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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

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<b>Manuscript Region of Origin:</b>	GERMANY
<b>Abstract:</b>	<p>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.</p> <p>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 <math>\geq 300</math> 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.</p> <p>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 (<math>p &lt; .001</math>). 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 (<math>p &lt; .001</math>). 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.</p> <p>Conclusion: Low-dose OIT is a promising, effective and safe treatment option for</p>

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

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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**

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48

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63

64 **Abstract**

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

69 **Methods:** 62 children aged 3-17 years with IgE-mediated, challenge-proven peanut  
70 allergy were randomized (1:1) to receive peanut-OIT with a maintenance dose of  
71 125-250 mg peanut protein or placebo. The primary outcome was the proportion of  
72 children tolerating  $\geq 300$  mg peanut protein at oral food challenge (OFC) after 16  
73 months of OIT. We measured occurrence of adverse events (AEs), immunological  
74 changes, QoL prior and post OIT and BoT during OIT.

75 **Results:** 23/31 (74.2%) children of the active group tolerated at least 300 mg peanut  
76 protein at final OFC compared to 5 /31 (16.1%) in the placebo group ( $p < .001$ ). 13/31  
77 (41.9%) children of the active versus 1/31 (3.2%) of the placebo group tolerated the  
78 highest dose of 4.5 g peanut protein at final OFC ( $p < .001$ ). There was no significant  
79 difference between the groups in the occurrence of AE-related drop-outs or in the  
80 number, severity and treatment of objective AEs. In the peanut-OIT group, we noted  
81 a significant reduction in peanut specific IL-4, IL-5, IL10 and IL-2 production by  
82 PBMCs compared to the placebo group, as well as a significant increase in peanut  
83 specific-IgG4 levels and a significant improvement of QoL. 86% of children evaluated  
84 the BoT positively.

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

88

89

90 **What is known already about the topic?**

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

94 **What does this article add to our knowledge?**

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

99 **How does this study impact current management guideline?**

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

102 **Short title**

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

105

106 **Abbreviations**

107 **AE** Adverse event

108 **BoT** Burden of treatment

109 **FAQLQ-PF/CF/TF** Food Allergy Quality of life Questionnaire-Parent/Child/Teenage  
 110 form

111 **GI** Gastrointestinal

112 **HRQL** Health-related quality of life

113 **IgE** Immunoglobulin E

114 **IL-4** Interleukin-4



115	<b>IQR</b>	Interquartile range
116	<b>IT</b>	Immunotherapy
117	<b>ITT</b>	Intention to treat
118	<b>MCID</b>	Minimum clinical important difference
119	<b>OAS</b>	Oral allergy syndrome
120	<b>OFC</b>	Oral food challenge
121	<b>OIT</b>	Oral immunotherapy
122	<b>PBMCs</b>	Peripheral blood mononuclear cells
123	<b>PP</b>	Per protocol
124	<b>QoL</b>	Quality of life
125	<b>SAE</b>	Severe adverse event
126	<b>SCORAD</b>	Scoring Atopic Dermatitis
127	<b>SPT</b>	Skin prick test
128	<b>Th2</b>	T helper 2
129	<b>URI</b>	Upper respiratory infection

130

### 131 **Keywords**

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

133

134

## 135 **Introduction**

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

145

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

154

155 Hypothetically, using a low maintenance dose and a long up-dosing period in peanut-  
156 OIT might lead to the same efficacy but better safety profile than using a higher  
157 maintenance dose for a shorter up-dosing period. The aim of this double-blind,  
158 placebo-controlled study was to assess efficacy for clinical desensitization and safety  
159 of OIT as well as possible changes in immunological parameters, in quality of life  
160 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

## 168 **Methods**

### 169 **Study overview**

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

178

### 179 **Study population**

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

190

### 191 **Study endpoints**

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

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

204

### 205 **Randomization**

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

210

### 211 **Study design**

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

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

226

### 227 **Open oral peanut challenges (OFC)**

228 Prior to the start of OIT - at initial OFC - as well as after the maintenance phase at  
229 final OFC children received an open oral peanut challenge using a modified  
230 PRACTALL protocol<sup>20</sup> with 2-hour time intervals between dose steps as previously  
231 described<sup>19</sup>. In summary, patients received whole crushed roasted peanuts in boiled  
232 apple sauce as a matrix in increasing titration steps for a maximum of three days  
233 (first day: 3 mg - 10 mg - 30 mg - 100 mg, second day: 100 mg - 300 mg – 1,000 mg  
234 – 3,000 mg, third day: 4,500 mg peanut protein). The procedure was stopped if  
235 objective clinical symptoms were observed. This dose was considered to be the  
236 eliciting dose. The last single dose the patient tolerated just before the eliciting dose  
237 was defined as the maximum tolerated single dose.

238

### 239 **Procedures for OIT:**

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

246 eliciting dose of 3 mg, 10 mg, 30 mg, 100 mg or  $\geq 300$  mg peanut protein at initial  
247 OFC they started OIT on a dose of 0.5 mg, 1 mg, 3 mg, 10 mg or 30 mg peanut  
248 protein, respectively. The same OIT dose was administered again the next day. After  
249 two hours of monitoring, patients were instructed to take this dose daily,  
250 approximately at the same time. Up-dosings were planned every two weeks under  
251 medical monitoring in the outpatient clinics of the study centers (see online repository  
252 **TABLE E1**). The up-dosing phase lasted a maximum of 14 months or shorter if the  
253 patients reached their individual planned maintenance dose. The planned final  
254 maintenance dose was determined by the eliciting dose patients had reacted to at  
255 initial OFC: Patients with an eliciting dose of 3 mg to 100 mg peanut protein at initial  
256 OFC were gradually increased to 125 mg whereas patients with an eliciting dose of  
257 300 mg to 4,500 mg peanut protein were dosed up to 250 mg peanut protein as an  
258 OIT-maintenance dose. The maintenance phase lasted for 8 weeks ( $\pm 2$  weeks).

259

### 260 **Safety outcomes**

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

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

276

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

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

287

288 The BoT questionnaire was sent out to the families three to four months after starting  
289 OIT. Mothers of children (3-12 years), children (8-12 years) and teenagers (13-17  
290 years) were asked to rate the advantages and disadvantages of OIT-treatment on a 7  
291 point-scale ranging from 1 (=extremely positive) through 4 (=neutral) to 7 (=extremely  
292 negative)<sup>25, 26</sup>. Mothers and patients were also asked if they would perform OIT  
293 again. Results are presented for each treatment group as numbers of mothers or  
294 children who reported on a positive (score 1-3) or a negative BoT (score 4-7) and  
295 who would and would not perform OIT once more. HRQL- and BoT data of teenagers  
296 were not included in data analysis due to the small number of teenagers within the  
297 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

## 322 **Results**

### 323 324 **Study population**

325 Of 186 children with suspected peanut allergy approached for the study, 119 refused  
326 to participate, four were tolerant to peanut at initial OFC and the youngest patient  
327 vigorously refused to eat the vehicle (chocolate pudding) (see **FIG 1**, CONSORT flow  
328 diagram). Thus sixty-two participants with a median age of 6.8 years (range: 3.2 to  
329 17.8 years), median peanut-specific IgE of 81.5 kU/l (range: 0.57-624 kU/l), median  
330 Ara h 2-specific IgE of 44.7 kU/l (range: 0.04- 256kU/l) and median maximum  
331 tolerated single dose at initial OFC of 30 mg peanut protein (range: 1-3,000 mg) were  
332 randomized to receive either active, peanut-OIT (n=31) or placebo-OIT (n=31). Ten  
333 of 62 patients discontinued during the study (see CONSORT diagram): One patient  
334 of the peanut-OIT group withdrew consent after randomization but before receiving  
335 the allocated intervention. This patient was still included in the ITT- analysis (n=62 in  
336 ITT). See **FIGURE 1** and the online repository (2.1) for further explanations of all  
337 drop-outs. There were no significant differences between the peanut-OIT and the  
338 placebo group in demographical and immunological baseline characteristics (**TABLE**  
339 **I**).

340

### 341 **Efficacy**

342 After a median of 13 months [10-14 months] of the up-dosing and 9.5 weeks [8.5-  
343 11.4 weeks] of the maintenance phase, 24 patients of the placebo-OIT and 28  
344 patients of the peanut-OIT group finished the study with a final OFC. 50% in each  
345 randomization group reached their planned maintenance dose. The median  
346 maintenance dose was 125mg peanut protein [50-250mg] in the peanut-OIT and  
347 “125mg placebo” [31.3-225mg] in the placebo-OIT group. Within the ITT population  
348 23 of 31 patients (74.2%) of the peanut-OIT group tolerated  $\geq 300$  mg peanut protein

349 whereas only 5 of 31 patients (16.1%) within the placebo-OIT group tolerated this  
350 dose at final OFC ( $p<.001$ ) (**TABLE II, FIG 2**). Also in the worst-case analysis  
351 ( $p=0.01$ ) as well as in the per protocol analysis ( $p<.001$ ) the primary endpoint was  
352 met (**TABLE II**). As a secondary endpoint, 13 patients of the peanut-OIT group  
353 (41.9% of the ITT population) tolerated the maximum dose of 4.5 g peanut protein at  
354 final OFC compared to only one patient (3.2%) within the placebo-OIT group  
355 ( $p<.001$ ). With a median of 20 mg [10-100] peanut protein, the maximum tolerated  
356 single dose at final OFC remained unchanged (fold change=1, [0.33-4.3]) when  
357 compared to the median maximum tolerated single dose at initial OFC (30 mg [8.3-  
358 100] peanut protein) within the placebo group. In comparison, the maximum tolerated  
359 single dose increased by a factor of 12.1 [4.3-97] from a median of 30 mg [10-300] to  
360 a median of 1,000 mg [825-4,500] peanut protein at final OFC within the peanut-OIT  
361 group.

362

363 Within the first six of eight dose steps of the final OFC the patients of the placebo  
364 group experienced more and more severe reactions than the peanut-OIT group  
365 (**FIG3**). However, comparing the number of grade IV reactions during all dose steps  
366 (3 mg to 4.5 g peanut protein) at final OFC, there was no difference between the  
367 peanut-OIT ( $n=7$ ) and placebo group ( $n=7$ ) (**FIG 3**).

368

### 369 **Safety**

370 Two patients in each group discontinued due to adverse events (6.5% of the total ITT  
371 population), one of these patients in each group due to a severe adverse event (SAE)  
372 being judged to be related to the OIT dose (**FIG 1**, for details see online repository  
373 **2.1 and TABLE E2**).

374

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

387

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

395

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

401 were more OIT doses associated with objective AEs during the up-dosing phase than  
402 during maintenance, but to a similar extent in both groups (**TABLE E2**). Dose  
403 reductions due to AEs could be a sign of more severe AEs during OIT. However,  
404 12/30 patients within the placebo-OIT group (40%) and 14/31 patients within the  
405 peanut-OIT group (45%) needed at least one dose reduction due to AEs during the  
406 course of OIT.

407

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

412

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

417

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

423

#### 424 **Immunological parameters**

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

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

440

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

442 Before start of OIT, baseline HRQL did not differ between the placebo and active  
443 group in all domains except for the domain of “risk of accidental exposure” in children  
444 (online repository **TABLE E4**). After final OFC, mothers of both study groups (n=38)  
445 filled out the FAQLQ-PF after a median of 9.5 weeks (IQR [5-15.3]), children (n=17)  
446 filled out the FAQLQ-CF after a median of 11 weeks (IQR [7-16]) after final OFC.  
447 Taking a minimum clinically important difference (MCID) of 0.5 for a significant  
448 clinical improvement of HRQL after an intervention<sup>27</sup> mothers of the peanut-OIT but  
449 not the placebo OIT group reported a median improvement of HRQL of greater than  
450 0.5 in score within the domain of “social and dietary limitations” post OIT (**FIG 5**).  
451 There was no meaningful median change in HRQL reported by mothers within the  
452 total score and the domains of emotional impact and food-related anxiety in the

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

456

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

464

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

473

## 474 **Discussion**

### 475 **Efficacy**

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

485

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

494

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



500 group tolerating at least 300 mg peanut protein at final challenge in comparison to  
501 19% of the placebo group<sup>18</sup>. Choosing an even higher maintenance dose of 800 mg  
502 peanut protein, Kukkonen et al were able to demonstrate in a non-controlled trial that  
503 67% of the children in their peanut-OIT group tolerated a maximum cumulative dose  
504 of 1,255 mg peanut protein at final challenge<sup>15</sup> which might be comparable to our  
505 results with 68% of the active group tolerating a cumulative dose of 1,443 mg peanut  
506 protein (data not shown) at final OFC. Recently published in a direct comparison,  
507 Vickery et al also demonstrated that using a very high maintenance dose (e.g. 3,000  
508 mg peanut protein) does not lead to a better efficacy than using a lower maintenance  
509 of 300 mg<sup>16</sup>.

510

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

519

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

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

535

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

548

549

550 **Safety**

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

558

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

569

570 Our excellent safety profile might result from the slow up-dosing and the low  
571 maintenance dose used in this protocol. Looking at the proportion of drop outs (13-  
572 21%) in other studies recruiting a similar study population but using faster up-dosing  
573 and a higher maintenance dose<sup>14, 15, 18</sup> the proportion of drop outs due to AEs (6.7%)  
574 in this study is much lower. There was no need of epinephrine treatment for AEs  
575 related to OIT and absence of development of eosinophilic oesophagitis. Moreover,  
576 antihistamine and steroid treatment was lower than previously reported by others<sup>15</sup>.

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<sup>8, 16-18</sup>. Uniquely, like in our pilot trial<sup>8</sup> 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- $\gamma$  and TNF- $\alpha$  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.<sup>30</sup>. They demonstrated a reduction of IL-5-, IL-13,  
588 but also of IFN- $\gamma$ -, IL-10- and TNF- $\alpha$ -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<sup>27</sup>, 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<sup>13, 31</sup> and in children and teenagers reports<sup>31</sup>  
602 using the same questionnaires but not comparing their results to a control group.

603

604 To our knowledge, this is the first time that BoT has been analyzed for an OIT study.

605 Although patients and parents had to cope with a daily therapy which might have also

606 elicited disgust, AEs and included also a daily two-hour interval of parental monitoring

607 of their children, the majority of mothers and children reported (after a median of four

608 months on OIT) being positive (=low BoT) about this treatment and would start this

609 kind of therapy again.

610

### 611 **Limitations of this study**

612 Since an unblinded OFC protocol was used in this study overreporting of allergic

613 reactions during baseline OFCs and underreporting at final OFCs due to change of

614 attitude of the children towards peanut ingestions might have occurred. However,

615 efficacy results are so robust and similar to other efficacy data on peanut-OIT

616 published so far<sup>15, 18</sup> this effect seems marginal. Additionally, the OFC protocol used

617 in this study- with a two-hour interval between dose steps<sup>19</sup>- differs from other OFC

618 protocols used in OIT trials possibly changing the sensitivity of threshold and severity

619 of reactions during OFC which might impact the efficacy data of this trial. However,

620 the eliciting dose for peanut-induced allergic reactions in 5% of this study population

621 ( $ED_{05}$ )<sup>19</sup> is comparable to the  $ED_{05}$  of other published peanut allergic populations

622 being challenged with 15 to 30 minute intervals<sup>32-34</sup>. Therefore the sensitivity for

623 threshold might not be too different to other published studies on peanut OIT. Due to

624 differences in the reporting of the severity of reaction during OFC the data of this

625 current study cannot be compared to others. Therefore it might well be that due to a

626 two hour interval between dose steps more severe reactions could have been

627 avoided.

628

## 629 **Summary**

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

642

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654 and their parents as well as the site personnel who assisted within the trial.



656  
657**TABLE I Baseline characteristics of study participants**

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

658

659 Continuous values are presented as medians with interquartile range. Kruskal-Wallis

660 test was used for statistical analysis. Categorical variables are presented as number

661 of participants and percentage using the chi-squared test for statistical analysis.

662 <sup>+</sup>defined as either historical or challenge proven systemic reaction to food allergens

663 other than peanut,

664 <sup>++</sup> for detailed definition of grading the severity of allergic reactions see Blumchen et665 al.<sup>19</sup>,



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

670

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

681

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
<b>Total number of adverse events (AEs), n=(%*)</b>	2,866 (20.7)	2,515 (20.3)	.71
<b>Total number of severe adverse events (SAEs), n=(%*)</b>	5 (0.04)	3 (0.02)	.73
<b>Number of SAEs related to OIT, n=(%*)</b>	1 (0.007)	1 (0.008)	1.0
<b>Number of patients who discontinued the study due to AEs, n=(%**)</b>	2(6.5)	2 (6.7)	1.0
<b>Number of patients with...../ receiving.....</b>			
<b>AEs related to OIT, n= (**)</b>	24 (77.4)	27 (90)	.3
<b><u>subjective</u> AEs related to OIT, n= (**)</b>	14 (45.2)	25 (83.3)	<b>.002</b>
OAS related to OIT, n= (**)	8 (25.8)	18 (60)	<b>.007</b>
abdominal pain related to OIT, n= (**)	6 (19.4)	20 (66.7)	<b>&lt;.001</b>
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
<b><u>objective</u> AEs related to OIT, n= (**)</b>	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
<b><u>lower respiratory tract symptoms</u> related to OIT (coughing, wheezing, shortness of breath), n= (**)</b>	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)	<b>.04</b>
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

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

684

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

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

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

692

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<sup>19, 21</sup>.

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- $\gamma$ - (J) and TNF- $\alpha$  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

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

726

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

735

736

737

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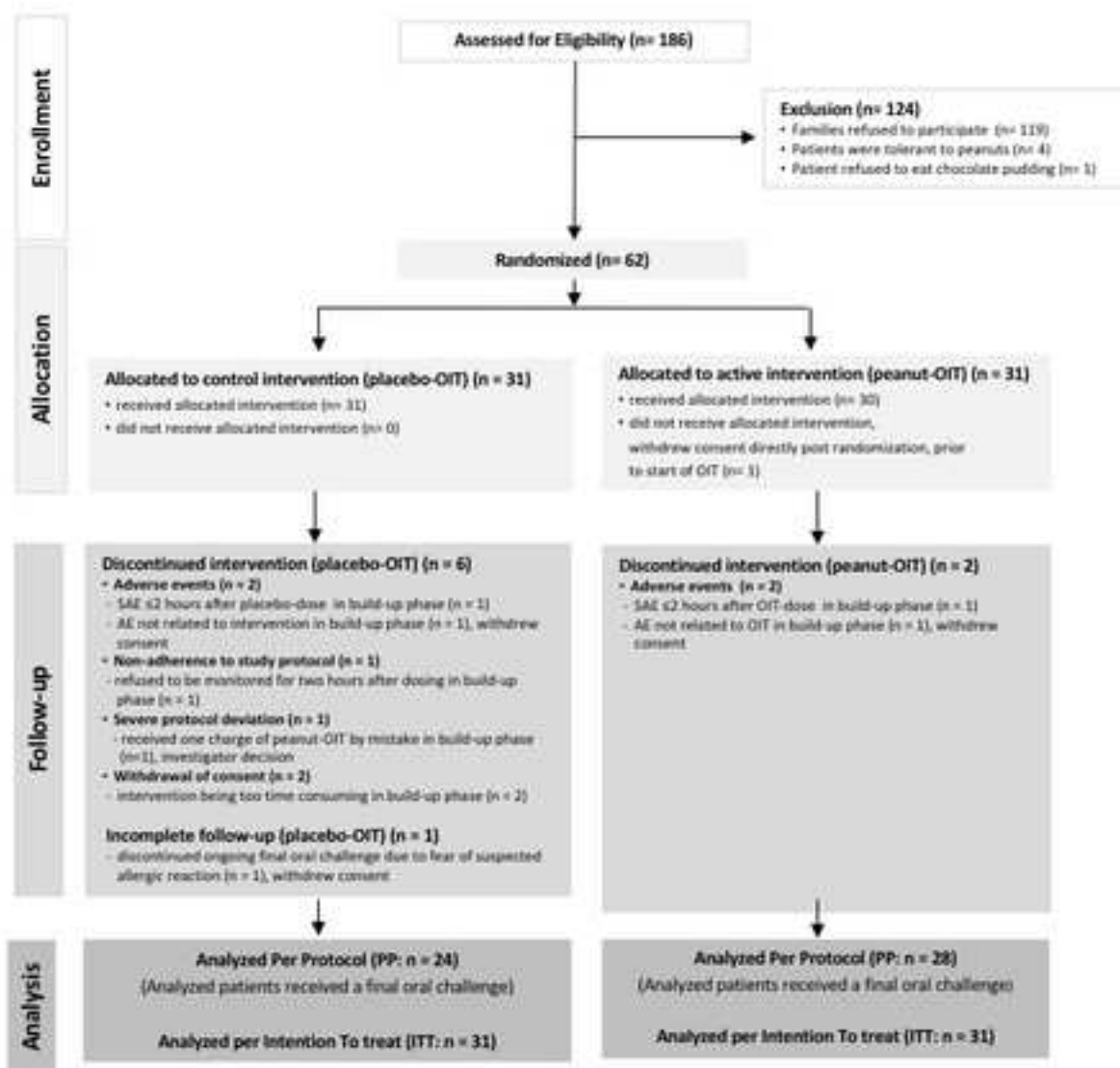
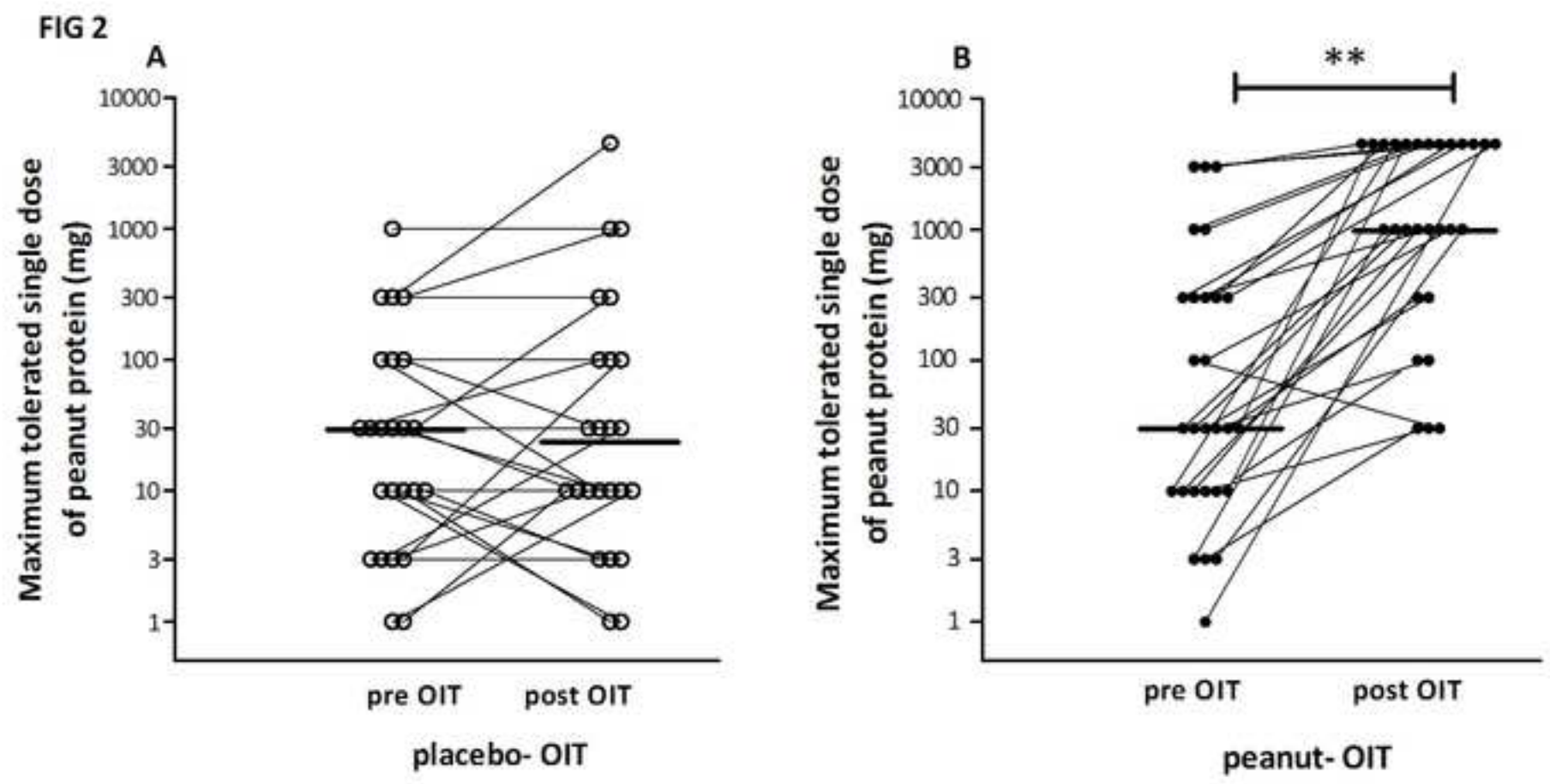


FIGURE 1 CONSORT diagram



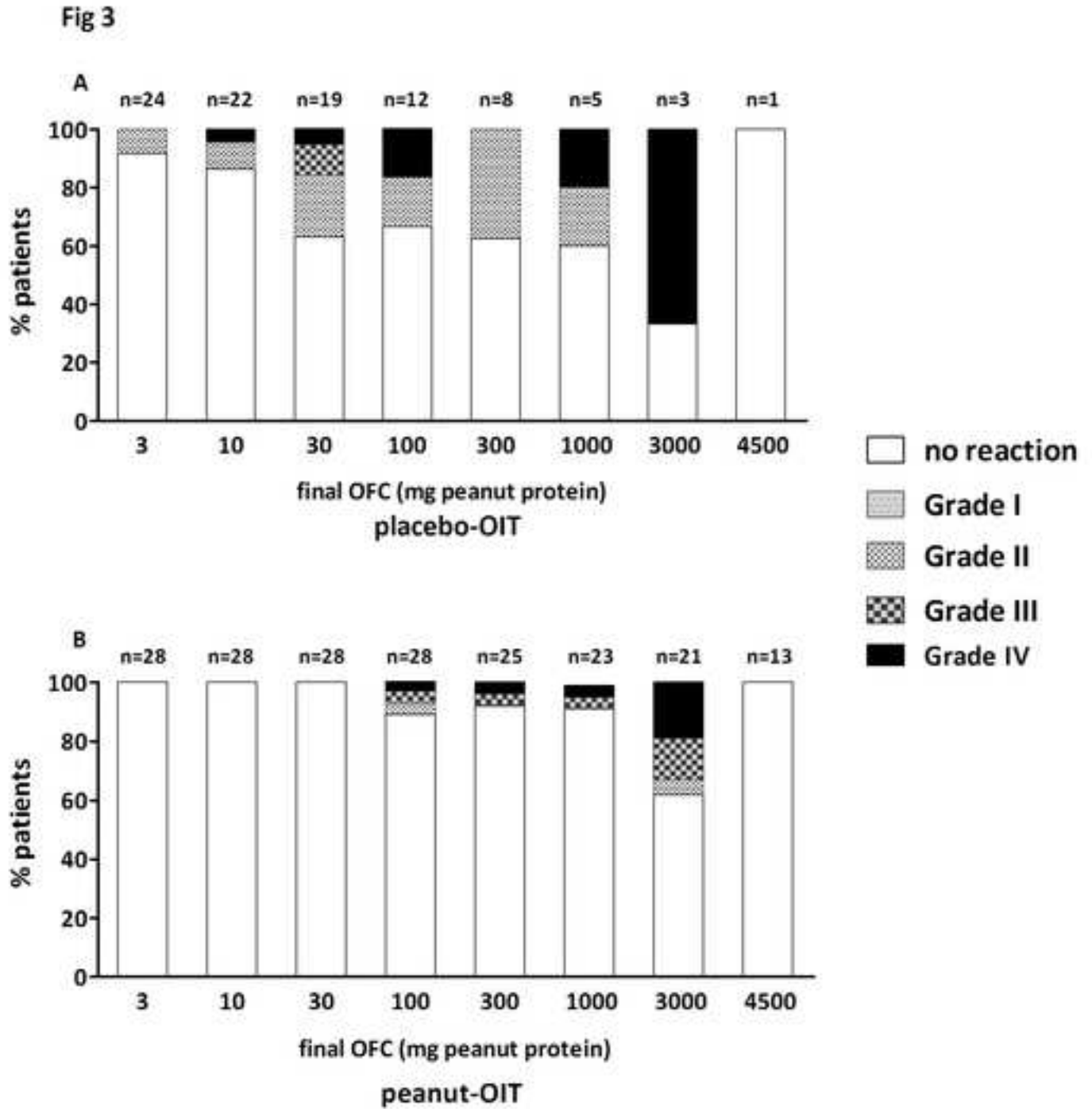


Fig 4

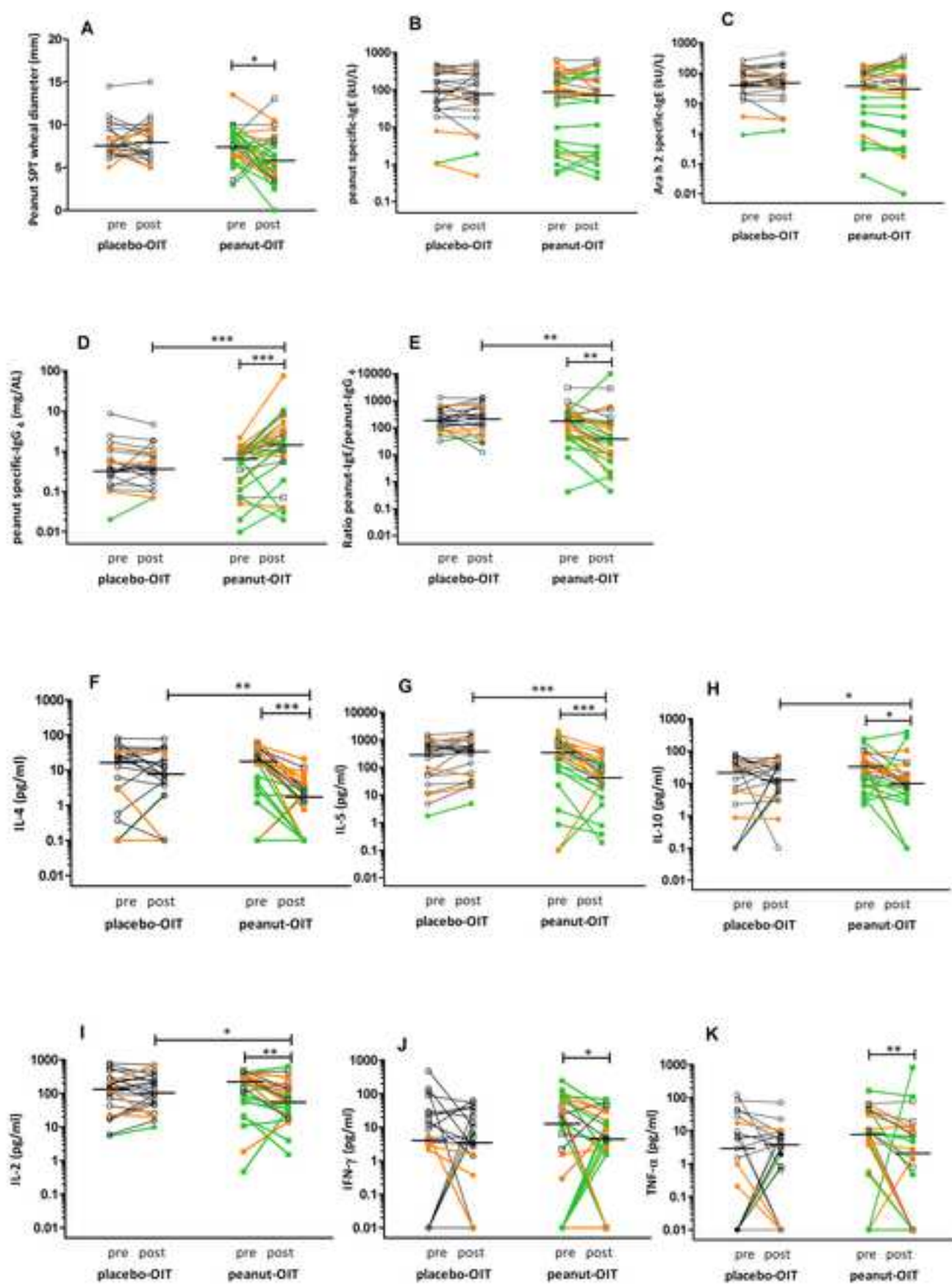
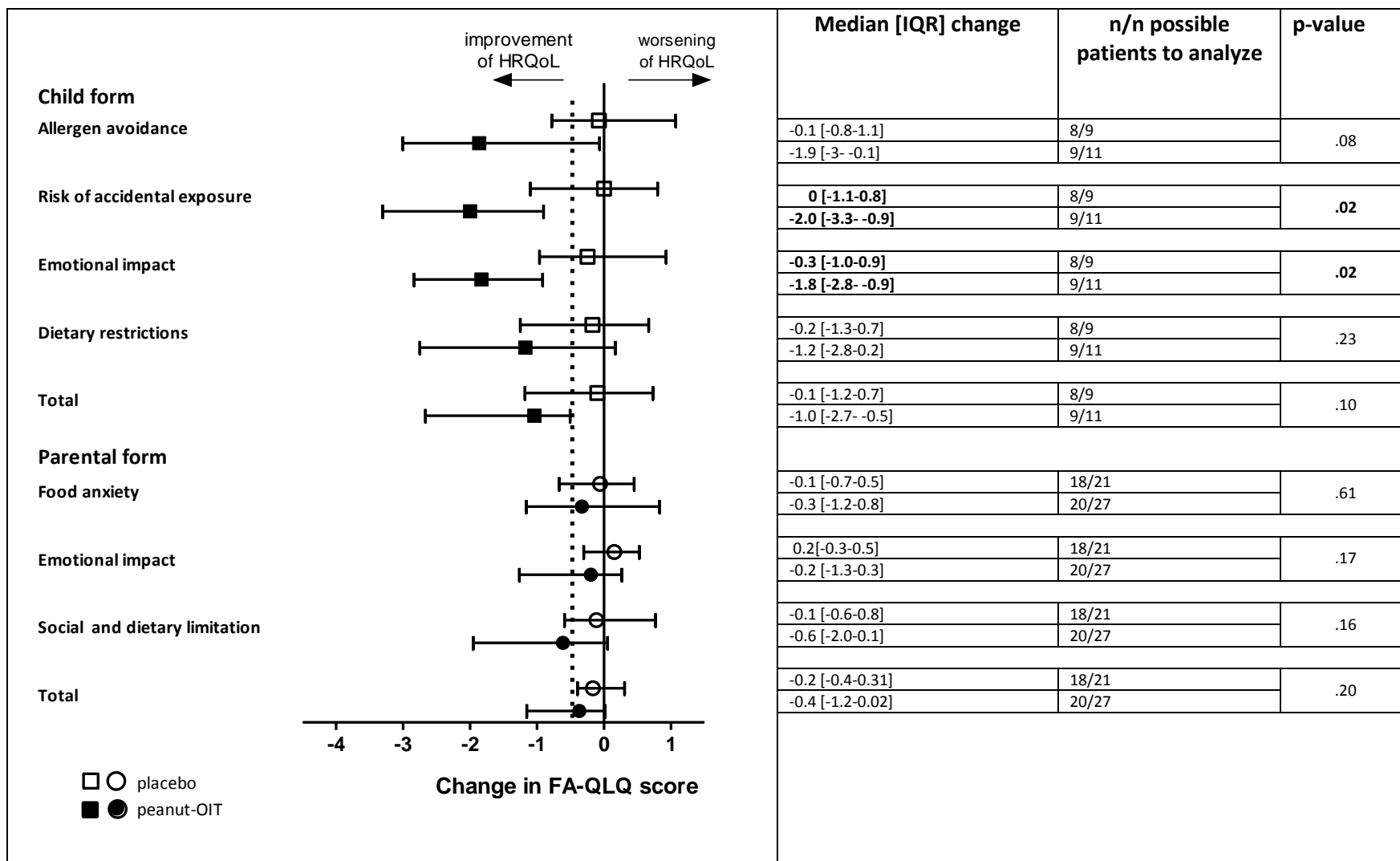


Fig. 5



**Online Repository ([www.jacionline.org](http://www.jacionline.org))**

**to**

**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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8 1.5 Applied Standard operating procedures for safety

9 1.6 Health related quality of life (HRQL) measures

10

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13 2.2 Safety

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

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

17 2.3 Health related quality of life (HRQL)

18 TABLE E4: Baseline median scores of FAQLQ prior to start of OIT

19

20 **3. Supplemental Discussion**

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

23 TABLE E5: Characteristics of patients who tolerated a maximum dose of  
24  $\geq 300$ mg peanut protein at baseline OFC

25

26 **4. Supplemental References**

27

## 28 **1. Supplemental Methods**

### 29 **1.1 Study centers**

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

38

### 39 **1.2 Preparation and dosing of oral immunotherapy**

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

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

63

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

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

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

85

86 **TABLE E1 Dosing schedule of “ready to eat”-chocolate peanut/placebo**  
 87 **pudding for OIT**

88

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

89

90

91 **1.3 Peanut protein quantification and homogeneity testing of**92 **pudding bases**93 Using a Kjeldahl total nitrogen method<sup>2</sup>, 51.0 % (0.6% CV) total protein was

94 determined in defatted peanut flour (Byrd Mill Company, Virginia, USA) used for the

95 preparation of low and high dose dessert bases, confirming the manufacturer's

96 information of a total protein content of 50% within the peanut flour. Accordingly, for

97 further investigation of peanut protein quantity and homogeneity using ELISA, 50%

98 peanut protein in peanut flour was assumed. Thus, according to the recipe, the high

99 and low dose pudding bases should contain 1.7 % or 0.34% peanut protein,

100 respectively. Using a previously described peanut-specific ELISA<sup>3</sup>, the presence and

101 quantity of peanut protein was investigated in 10 placebo batches, 11 low dose

102 pudding bases, and 15 high dose pudding bases. From each of these, a total of ten

103 4 g sub-samples were randomly drawn, individually extracted, and analysed in each

104 triplicate dilution and triplicate wells per dilution. In placebo pudding bases, peanut

105 protein was not detectable (with a limit of detection of 0.1 ppm or 0.00001 % peanut

106 protein). Considering that final, "ready to eat" chocolate pudding portions are made of

107 1:3.333 dilution of pudding base in water, peanut protein is absent or below 0.03 ppm

108 in placebo meals. As a worst case calculation, peanut protein at the limit of detection

109 would theoretically translate to a peanut protein dose of 1.5 µg for the largest portion  
110 (50 ml) given to patients.

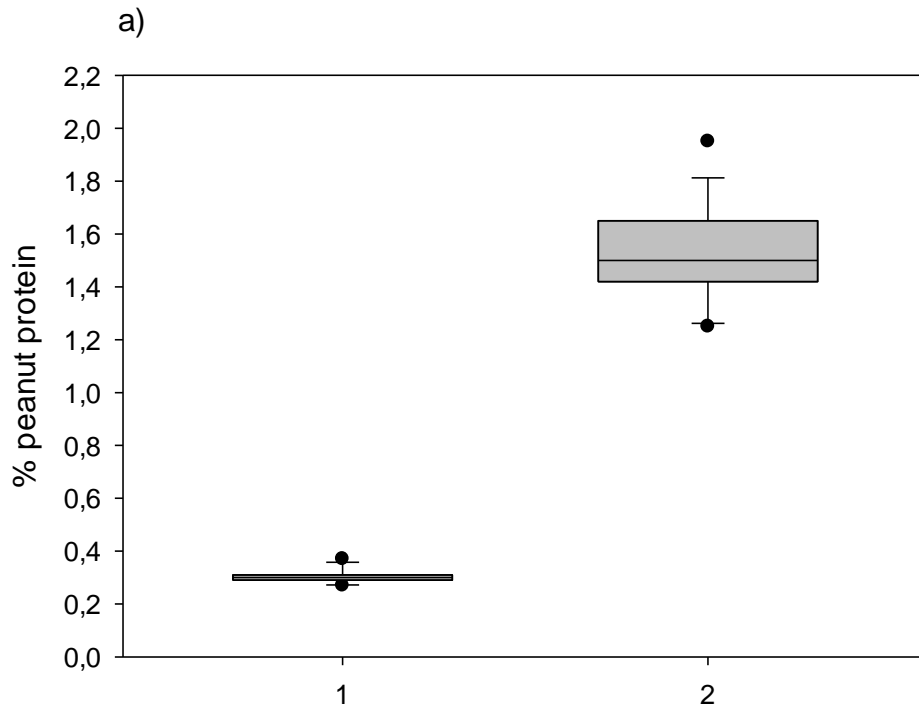
111 With a median recovery (ratio of protein quantified and protein added according to  
112 recipe) of 88% (range 73 – 115 %), the amount of peanut protein of 0.34% and 1.7%  
113 was analytically confirmed in all batches of low and high dose pudding (**FIG E1 a**).

114 Further, all batches of high dose pudding showed homogeneous distribution of  
115 peanut protein with mean CV < 15 % (**FIG E1 b**). In 10/11 low dose pudding bases a  
116 homogeneous peanut protein distribution with mean CV < 15 % was determined.

117 With 15.3 % CV in 1/11 low dose dessert bases the upper limit of the 95%-  
118 confidence interval slightly exceeded the set limit of 15 % mean CV but was still  
119 interpreted as presenting acceptable homogeneity. Statistical significance was  
120 achieved in the majority of cases with  $p < .0001$ . For this above described batch and  
121 all following batches patients had to step one step down in their dosing schedule and  
122 be monitored when consuming a new batch for the first time. Ready-to eat chocolate  
123 pudding stored frozen showed 91.5 % of detectable peanut protein compared to  
124 freshly made chocolate pudding.

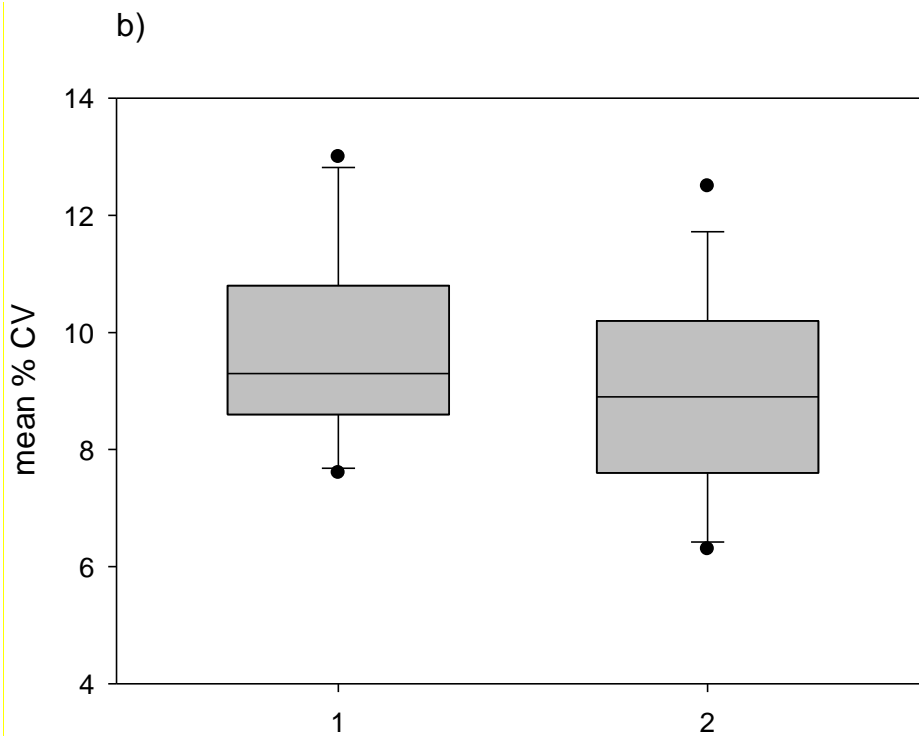
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126 **Fig E1**



127

128



129

130 **FIG E1 Peanut protein quantification and homogeneity in different batches of**

131 **low dose and high dose pudding bases**



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

135

#### 136 **1.4 Statistical analyses: power calculation**

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

142

143

## 144 **1.5 Standard operating procedures (SOPs) for safety**

145 During the inpatient phase (at challenge and start of OIT) emergency training for the  
146 emergency kit was repeated. The emergency kit included two epinephrine auto-  
147 injectors, oral antihistamine, an oral corticosteroid or suppository, and beta2-agonist  
148 for inhalation. Families were advised that the kit should be available at all times. A  
149 24-hour-telephone hotline by medical doctors was available to answer questions  
150 regarding dosing and AEs. Parents were instructed to monitor their children for two  
151 hours after the intake of peanut- /placebo-OIT. Patients were advised to avoid  
152 strenuous physical activity during this time, to carry the emergency medication (e.g.  
153 two epinephrine auto-injectors) at all times and to strictly avoid peanuts otherwise.

154

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

- 156 • If symptoms were considered to be a viral or bacterial infection, the advice  
157 was to continue with 50% of the previous daily OIT dose. This 50% of the dose  
158 was given for three days, followed by another three days with 75% of the  
159 dose. After that the full dose could be ingested again (i.e. “dosing scheme for  
160 infections”). This reduction and up-dosing was done at home. Patients had to  
161 be stable on this dose for further 14 days until another up-dosing to a new  
162 dose could occur in the clinic.
- 163 • If symptoms were considered to be due to an accidental reaction or the patient  
164 received a vaccination or an elective operation, the OIT-dose was skipped that  
165 day and for the following days the “dosing scheme for infections” was applied.

- 166 • For safety reasons doses were reduced by one dose- step if a new charge of  
167 chocolate peanut/placebo- pudding bases was used.
- 168 • If a mild related or non-related AE (severity grade I) or a mild AE at up-dosing  
169 or a subjective AE occurred, the same dose was given the next day at home.
- 170 • If an objective, moderate related AE (severity grade II-IV) or an objective,  
171 moderate AE at up-dosing occurred, either the “dosing scheme for infections”  
172 or a reduction of dose (one step down) was applied at home as determined by  
173 the study physician. The subsequent up-dosing was performed in the study  
174 center.
- 175 • If a moderate non-related AE (severity grade II-IV) occurred, either the “dosing  
176 scheme for infections” or a reduction of dose (one step down) was applied or  
177 the same dose was given for another 14 days before up-dosing.
- 178 • If recurrent mild related or recurrent non-related AEs (especially GI symptoms)  
179 were detected, the dose was reduced by one step.
- 180 • If recurrent related or non-related pulmonary symptoms at a certain dose  
181 occurred a more vigorous dose reduction was performed (2 to 12 dose steps  
182 down) as was determined by the study physician. The doses were then  
183 increased every 14 days at home until the former dose was reached.

184

185 **Early termination of the treatment:**

- 186 • OIT-treatment was terminated if a patient showed objective symptoms related  
187 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<sup>5</sup>, parental form, proxy measurement), children (FAQLQ-CF<sup>6</sup>, child form) and  
205 teenagers (FAQLQ-TF<sup>7</sup>, 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,

211 food-related anxiety, social and dietary limitations). It measured the parent's report on  
212 the child's HRQL from the child's perspective. The FAQLQ-CF included 24 items in  
213 four domains (allergen avoidance, risk of accidental exposure, emotional impact,  
214 dietary restrictions). The scoring system was a 7-point Likert scale ranging from  
215 either 0 in the FAQLQ-PF or from 1 in the FAQLQ-CF (= no impact on HRQL) to 6 in  
216 the FAQLQ-PF or 7 in the FAQLQ-CF (= extreme impact on HRQL). To harmonize  
217 both scales in data analysis the raw scores 0-6 in the FAQLQ-PF were recorded as  
218 1-7, as in other studies<sup>6, 8</sup>. For comparison of changes in HRQL before and after OIT  
219 in both study groups only complete data sets were considered for analysis (PP  
220 analysis). The mean total and mean domain scores were calculated for each  
221 child/mother.

222

## 223 **2. Supplemental Results**

### 224 **2.1 Drop outs**

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

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

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

259 be excluded.

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273 **2.2 Safety**

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**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	
<b>Number of OIT doses associated with.....</b>			<b>p values*</b>
AEs in total, n= (%)	170 (1.2)	534 (4.3)	<b>.001</b>
<b>Subjective</b> AEs, n= (%)	72 (0.52)	459 (3.7)	<b>&lt;.001</b>
OAS, n= (%)	29 (0.21)	281 (2.26)	<b>.003</b>
Abdominal pain, n= (%)	20 (0.14)	111 (0.89)	<b>&lt;.001</b>
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
<b>Objective</b> AEs, n= (%)	107 (0.77)	85 (0.68)	.98
<b>Objective</b> AEs during up-dosing, n= (%)	99 (0.84)	81 (0.78)	.99
<b>Objective</b> 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)	<b>.045</b>
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 <u>severity grade</u> I, n= (%)	6 (0.04)	12 (0.1)	.46
AEs of <u>severity grade</u> II, n= (%)	54 (0.39)	12 (0.1)	.81
AEs of <u>severity grade</u> III, n= (%)	28 (0.2)	50 (0.4)	.73
AEs of <u>severity grade</u> IV, n= (%)	19 (0.14)	11 (0.1)	.28
AEs of <u>severity grade</u> V, n= (%)	1 (0.01)	0 (0)	.33
<u>Treatment</u> 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

277



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**

283

284 Table is in landscape format, attached in extra file

285

286 **2.3 Health related quality of life (HRQL)**

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288 **TABLE E4: Baseline median scores of FAQLQ prior to start of OIT as measure**289 **of HRQL**

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

290 Results are reported as the median [IQR] baseline scores of total and specific

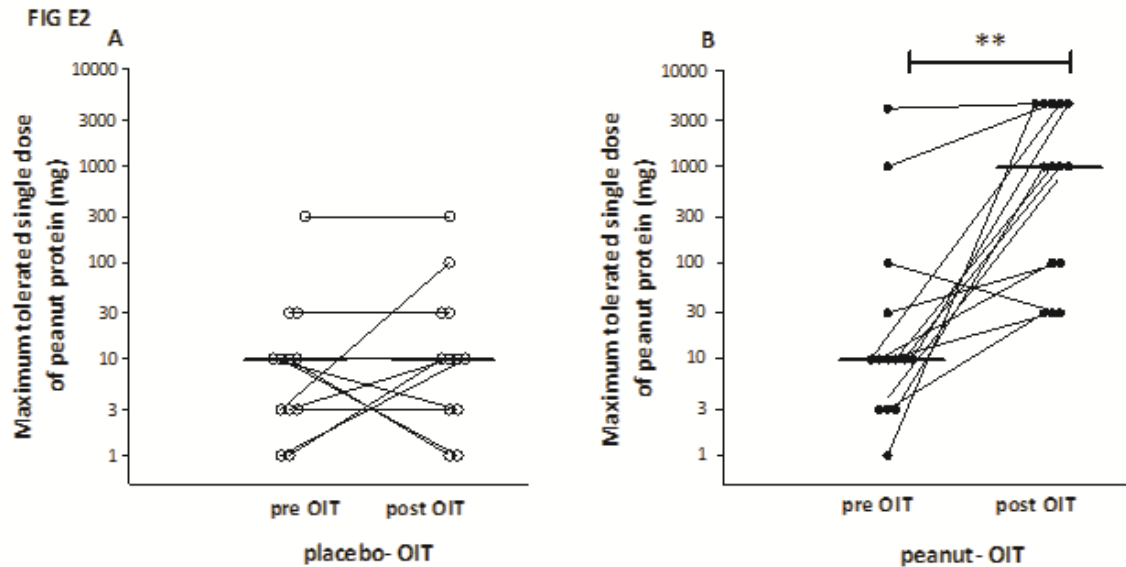
291 domains of the distributed questionnaire for HRQL (FAQLQ).

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

293

294 **3. Supplemental Discussion**

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296

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

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

307 \*\*p&lt;.01

308

309 **TABLE E5: Characteristics of patients who tolerated a maximum dose of**  
 310 **≥300mg peanut protein at baseline OFC**

311 Table is in landscape format, attached in extra file

312

313 **4. Supplemental References**

314

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- 343

**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

<b>peanut-OIT</b>	15	1	23 hours	Possible accidental reaction after Sushi meal	OAS, coughing, allergic rhinoconjunctivitis, itching	III	Antihistamine i.v., corticosteroid i.v. → hospitalization
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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  $\geq 300$ mg peanut protein at baseline OFC (PP population)

Patient number	Randomization 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)