Data presents the primary and secondary clinical endpoints within the intention to treat (ITT) and per protocol (PP) population. For primary and secondary clinical endpoints data is presented as proportions of patients within one randomization arm at final OFC post OIT and was statistically analyzed by using the chi-squared test for contingency tables (Fisher exact test). Within the PP population the median change of maximum tolerated peanut dose between initial OFC and final OFC was calculated and statistically analyzed using the Kruskal-Wallis test. For analysis of the primary endpoint a worst-case imputation was also used. * Number of patients who did not tolerate ≥ 300 mg peanut protein (single eliciting dose of 3-300 mg) at initial OFC but tolerated it at final OFC.

682 **TABLE III: Patients with adverse events (AEs) related to OIT in the placebo-OIT**

683 **and peanut-OIT group**

| | placebo-OIT n=31 | peanut-OIT n=30 | p values |
|---|---|---|---|
| **Total number of adverse events (AEs), n=(%*)** | 2,866 (20.7) | 2,515 (20.3) | .71 |
| **Total number of severe adverse events (SAEs), n=(%*)** | 5 (0.04) | 3 (0.02) | .73 |
| **Number of SAEs related to OIT, n=(%*)** | 1 (0.007) | 1 (0.008) | 1.0 |
| **Number of patients who discontinued the study due to AEs, n=(%**)** | 2(6.5) | 2 (6.7) | 1.0 |
| **Number of patients with…../ receiving…….** | | | |
| **AEs related to OIT, n= (%**)** | 24 (77.4) | 27 (90) | .3 |
| **subjective AEs related to OIT, n= (%**)** | 14 (45.2) | 25 (83.3) | **.002** |
| OAS related to OIT, n= (%**) | 8 (25.8) | 18 (60) | **.007** |
| abdominal pain related to OIT, n= (%**) | 6 (19.4) | 20 (66.7) | **<.001** |
| nausea related to OIT, n= (%**) | 2 (6.5) | 7 (23.3) | .06 |
| skin itching related to OIT, n= (%**) | 7 (22.6) | 7 (23.3) | .94 |
| joint pain/ headache/ throat pain related to OIT, n= (%**) | 2 (6.5) | 6 (20) | .12 |
| **objective AEs related to OIT, n= (%**)** | 21 (67.7) | 19 (63.3) | .72 |
| skin symptoms related to OIT (contact urticarial, flush, generalized hives, angioedema), n= (%**) | 8 (25.8) | 12 (40) | .24 |
| GI symptoms related to OIT (vomiting, diarrhea), n= (%**) | 7(22.6) | 8 (26.7) | .71 |
| URT symptoms related to OIT (conjunctivitis, rhinitis, sneezing, rhinoconjunctivitis), n= (%**) | 10 (32.3) | 9 (30) | .85 |
| laryngeal symptoms related to OIT (hoarseness, stridor), n= (%**) | 1 (3.2) | 1 (3.3) | .98 |
| lower respiratory tract symptoms related to OIT (coughing, wheezing, shortness of breath), n= (%**) | 9 (29) | 13 (43.3) | .25 |
| coughing related to OIT, n= (%**) | 6 (19.4) | 11 (36.7) | .13 |
| wheezing related to OIT, n= (%**) | 1 (3.2) | 6 (20) | **.04** |
| shortness of breath related to OIT, n= (%**) | 4 (12.9) | 3 (10) | .72 |
| cardio-vascular symptoms (drop in blood pressure, unconsciousness) related to OIT, n= (%**) | 1 (3.2) | 0 (0) | 1.0 |

| | | | |
|---|---|---|---|
| **AEs related to OIT of severity grade I, n= (%**)** | 5 (16.1) | 7 (23.3) | .48 |
| **AEs related to OIT of severity grade II, n= (%**)** | 10 (32.2) | 11 (36.7) | .72 |
| **AEs related to OIT of severity grade III, n= (%**)** | 13 (41.9) | 10 (33.3) | .49 |
| **AEs related to OIT of severity grade IV, n= (%**)** | 4 (12.9) | 7 (23.3) | .29 |
| **AEs related to OIT of severity grade V, n= (%**)** | 1 (3.2) | 0 (0) | .32 |
| **Treatment for AEs related to OIT, n= (%**)** | 9 (29) | 12 (40) | .37 |
| **Systemic antihistamines for AEs related to OIT, n= (%**)** | 6 (19.4) | 8 (26.7) | .55 |
| **Systemic steroids for AEs related to OIT, n= (%**)** | 4 (12.9) | 4 (13.3) | 1.0 |
| **Inhalant salbutamol for AEs related to OIT, n= (%**)** | 5 (16.1) | 6 (20) | .69 |
| **Adrenalin for AEs related to OIT, n= (%**)** | 0 (0) | 0(0) | 1.0 |
| **New sensitization to inhalant allergens post OIT, n=** | (n=24)<br>10 | (n=28)<br>11 | 1.0 |
| **New diagnosed atopic diseases post OIT, n=** | (n=24)<br>4 | (n=28)<br>5 | 1.0 |
| **Worsening of atopic diseases post OIT, n=** | (n=24)<br>8 | (n=28)<br>4 | 0.1 |
| **Accidental reactions, total n= (average per person)** | 24 (0.77) | 8 (0.27) | **<.001** |
| **Number of patients with accidental reactions, n= (%**)** | 14 (45.2) | 5 (16.7) | **.026** |

Data presents the occurrence of adverse events and the number of patients with AEs related to OIT during the study in both randomization groups. Severity was graded using a modified grading system for food-induced anaphylaxis[19, 21].

Adverse event (AE), Severe Adverse Event (SAE), Oral allergy syndrome (OAS), GI (Gastro-intestinal), Upper respiratory tract (URT).

*% of all OIT doses within randomization group, ** % of all patients within the randomization group.

693 ## Figure Legends

694

695 **Fig 1 CONSORT diagram.**

696

697 **Fig 2 Maximum tolerated single dose of peanut protein prior and post OIT.**
698 Shown are the maximum tolerated single doses of peanut protein at initial and final
699 OFC in (**A**) individual placebo-OIT patients and (**B**) peanut-OIT patients of the per
700 protocol population. The horizontal lines represent the median of the maximum
701 tolerated single dose in each group. For the statistical analysis of the comparison of
702 data pre and post treatment within one randomization group, the Wilcoxon-test was
703 used. **p<.01

704

705 **Fig 3 Grade of severity of allergic reactions during final OFC at individual dose**
706 **steps.** Shown are the proportions of patients of (**A**) the placebo-OIT and (**B**) the
707 peanut-OIT group with their individual severity of symptoms at each dose step during
708 final OFC within the per protocol population. Severity was graded using a modified
709 grading system for food-induced anaphylaxis[19, 21].
710 OFC, oral food challenge

711

712 **Fig 4 Immunological changes from baseline (pre OIT) to post OIT at final OFC**
713 **of the per protocol population.** Shown are wheal size diameter of peanut SPT (A),
714 peanut specific-IgE (B), Ara h 2 specific-IgE (C), peanut specific-IgG4 (D), ratio of
715 peanut specific-IgE/peanut specific-IgG4 (E), IL-4- (F), IL-5- (G), IL-10- (H), IL-2- (I),
716 IFN-γ- (J) and TNF-α production (K) after *in vitro* stimulation of PBMCs with peanut
717 extract minus the amount of cytokine production after stimulation with medium. Black
718 lines represent median values. Black circles/ squares represent patients tolerating a

maximum dose of up to 100mg, orange circles/squares represent patients tolerating a maximum dose of 300 to 3,000mg and green circles/squares represent patients tolerating a maximum dose of 4,500mg peanut protein at final OFC. For the statistical analysis of the comparison of data pre and post treatment within one randomization group the Wilcoxon-test was used. For the intergroup comparison the median changes from baseline in each group were calculated and analyzed by the Kruskal-Wallis test (*p<.05; **p<.01; ***p<.001).

**FIGURE 5 Change in HRQL after OIT.** Presented is the median change in total and each domain score for the FA-QLQ CF (child form) and FA-QLQ PF (parent form) for each study group after OIT in the per protocol population. Open symbols represent the placebo group, filled symbols the peanut-OIT group, dotted the minimum clinical important difference (MCID). The greater the negative change in score the better is the improvement of HRQL. The Kruskal-Wallis test was used for a group comparison. p= statistical significance, bold values represent a significant change in HRQL after OIT when placebo and active group are compared.

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Figure No.1

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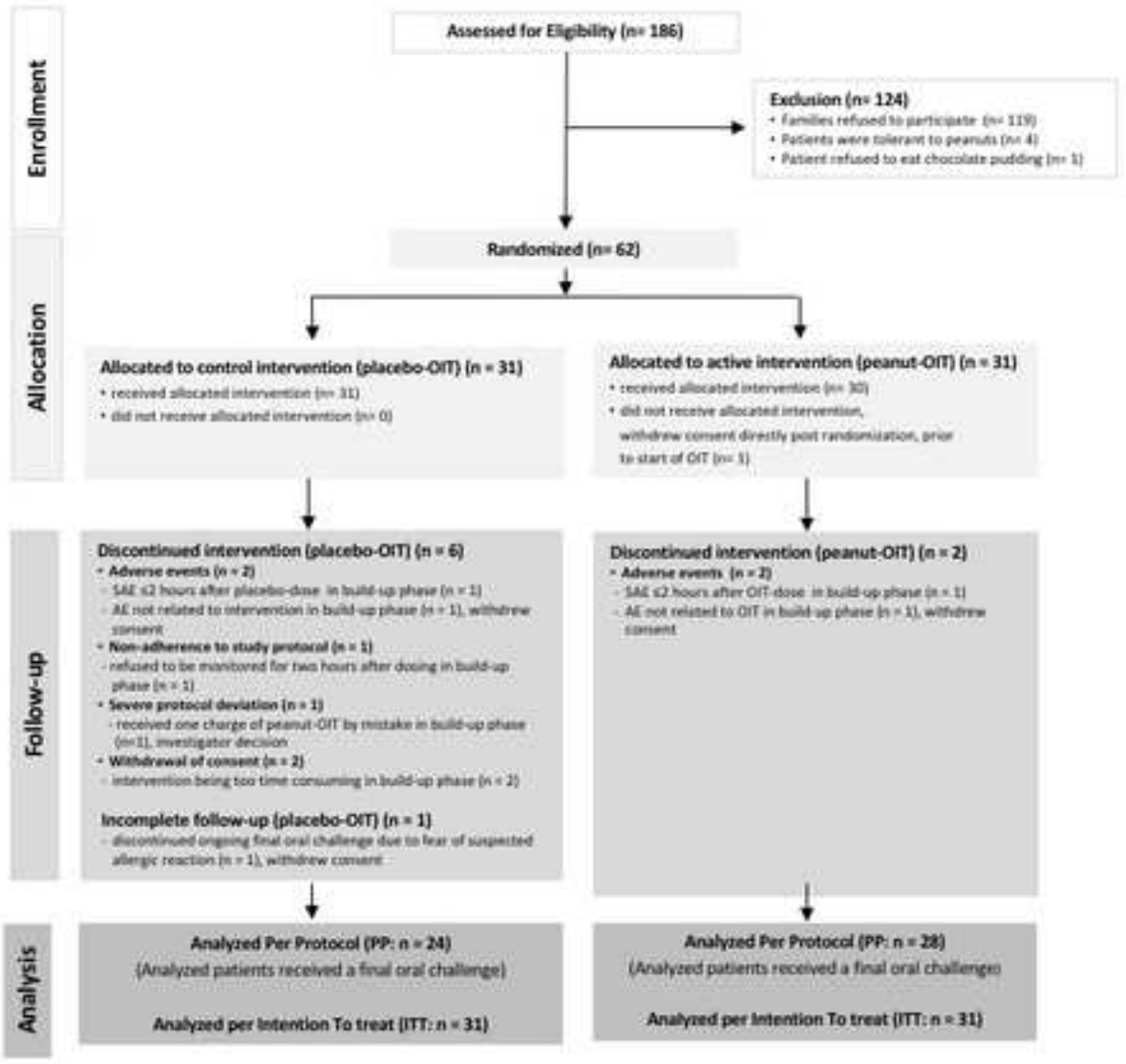



**FIGURE 1 CONSORT diagram**

Figure No.2

Click here to access/download;Figure No.;Fig 2 Change in max tol dose.tif

FIG 2

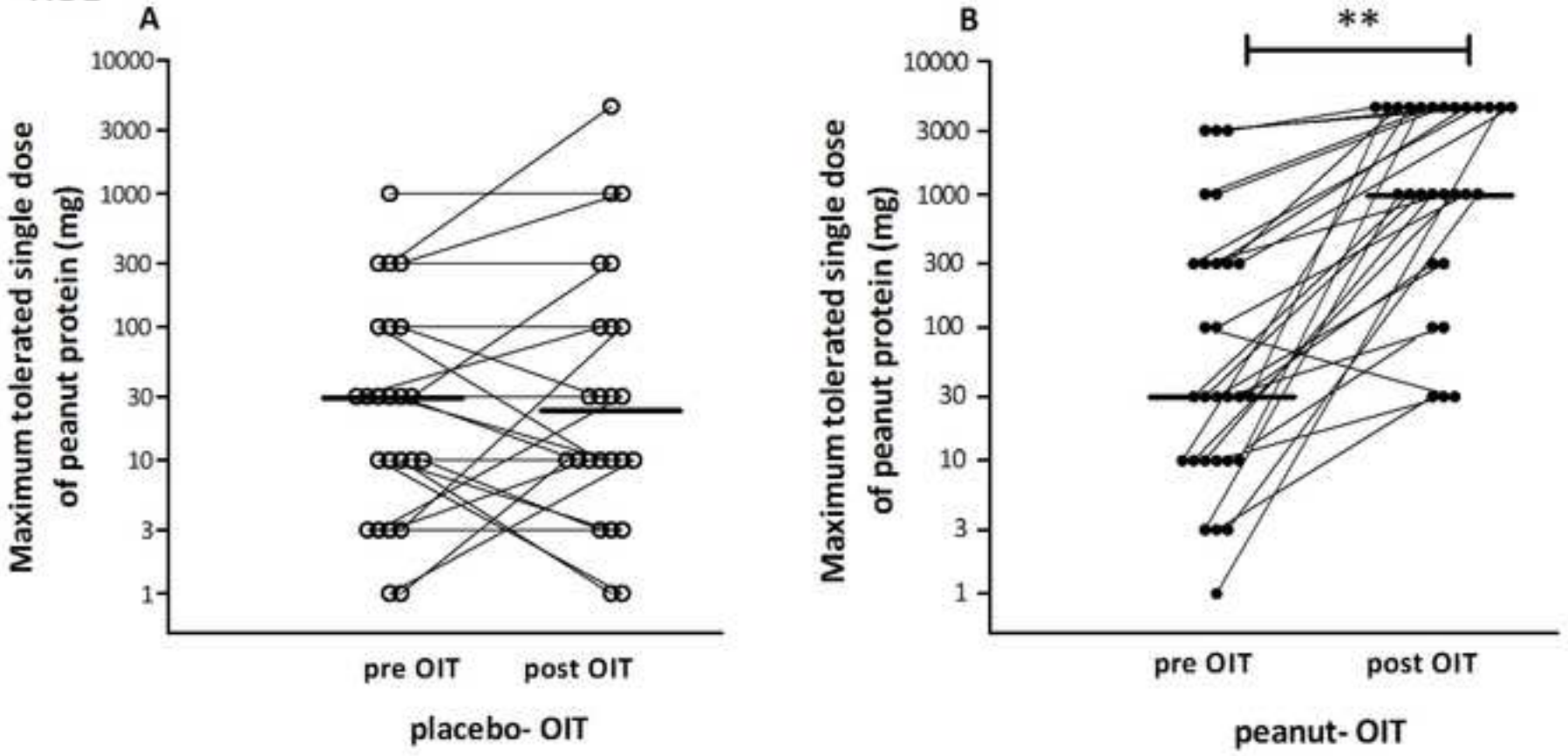
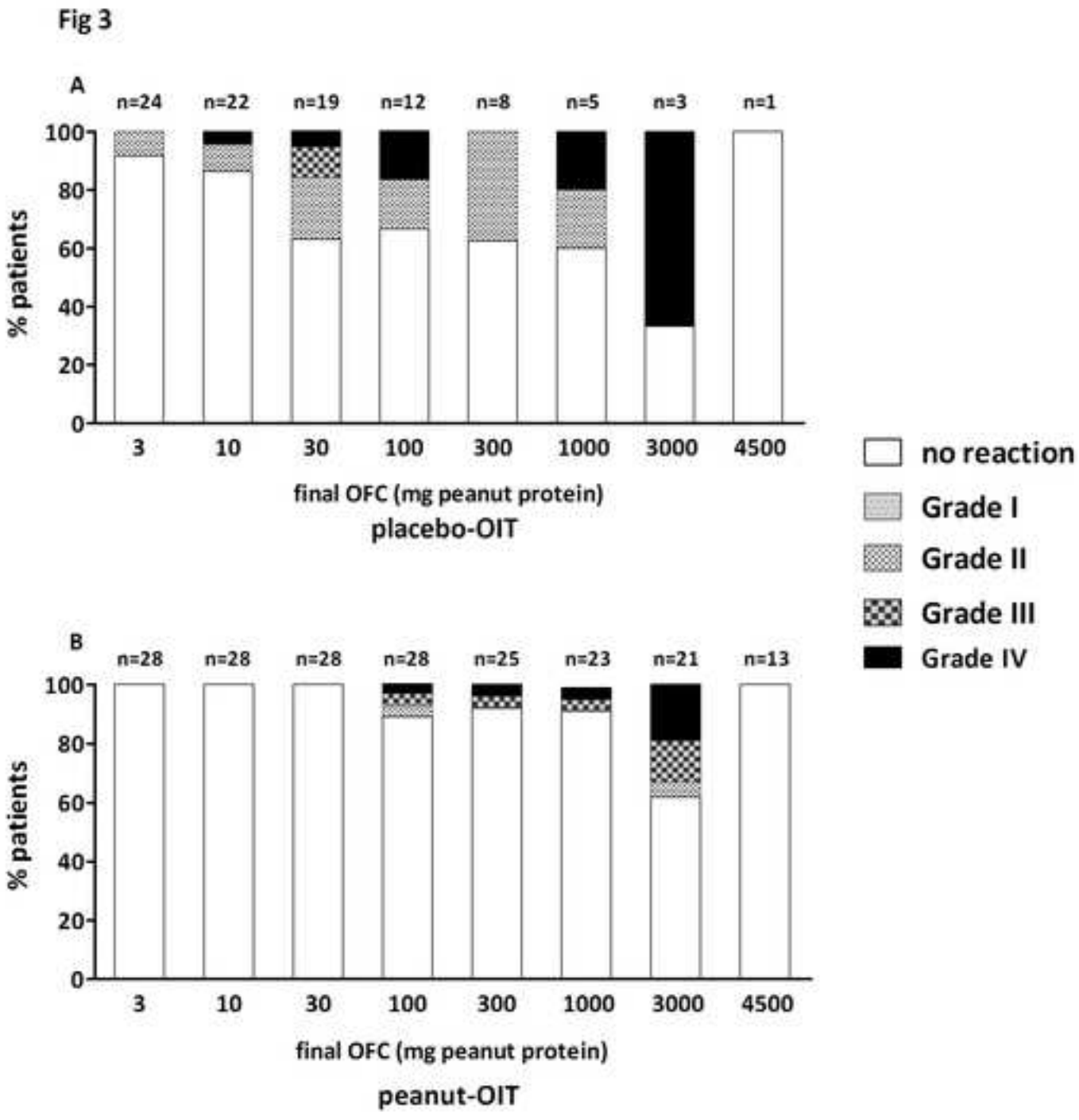


Figure No.3

Click here to access/download;Figure No.;Fig 3 Severity of reaction at final OFC.tif

Fig 3



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# Fig 4

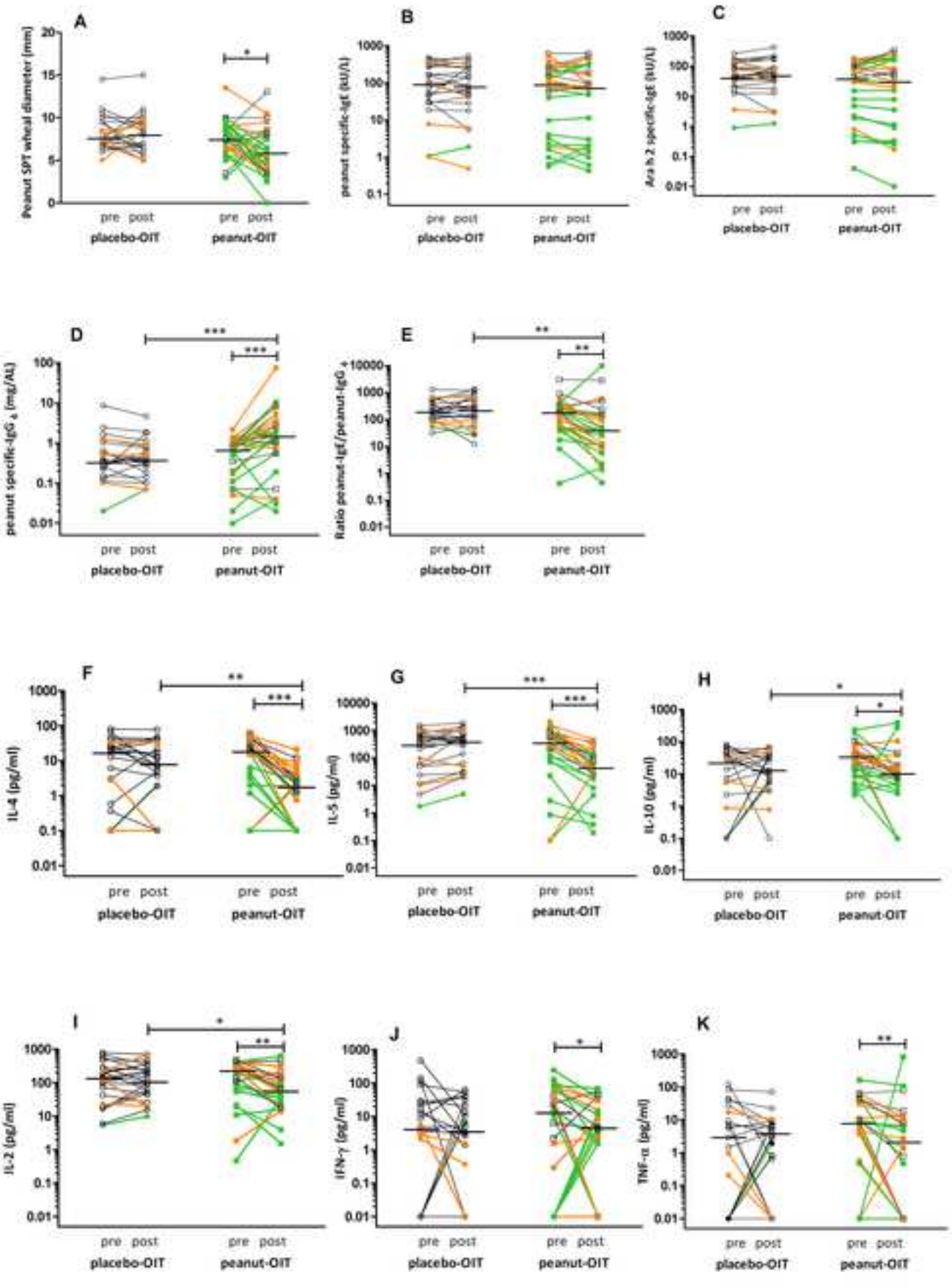
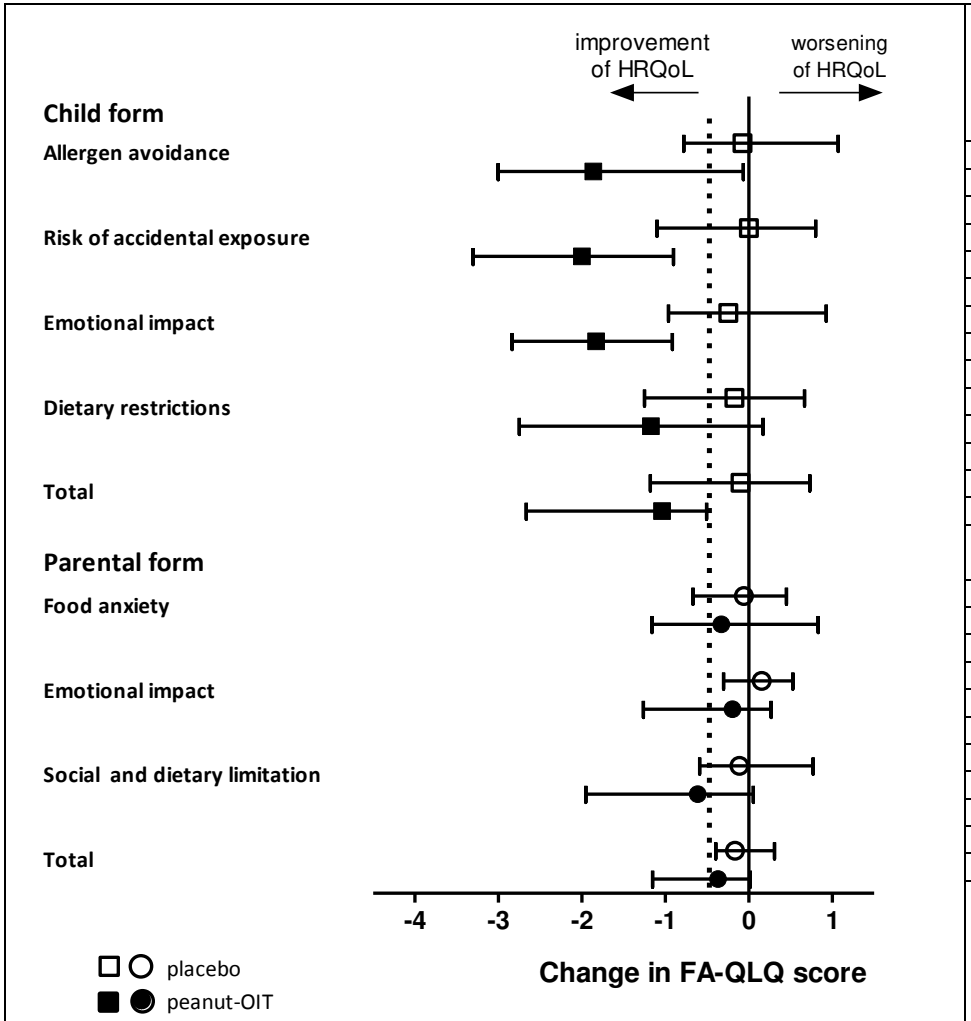


Figure No.5

**Fig. 5**




| Median [IQR] change | n/n possible patients to analyze | p-value |
|---|---|---|
| -0.1 [-0.8-1.1] | 8/9 | .08 |
| -1.9 [-3- -0.1] | 9/11 | |
| | | |
| **0 [-1.1-0.8]** | 8/9 | **.02** |
| **-2.0 [-3.3- -0.9]** | 9/11 | |
| | | |
| **-0.3 [-1.0-0.9]** | 8/9 | **.02** |
| **-1.8 [-2.8- -0.9]** | 9/11 | |
| | | |
| -0.2 [-1.3-0.7] | 8/9 | .23 |
| -1.2 [-2.8-0.2] | 9/11 | |
| | | |
| -0.1 [-1.2-0.7] | 8/9 | .10 |
| -1.0 [-2.7- -0.5] | 9/11 | |
| | | |
| -0.1 [-0.7-0.5] | 18/21 | .61 |
| -0.3 [-1.2-0.8] | 20/27 | |
| | | |
| 0.2[-0.3-0.5] | 18/21 | .17 |
| -0.2 [-1.3-0.3] | 20/27 | |
| | | |
| -0.1 [-0.6-0.8] | 18/21 | .16 |
| -0.6 [-2.0-0.1] | 20/27 | |
| | | |
| -0.2 [-0.4-0.31] | 18/21 | .20 |
| -0.4 [-1.2-0.02] | 20/27 | |

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# Efficacy, safety and quality of life in a multi-center, randomized, placebo-controlled trial on low-dose peanut oral immunotherapy in peanut allergic children

# Table of contents

## 4. Supplemental References

# 1. Supplemental Methods

## 1.1 Study centers

Dept. of Pediatric Pneumology, Immunology and Intensive Care Medicine, Charité Universitätsmedizin Berlin, Berlin, Germany; Children's Hospital "Altona", Hamburg; Dept. of Pediatrics, Technical University Munich, Munich; Dept. of Pediatrics, Ruhr-University Bochum, Bochum; Dept. of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover; Department of Pediatrics and Adolescent Medicine, University Medical Center, Medical Faculty, University of Freiburg, Freiburg, Germany; Dept. of Pediatrics, University Hospital Carl Gustav Carus, Technical University of Dresden, Dresden

## 1.2 Preparation and dosing of oral immunotherapy

For proper blinding of OIT, a special chocolate pudding vehicle was used during this study developed by the EuroPrevall project[1]. Initially it was developed by the Institute of Food Research (Norwich, UK) based on a system devised by Unilever R&D BV (Vlaardingen, Netherlands). It is a well standardized vehicle and shows good reproducibility, homogenicity, blinding capacities and a long shelf life (up to 6 months as the pudding base). Three sets of chocolate peanut/placebo pudding bases were produced every 4-5 months :((1) "high dose"- recipe (5mg peanut protein/ml in final "ready to eat" pudding), (2) "low dose"- recipe (1mg peanut protein/ml in final "ready to eat" pudding) and (3) an allergen free recipe (placebo)). All materials for the pudding bases were stored in a microbiologically stable manner. The pudding bases during the study were prepared in a food-grade environment (the main hospital kitchen of the Charité, Berlin). All equipment was thoroughly washed with detergent

before use so that any dust or allergenic material was removed. The following ingredients were used for the chocolate pudding bases: cold swelling starch (ULTRA-TEX 4, National Starch, Hamburg, Germany), cocoa powder (Cebe Cacao, lightly defatted, Wilhelm Reuss, Berlin, Germany), rapeseed oil (Karl Heidenreich GmbH; Mannheim, Germany), icing sugar (sweet family Nordzucker, Braunschweig, Germany), sweetener (Huxol, Nutrisun, Seevetal, Germany), Tween™ 60 (Croda, Singapore). As the vehicle contains Tween™ 60 (polysorbat 60, E435), therefore all study participants had to weigh at least 13 kg. Peanut flour (light roasted, 12 % fat, 50% protein) from Byrd Mill Company, Virginia, USA was used as the peanut protein source. 128.57g of the chocolate peanut/placebo pudding bases was transferred into a container and was stored in a cool dark condition (20°C) for up to 6 months.

Before distribution to the patient, the microbiological safety of each batch of the chocolate peanut/placebo pudding base was checked by Institute Fresenius (Berlin, Germany) according to national and international standards. Testing included total bacterial counts, tests for yeasts, moulds and lactic acid bacteria as well as surveillance for indicator microorganisms (Enterobactereriacae, sulphite-reducing clostridia) and pathogens (*Salmonella spp*, *Bacilli spp, S. aureus*, *C. perfringens*, *Listeria monocytogenes*, *E. coli*, *Campylobacter spp.*, *Vibrio cholerae*, *Y. enterocolitica*). Each batch was also tested for peanut protein quantification and homogeneity verification determining the allergen-free status of the chocolate placebo pudding, the homogeneity of the chocolate peanut pudding and the stability of protein doses between batches.

Every 7 to 14 days, parents had to prepare the “ready to eat”, hydrated pudding themselves. Parents were thoroughly instructed and taught how to prepare the “ready

to eat pudding" to ensure good homogeneity. They had to pour 300 ml of bottled water into each container of the pudding bases and mix it thoroughly with an electric mixer. This freshly made pudding was divided into smaller portions which were then frozen at home for a maximum of 40 days. Every two days one storage box of frozen "ready to eat" pudding was defrosted. The patient's individual dose of pudding was measured using different sized spoons. The volumes were exact if the whole spoon was filled with the pudding, and levelled off by a flat edge knife and extra pudding being removed from the sides. The exact dosing schedule is outlined in **TABLE E1**.

**TABLE E1 Dosing schedule of "ready to eat"-chocolate peanut/placebo pudding for OIT**

| | **Active** | | | | **Placebo** |
|---|---|---|---|---|---|
| **Incremental-steps** | **Peanut protein concentration of "ready to eat" pudding (mg protein/ml)** | **Whole peanut (mg)** | **Peanut protein (mg)** | **Volume of pudding (ml)** | **Peanut protein concentration of "ready to eat" pudding (mg protein/ml)** |
| **1** | 1.0 | 2.0 | 0.5 | 0.5 | 0 |
| **2** | 1.0 | 4.0 | 1.0 | 1.0 | 0 |
| **3** | 1.0 | 6.0 | 1.5 | 1.5 | 0 |
| **4** | 1.0 | 10.0 | 2.5 | 2.5 | 0 |
| **5** | 1.0 | 12.0 | 3.0 | 3.0 | 0 |
| **6** | 1.0 | 14.0 | 3.5 | 3.5 | 0 |
| **7** | 1.0 | 18.0 | 4.5 | 4.5 | 0 |
| **8** | 1.0 | 22.0 | 5.5 | 5.5 | 0 |
| **9** | 1.0 | 26.0 | 6.5 | 6.5 | 0 |
| **10** | 1.0 | 32.0 | 8.0 | 8.0 | 0 |
| **11** | 1.0 | 38.0 | 9.5 | 9.5 | 0 |
| **12** | 1.0 | 44.0 | 11.0 | 11.0 | 0 |
| **13** | 1.0 | 52.0 | 13.0 | 13.0 | 0 |
| **14** | 1.0 | 60.0 | 15.0 | 15.0 | 0 |
| **15** | 1.0 | 70.0 | 17.5 | 17.5 | 0 |
| **16** | 1.0 | 80.0 | 20.0 | 20.0 | 0 |
| **17** | 1.0 | 90.0 | 22.5 | 22.5 | 0 |
| **18** | 1.0 | 100.0 | 25.0 | 25.0 | 0 |
| **19** | 1.0 | 120.0 | 30.0 | 30.0 | 0 |
| **20** | 5.0 | 140.0 | 35.0 | 7.0 | 0 |
| **21** | 5.0 | 160.0 | 40.0 | 8.0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| **22** | 5.0 | 180.0 | 45.0 | 9.0 | 0 |
| **23** | 5.0 | 200.0 | 50.0 | 10.0 | 0 |
| **24** | 5.0 | 240.0 | 60.0 | 12.0 | 0 |
| **25** | 5.0 | 280.0 | 70.0 | 14.0 | 0 |
| **26** | 5.0 | 340.0 | 85.0 | 17.0 | 0 |
| **27** | 5.0 | 420.0 | 105.0 | 21.0 | 0 |
| **28** | 5.0 | 500.0 | 125.0 | 25.0 | 0 |
| **29** | 5.0 | 600.0 | 150.0 | 30.0 | 0 |
| **30** | 5.0 | 700.0 | 175.0 | 35.0 | 0 |
| **31** | 5.0 | 800.0 | 200.0 | 40.0 | 0 |
| **32** | 5.0 | 900.0 | 225.0 | 45.0 | 0 |
| **33** | 5.0 | 1,000.0 | 250.0 | 50.0 | 0 |

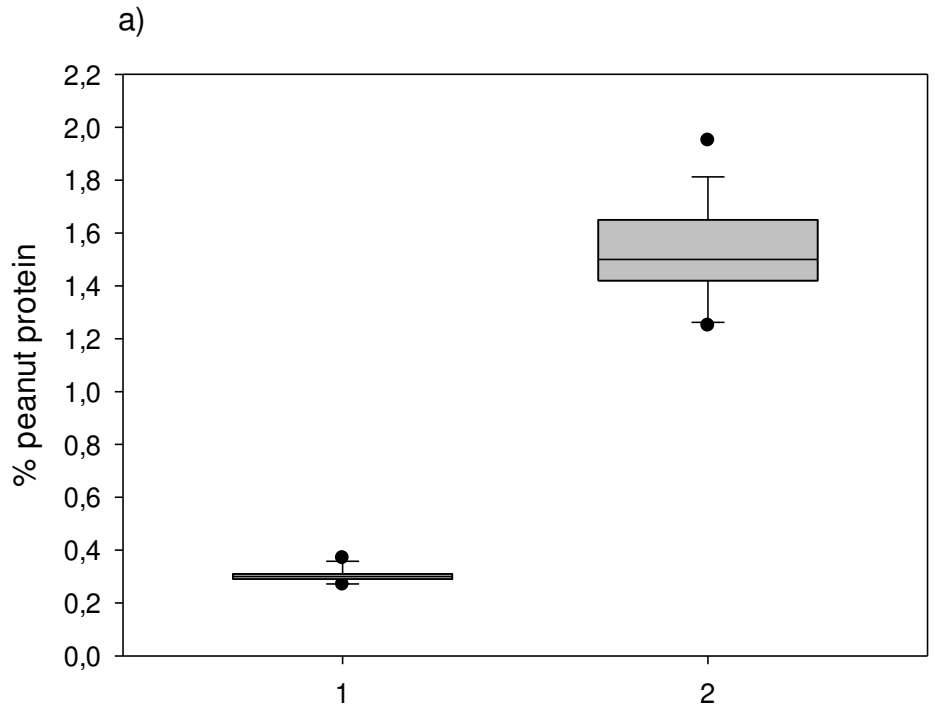
## 1.3 Peanut protein quantification and homogeneity testing of pudding bases

Using a Kjeldahl total nitrogen method[2], 51.0 % (0.6% CV) total protein was determined in defatted peanut flour (Byrd Mill Company, Virginia, USA) used for the preparation of low and high dose dessert bases, confirming the manufacturer's information of a total protein content of 50% within the peanut flour. Accordingly, for further investigation of peanut protein quantity and homogeneity using ELISA, 50% peanut protein in peanut flour was assumed. Thus, according to the recipe, the high and low dose pudding bases should contain 1.7 % or 0.34% peanut protein, respectively. Using a previously described peanut-specific ELISA[3], the presence and quantity of peanut protein was investigated in 10 placebo batches, 11 low dose pudding bases, and 15 high dose pudding bases. From each of these, a total of ten 4 g sub-samples were randomly drawn, individually extracted, and analysed in each triplicate dilution and triplicate wells per dilution. In placebo pudding bases, peanut protein was not detectable (with a limit of detection of 0.1 ppm or 0.00001 % peanut protein). Considering that final, "ready to eat" chocolate pudding portions are made of 1:3.333 dilution of pudding base in water, peanut protein is absent or below 0.03 ppm in placebo meals. As a worst case calculation, peanut protein at the limit of detection

would theoretically translate to a peanut protein dose of 1.5 µg for the largest portion (50 ml) given to patients.

With a median recovery (ratio of protein quantified and protein added according to recipe) of 88% (range 73 – 115 %), the amount of peanut protein of 0.34% and 1.7% was analytically confirmed in all batches of low and high dose pudding (**FIG E1 a**). Further, all batches of high dose pudding showed homogeneous distribution of peanut protein with mean CV < 15 % (**FIG E1 b**). In 10/11 low dose pudding bases a homogeneous peanut protein distribution with mean CV < 15 % was determined. With 15.3 % CV in 1/11 low dose dessert bases the upper limit of the 95%-confidence interval slightly exceeded the set limit of 15 % mean CV but was still interpreted as presenting acceptable homogeneity. Statistical significance was achieved in the majority of cases with $p < .0001$. For this above described batch and all following batches patients had to step one step down in their dosing schedule and be monitored when consuming a new batch for the first time. Ready-to eat chocolate pudding stored frozen showed 91.5 % of detectable peanut protein compared to freshly made chocolate pudding.

## Fig E1



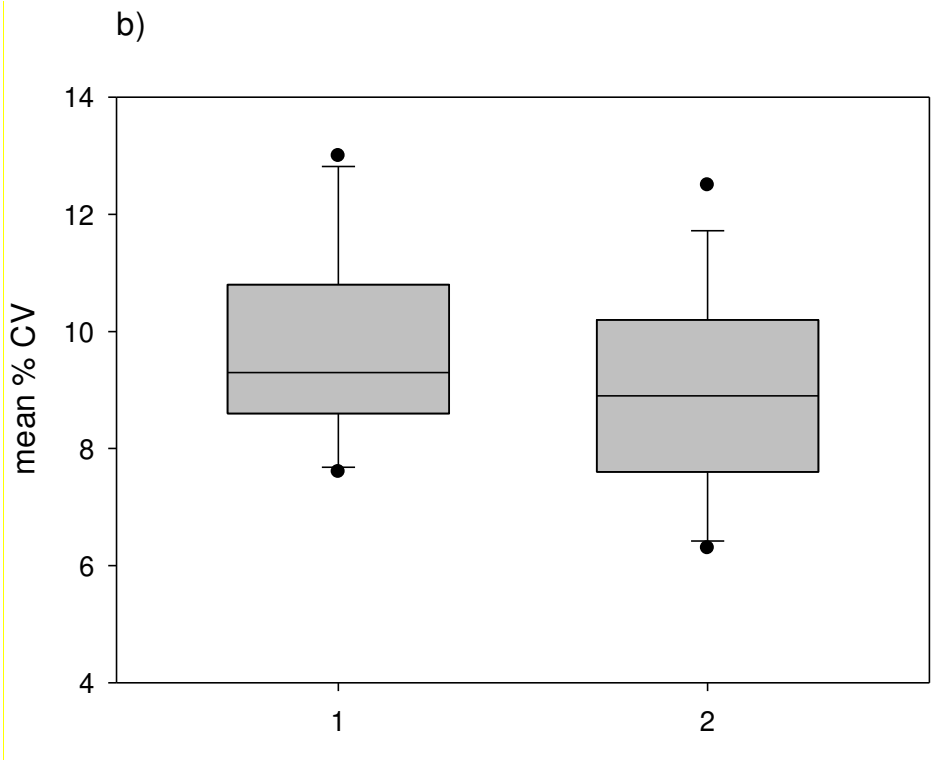




**FIG E1 Peanut protein quantification and homogeneity in different batches of low dose and high dose pudding bases**

Box plot analysis of low dose (1) and high dose (2) pudding bases. Percentage of peanut protein quantified (a), and variation of homogeneity expressed as mean % CV (b). CV= coefficient of variation

## 1.4 Statistical analyses: power calculation

For the primary endpoint, efficacy rates were assumed to be 65% in the peanut-OIT group and 15% in the placebo-OIT group on the basis of our previously published data[4]. With an assumed drop out-rate of 20%, a target sample size of 56 patients (randomized 1:1 to both groups) was calculated to provide at least 90% power ($\alpha/2=0.025$, one-sided Fisher-exact test).

## 1.5 Standard operating procedures (SOPs) for safety

During the inpatient phase (at challenge and start of OIT) emergency training for the emergency kit was repeated. The emergency kit included two epinephrine auto-injectors, oral antihistamine, an oral corticosteroid or suppository, and beta2-agonist for inhalation. Families were advised that the kit should be available at all times. A 24-hour-telephone hotline by medical doctors was available to answer questions regarding dosing and AEs. Parents were instructed to monitor their children for two hours after the intake of peanut- /placebo-OIT. Patients were advised to avoid strenuous physical activity during this time, to carry the emergency medication (e.g. two epinephrine auto-injectors) at all times and to strictly avoid peanuts otherwise.

**Reasons and procedure for OIT-dose reductions:**

- If symptoms were considered to be a viral or bacterial infection, the advice was to continue with 50% of the previous daily OIT dose. This 50% of the dose was given for three days, followed by another three days with 75% of the dose. After that the full dose could be ingested again (i.e. “dosing scheme for infections”). This reduction and up-dosing was done at home. Patients had to be stable on this dose for further 14 days until another up-dosing to a new dose could occur in the clinic.

- If symptoms were considered to be due to an accidental reaction or the patient received a vaccination or an elective operation, the OIT-dose was skipped that day and for the following days the “dosing scheme for infections” was applied.

- For safety reasons doses were reduced by one dose- step if a new charge of chocolate peanut/placebo- pudding bases was used.

- If a mild related or non-related AE (severity grade I) or a mild AE at up-dosing or a subjective AE occurred, the same dose was given the next day at home.

- If an objective, moderate related AE (severity grade II-IV) or an objective, moderate AE at up-dosing occurred, either the “dosing scheme for infections” or a reduction of dose (one step down) was applied at home as determined by the study physician. The subsequent up-dosing was performed in the study center.

- If a moderate non-related AE (severity grade II-IV) occurred, either the “dosing scheme for infections” or a reduction of dose (one step down) was applied or the same dose was given for another 14 days before up-dosing.

- If recurrent mild related or recurrent non-related AEs (especially GI symptoms) were detected, the dose was reduced by one step.

- If recurrent related or non-related pulmonary symptoms at a certain dose occurred a more vigorous dose reduction was performed (2 to 12 dose steps down) as was determined by the study physician. The doses were then increased every 14 days at home until the former dose was reached.

**Early termination of the treatment:**

- OIT-treatment was terminated if a patient showed objective symptoms related to OIT (within two hours of OIT dose) repeatedly, every time the dose was

188 increased above a certain level. A maximum of three trials for up-dosings were
189 tried before early termination was considered.

190 • OIT-treatment was terminated if there was a serious adverse event (SAE)
191 related or possibly related to OIT. This was defined as an SAE occurring within
192 two hours of OIT ingestion. Events were categorized as SAEs if they were life-
193 threatening, resulted in any kind of hospitalization (included if only for
194 monitoring), disability, congenital anomaly and death or were otherwise
195 deemed an important medical event.

196 • OIT-treatment was terminated by or on behalf of the patient´s decision.

197 • OIT-treatment was terminated by the study physician due to safety concerns
198 (e.g. insufficient adherence to protocol, or recurrent gastrointestinal AEs
199 possibly related to OIT).

200

201 **1.6 Health related quality of life (HRQL) measures**

202 To measure changes in health-related quality of life (HRQL) the German translation
203 of the Food Allergy Quality of Life Questionnaire was sent out to parents (FAQLQ-
204 PF[5], parental form, proxy measurement), children (FAQLQ-CF[6], child form) and
205 teenagers (FAQLQ-TF[7]), teenage form) 4 weeks before initial OFC and 4 weeks after
206 final OFC. Mothers of children 3-12 years old, children 8-12 years old and teenager
207 13-17 years old were asked to fill out the forms at home and to send them back to the
208 study unit. FAQLQ-TFs were not included in data analysis due to the small number of
209 teenagers in the study (active group n=1, placebo group n=5). Depending on the age
210 of the child, the FAQLQ-PF included 26-30 items in three domains (emotional impact,

food-related anxiety, social and dietary limitations). It measured the parent's report on the child's HRQL from the child's perspective. The FAQLQ-CF included 24 items in four domains (allergen avoidance, risk of accidental exposure, emotional impact, dietary restrictions). The scoring system was a 7-point Likert scale ranging from either 0 in the FAQLQ-PF or from 1 in the FAQLQ-CF (= no impact on HRQL) to 6 in the FAQLQ-PF or 7 in the FAQLQ-CF (= extreme impact on HRQL). To harmonize both scales in data analysis the raw scores 0-6 in the FAQLQ-PF were recorded as 1-7, as in other studies[6, 8]. For comparison of changes in HRQL before and after OIT in both study groups only complete data sets were considered for analysis (PP analysis). The mean total and mean domain scores were calculated for each child/mother.

## 2. Supplemental Results

### 2.1 Drop outs

Ten of 62 patients discontinued during the study (see FIG 1): One patient of the peanut-OIT group withdrew consent after randomization but before receiving the allocated intervention. Two patients of each randomization group discontinued due to adverse events: Within the placebo-OIT group one patient suffered from sudden abdominal pain, sleepiness, followed by rhinoconjunctivitis, vomiting and unconsciousness 75 minutes after intake of the placebo-OIT dose and 15 minutes after eating a cookie from a friend. Due to the severity of symptoms (severity grade V) this event was considered a severe adverse event (SAE) related to OIT (and thus- following the protocol- the patient had to be excluded. Another patient in the placebo-

OIT group experienced a worsening of known episodes of recurrent obstructive bronchitis in the winter not related to OIT. Although the pulmonary situation stabilized after a reduction of the placebo-OIT dose the mother decided to discontinue the study. One patient of the peanut-OIT group suffered from abdominal pain, rhinoconjunctivitis, swelling of the eyes and lips, generalized hives and dry cough (severity grad III) 45 minutes after ingestion of 500 mg peanut protein-OIT during physical activity outside during the summer. After treatment with inhalant salbutamol, systemic antihistamines and steroids the patient was admitted to hospital for monitoring, and per protocol, the patient had to be excluded from the study. The patient was known to suffer from seasonal allergic rhinoconjunctivitis due to grass pollen sensitization and bronchial asthma. Prior to this event the patient had had to be down-dosed due to GI symptoms. In the winter, one patient in the peanut-OIT group experienced recurrent infections of the upper airways and coughs, rhinoconjunctivitis and shortness of breath not related to OIT. After a 50% reduction of OIT dose symptoms remained. The family decided to stop OIT. The patient was highly sensitized to house dust mite and suffered from bronchial asthma and perennial rhinoconjunctivitis before starting OIT.

In the placebo-group one patient did not adhere to the study protocol; two patients withdrew consent during the build-up phase; one patient refused to finish the OFC due to fear of allergic reactions during final oral OFC and one patient experienced a severe protocol deviation. This patient suffered from worsening of GI symptoms during the build-up phase of the placebo-OIT. Although receiving a 75%-step-down in dosing, the symptoms remained. The chocolate pudding vehicle was sent back to the study center, where it was noticed that the patient received one charge of the wrong peanut-chocolate pudding vehicle. The investigator decided that the patient should

be excluded.

## 2.2 Safety

**TABLE E2: Number of OIT doses associated with adverse events (AEs) in the placebo- and peanut-OIT group**

| | placebo-OIT | peanut-OIT | |
|---|---|---|---|
| Total OIT doses, n= | 13,813 | 12,412 | |
| OIT doses during up-dosing, n= | 11,838 | 10,323 | |
| OIT doses during maintenance, n= | 1,975 | 2,089 | |
| **Number of OIT doses associated with…..** | | | **p values*** |
| AEs in total, n= (%) | 170 (1.2) | 534 (4.3) | **.001** |
| S**ubjective** AEs, n= (%) | 72 (0.52) | 459 (3.7) | **<.001** |
| OAS, n= (%) | 29 (0.21) | 281 (2.26) | **.003** |
| Abdominal pain, n= (%) | 20 (0.14) | 111 (0.89) | **<.001** |
| Nausea, n= (%) | 8 (0.06) | 13 (0.1) | .07 |
| Skin itching, n= (%) | 23 (0.17) | 10 (0.1) | 1.0 |
| Joint pain/ headache/ throat pain, n= (%) | 5 (0.04) | 70 (0.56) | .11 |
| **Objective** AEs, n= (%) | 107 (0.77) | 85 (0.68) | .98 |
| Objective AEs during up-dosing, n= (%) | 99 (0.84) | 81 (0.78) | .99 |
| Objective AEs during maintenance, n= (%) | 8 (0.41) | 4 (0.19) | .79 |
| Skin symptoms (contact urticarial, flush, generalized hives, angioedema), n= (%) | 23 (0.17) | 24 (0.19) | .44 |
| GI symptoms (vomiting, diarrhoea), n= (%) | 10 (0.07) | 26 (0.21) | .63 |
| URT symptoms (conjunctivitis, rhinitis, sneezing, rhino-conjunctivitis), n= (%) | 58 (0.42) | 10 (0.08) | .64 |
| Laryngeal symptoms (hoarseness, stridor), n= (%) | 14 (0.1) | 1 (0.01) | 1.0 |
| Lower respiratory tract symptoms (coughing, wheezing, shortness of breath), n= (%) | 20 (0.14) | 52 (0.42) | .11 |
| Coughing, n= (%) | 15 (0.11) | 41 (0.33) | .09 |
| Wheezing, n= (%) | 1 (0.01) | 8 (0.06) | **.045** |
| Shortness of breath related to OIT, n= (%) | 4 (0.03) | 3 (0.02) | .8 |
| Cardio-vascular symptoms (drop in blood pressure, unconsciousness), n= (%) | 1 (0.01) | 0 (0) | .33 |
| AEs of severity grade I, n= (%) | 6 (0.04) | 12 (0.1) | .46 |
| AEs of severity grade II, n= (%) | 54 (0.39) | 12 (0.1) | .81 |
| AEs of severity grade III, n= (%) | 28 (0.2) | 50 (0.4) | .73 |
| AEs of severity grade IV, n= (%) | 19 (0.14) | 11 (0.1) | .28 |
| AEs of severity grade V, n= (%) | 1 (0.01) | 0 (0) | .33 |
| Treatment for AEs related to OIT, n= (%) | 12 (0.09) | 23 (0.19) | .261 |
| Application of systemic antihistamines for AEs related to OIT, n= (%) | 9 (0.07) | 12 (0.1) | .531 |
| Application of systemic steroids for AEs related to OIT, n= (%) | 4 (0.03) | 5 (0.04) | .922 |
| Application of inhalant salbutamol for AEs related to OIT, n= (%) | 6 (0.04) | 10 (0.08) | .561 |
| Application of adrenalin for AEs related to OIT, n= (%) | 0 (0) | 0(0) | 1.0 |

278 ***p-value comparing the percentage of OIT doses associated with AEs per**
279 **patient between groups using the Kruskal-Wallis test**

280

281

282 **TABLE E3: Serious adverse events during placebo-/peanut-OIT**

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284 Table is in landscape format, attached in extra file

285

## 2.3 Health related quality of life (HRQL)

**TABLE E4: Baseline median scores of FAQLQ prior to start of OIT as measure of HRQL**

| | placebo-OIT | peanut-OIT |
|---|---|---|
| **Parent form (FAQLQ-PF) [IQR]** | | |
| Median total score | n=23/26<br>3.6 [3.1-5.2] | n=22/30<br>4.0 [3.4-4.7] |
| Median emotional impact score | n=23/26<br>3.4 [2.8-5.2] | n=22/30<br>3.8 [2.9-4.4] |
| Median food anxiety score | n=23/26<br>4.7 [2.6-5.1] | n=22/30<br>4.0 [3.2-4.3] |
| Median social and dietary limitation score | n=23/26<br>4.7 [3.1-5.2] | n=22/30<br>4.6 [3.6-5.7] |
| **Child form (FAQLQ-CF) [IQR]** | | |
| Median total score | n=10/10<br>5.0 [4.1-5.4] | n=9/13<br>5.3 [4.7-5.8] |
| Median allergen avoidance score | n=10/10<br>4.0 [3.4-5.2] | n=9/13<br>4.7 [4.0-5.6] |
| Median risk of accidental exposure score | ***n=10/10<br>4.9 [4.3-5.7]*** | ***n=9/13<br>6.2 [5.3-6.4]**** |
| Median emotional impact score | n=10/10<br>6.3 [4.5-6.7] | n=9/13<br>5.7 [5.5-6.2] |
| Median dietary restrictions score | n=10/10<br>4.7 [3.6-5.8] | n=9/13<br>4.7 [4.1-5.7] |

Results are reported as the median [IQR] baseline scores of total and specific domains of the distributed questionnaire for HRQL (FAQLQ).

PF Parent form, CF child form. *p= .035

## 3. Supplemental Discussion

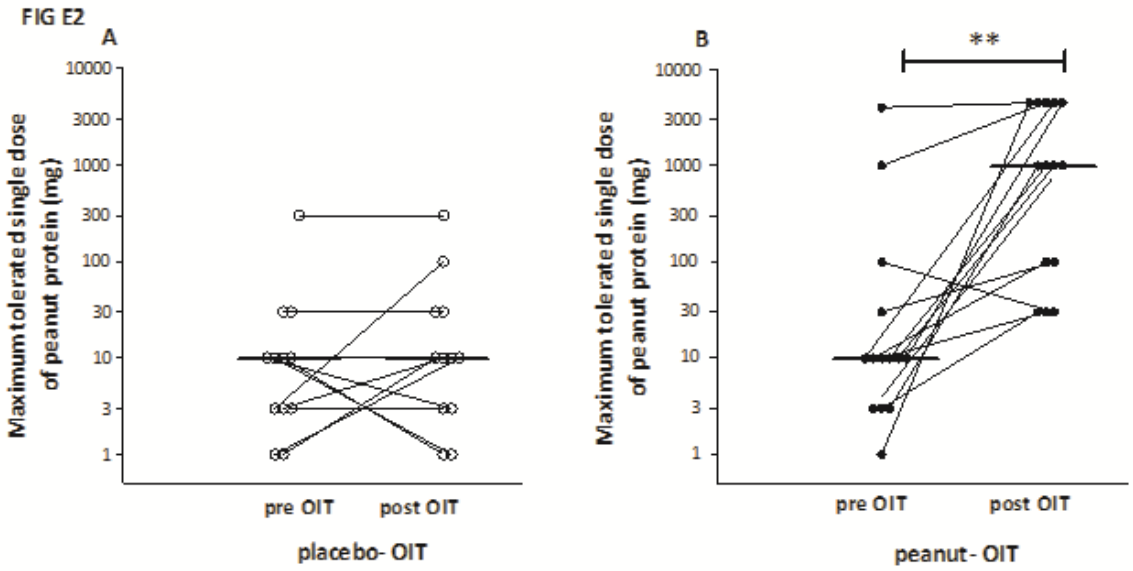


**FIG E2: Maximum tolerated single dose of peanut protein prior and post OIT of patients not reaching their planned maintenance dose.**

Shown are the maximum tolerated single doses of peanut protein at initial and final OFC in (**A**) individual placebo-OIT patients not reaching the planned maintenance dose with a median maintenance dose of “32.5mg Placebo (range: 3.5- 150mg)” and (**B**) peanut-OIT patients not reaching the planned maintenance dose with a median maintenance dose of 50mg peanut protein (range: 2.5- 225mg) of the per protocol population. The horizontal lines represent the median of the maximum tolerated single dose in each group. For the statistical analysis of the comparison of data pre and post treatment within one randomization group, the Wilcoxon-test was used.

**p<.01

**TABLE E5: Characteristics of patients who tolerated a maximum dose of ≥300mg peanut protein at baseline OFC**

Table is in landscape format, attached in extra file

## 4. Supplemental References


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**TABLE E3:** Serious adverse events during placebo-/peanut-OIT*

| Patient/ Randomi-zation | Days on OIT | OIT dose (mg) | Time to reaction after OIT dose | Cause for SAE/ possible augmentation factor | Symptoms | Severity | Treatment/ early termination |
|---|---|---|---|---|---|---|---|
| **placebo-OIT** | 118 | 4.5 | 75 minutes | Possible accidental reaction after cookie from friend | Abdominal pain, tiredness, allergic rhinoconjunctivitis, vomiting, unconsciousness | V | Antihistamine p.o., corticosteroid rec.→ monitoring for 2 hours in ER→ per protocol early termination: SAE related to OIT |
| **placebo-OIT** | 270 | 150 | 24 hours | Accidental reaction after peanut sauce at barbeque | OAS, angioedema, abdominal pain, coughing, generalized hives | III | Antihistamine p.o., corticosteroid rec., inhalative Salbutamol → hospitalization |
| **placebo-OIT** | 106 | 3.5 | 24 hours | Possible accidental reaction after mucosal contact with peanut/ physical activity | Conjunctivitis, hoarseness, coughing, shortness of breath, flush | IV | No medication applied → hospitalization |
| **placebo-OIT** | 88 | 20 | 17 hours | Possible accidental reaction after eating snack | Abdominal pain, generalized hives, coughing, wheezing | IV | Antihistamine p.o., corticosteroid p.o., inhalative adrenalin → hospitalization |
| **placebo-OIT** | 168 | 1.75 | 16 hours | Ingestion of raw carrot/ viral infection | OAS, coughing | III | Antihistamine p.o., corticosteroid p.o. → hospitalization |
| **peanut-OIT** | 342 | 125 | 45 minutes | Physical activity (running around in garden) | Abdominal pain, rhinoconjunctivitis, angioedema, pruritus, generalized hives, coughing | III | Inhaled Salbutamol, antihistamine p.o., corticosteroid i.v. →hospitalization → per protocol early termination: SAE related to OIT and hospitalization |
| **peanut-OIT** | 230 | 4.5 | 3.5 hours | Possible accidental reaction after Chinese meal/ URI | Coughing, somnolence | V | Adrenaline i.m., antihistamine p.o., corticosteroid rec.→ hospitalization |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **peanut-OIT** | 15 | 1 | 23 hours | Possible accidental reaction after Sushi meal | OAS, coughing, allergic rhinoconjunctivitis, itching | III | Antihistamine i.v., corticosteroid i.v.→ hospitalization |

p.o. (per os), i.v. (intravenous), i.m. (intramuscular), rec. (rectal), URI (upper respiratory infection), OAS (oral allergy syndrome), emergency room (ER)

*defined as adverse events that were leading to death, hospitalization, disability, were life-threatening or otherwise deemed an important medical event

**TABLE E5:** Characteristics of patients who tolerated a maximum dose of ≥300mg peanut protein at baseline OFC (PP population)

| Patient number | Randomi-zation of OIT | Maximum tolerated dose *at baseline OFC | Baseline peanut SPT (mm) | Baseline peanut specific-IgE (kU/l) | Baseline peanut specific-IgG4 (mgA/l) | Reached maintenance dose * | Maximum tolerated dose *at final OFC | Delta peanut SPT (mm) | Delta peanut specific-IgE (kU/l) | Delta peanut specific-IgG4 (mgA/l) | Number of wheezing episodes related to OIT, n= | Number accidental reactions, n= |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #5 | placebo | 300mg | 7.5 | 1.08 | 0.02 | "250mg" | 4,500mg | +0.5 | +0.79 | +0.05 | 0 | 1 |
| #28 | placebo | 1,000mg | 5 | 1.02 | ND | "250mg" | 1,000mg | +3.5 | -0.53 | ND | 0 | 1 |
| #53 | placebo | 300mg | 7 | 7.79 | 0.1 | "150mg" | 300mg | +3 | -1.7 | -0.03 | 0 | 0 |
| #62 | placebo | 300mg | 7.5 | 93.8 | 1.22 | "250mg" | 1,000mg | -2.5 | -50.4 | -0.3 | 0 | 1 |
| #1 | peanut | 1,000 mg | 5 | 4.1 | 0.11 | 250mg | 4,500mg | +0.5 | -1.09 | +0.43 | 0 | 0 |
| #2 | peanut | 3,000mg | 3 | 2.21 | 0.01 | 250mg | 4,500mg | +4.5 | -0.15 | +0.03 | 0 | 0 |
| #7 | peanut | 300mg | 5.5 | 68.9 | 1.39 | 250mg | 4,500mg | -2.5 | -20.2 | +0.52 | 0 | 0 |
| #16 | peanut | 300mg | 6 | 1.86 | 0.05 | 250mg | 1,000mg | +2.5 | -0.27 | -0.01 | 1 | 0 |
| #29 | peanut | 1000mg | 8 | 0.98 | 0.02 | 225mg | 4,500mg | -4.5 | -0.55 | +0.17 | 0 | 0 |
| #46 | peanut | 300mg | 7 | 40.2 | 0.62 | 250mg | 4,500mg | +1 | +10.9 | +4.7 | 0 | 0 |
| #50 | peanut | 3,000mg | 6 | 1.63 | 0.2 | 225mg | 4,500mg | -6 | -1.02 | +1.17 | 0 | 0 |
| #55 | peanut | 300mg | 9 | 0.57 | 1.32 | 250mg | 4,500mg | -6.5 | +0.86 | -0.43 | 0 | 0 |
| #64 | peanut | 300mg | 8.5 | 3.15 | 0.07 | 250mg | 4,500mg | -4 | -2.15 | -0.05 | 0 | 0 |
| #69 | peanut | 3,000mg | 9.5 | 0.63 | ND | 250mg | 4,500mg | -4.5 | +1.41 | ND | 0 | 0 |

* Dose of peanut protein

** “Delta” represents the change from baseline to post treatment (post-OIT value minus pre-OIT value)