Supplementary Figure E1 – Cumulative dose ingested during entry open OFCs in the E-OIT ITT population

Supplementary Figure E2 – Distribution of all allergic AEs during build-up and maintenance phases. Multiple symptoms included any single reaction that involved multiple systems (skin/gastrointestinal/upper respiratory/ lower respiratory). This group does not overlap with the other groups which involved isolated symptoms in each specified category. The "Other" category included isolated symptoms that occurred with <5% frequency (isolated cough at 2%, isolated angioedema at 1%, and isolated eye-tearing at 0.5%).

SUPPLEMENTAL METHODS

## Intervention

Participants were randomly assigned to receive peanut OIT at 300 mg or 3000 mg per day target maintenance doses. To maintain allocation concealment, unblinded laboratory personnel kept the randomization table and manufactured and labeled the study product. We purchased 12% lightly roasted, partially defatted peanut flour (Golden Peanut Co., Alpharetta GA) and manufactured it at the MSRB Duke Manufacturing Facility and subsequently at the University of North Carolina Manufacturing Facility under 21 CFR 211 Good Manufacturing Practice. Release testing of the investigational drug product was performed in accordance with U.S. Pharmacopeia standards and described in the Chemistry, Manufacturing, and Control section of our Investigational New Drug Application filing with the Food and Drug Administration (IND-13665, PI: Burks). For the low-dose arm, oat flour was purchased (Arrowhead Mills) and toasted and mixed in with peanut at all dose steps above 300 mg to maintain blinding. Doses were packaged in polystyrene cups (Solo Corp) and individually labeled with the subject's study ID. Participants consumed one dose per day by mixing the investigational product in a vehicle food of their choosing (e.g. applesauce, pudding). They otherwise maintained a peanut-free diet. We advised but did not require participants to dose at approximately the same time every day on a full stomach, and we recommended limited activity such as quiet play for approximately two hours after dosing. On the basis of previously published work, we provided standard anticipatory guidance about the withholding of doses for illnesses common to this age group such as febrile infections, gastroenteritis, etc[1]. A caregiver for each participant filled out a daily dosing log, noting whether the dose was given or held and any adverse events. Each participant had an up-to-date food allergy action plan, an in-date epinephrine autoinjector, and around-the-clock access to an on-call allergy physician.

## Dosing Schedule

All participants underwent an initial-day escalation (IDE) phase, and those able to tolerate a minimum of 3 mg proceeded to an approximately 42 week buildup phase, to a goal maintenance dose of 3000 mg/day, which was continued until the end of the maintenance period. The following tables show the schedules for Initial Day Escalation and Buildup Phases (Table E1 and Table E2).

**Food challenge assessments**

At screening, eligible participants underwent an open oral food challenge to 4 grams of peanut protein, using peanut butter. Challenges were judged positive only when participants demonstrated clear objective evidence of an allergic reaction (urticaria, angioedema, respiratory distress/wheeze/cough, vomiting/diarrhea, anaphylaxis).

At the end of the maintenance period upon qualifying for endpoint assessment, subjects presented to the clinical research unit to assess clinical desensitization with a double-blinded, placebo-controlled food challenge (DBPCFC) to a cumulative total of 5 grams of peanut protein. Prior to the DBPCFC, subjects were asked to restrict the use of antihistamines (short acting, 72 hours: long acting, 7 days), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours). One part of the DBPCFC consisted of six doses of peanut given every 10-20 minutes in increasing amounts up to a total weight of 5 grams of peanut protein. The other part of the challenge consisted of equal amounts of placebo (oat) material given also in six doses. The cumulative dose of peanut protein given is 5 grams (10 gram weight of peanut flour), and all doses were mixed thoroughly and delivered in a non-allergenic vehicle of the subject's choosing, usually applesauce or pudding. Both challenges started by first touching the patient's lip/tongue with a small amount of the test material. The first ingested dose was 0.5 grams (5 %), then increasing to 1 gram (10%), 2 grams (20%), 2.0 grams (20%), 2 grams (20%), and 2.5 grams

(25%). Randomization and preparation of the challenge materials were performed by an unblinded research nutritionist or a representative from Dr. Burks' laboratory. If all of the challenge material was consumed without dose-limiting symptoms, OIT was stopped for 4 weeks, and the DBPCFC was repeated, using the same procedure, to assess for SU. SU was confirmed with an open feeding of 5-8 grams of peanut butter in one serving 1-2 hours after the completion of the DBPCFC. Such subjects were then instructed to add peanut to their diets *ad libitum*.

**Mechanistic studies**

*Serologic and cell-based assays*

Peripheral blood was collected in serum-separator or sodium-heparin tubes. Serum and plasma were collected by whole-blood centrifugation, and stored frozen until analysis. Peripheral blood mononuclear cells were isolated and cultured under various conditions; the results of these experiments will be reported separately. A subset of subjects had blood drawn in sodium-heparin tubes to assess basophil activation at the time of both exit DBPCFCs, using previously described assay methods[2].

*Total-IgE and peanut-specific IgE and IgG4 quantification*

Peanut-specific IgE and IgG4, as well as total-IgE were measured via ImmunoCAP 100 (Thermo Fisher, Uppsala, Sweden) according to manufacturer's specifications.

**Power/Statistical Considerations**

No placebo-controlled studies have been published that evaluated the development of tolerance/sustained unresponsiveness after years of treatment with peanut OIT. Published data from our own uncontrolled pilot study in older children with long-standing disease suggest that suggested that SU developed in 12/24

(50%) of peanut-allergic subjects completing high-dose OIT[3]. Based on the preliminary data that were available to us at the time this study was conceived in 2008, and according to our hypothesis, we predicted prior to the study that 70% of low-dose subjects would develop sustained unresponsiveness, compared to the expected rate of spontaneous peanut allergy resolution of 20% as shown in multiple cohort studies. At a two-sided significance level of 0.05, 15 subjects in each treatment arm would have at least 80% power to detect a 50% absolute average difference between the proportion of subjects in each arm passing the exit SU OFC and the 20% rate of spontaneous tolerance expected in untreated controls. Based on a prestudy assumption of 15-20% dropout, we enrolled 20 subjects per arm to ensure adequate power.

Practical considerations prevented a trial large enough to show definitive comparisons of high and low dose therapy directly. Twenty subjects in each arm would have 63% power to identify a 40% difference in SU acquisition between regimens. Even if underpowered to show a difference between low and high dose therapy, we reasoned *a priori* that a Type II error in this setting may still be clinically meaningful so long as the proportion achieving SU in the low-dose group significantly exceeded 20%. This would be especially true if low-dose therapy offers other advantages (fewer visits, better safety profile, improved palatability, etc.).

**Control cohort**

We collaborated with the pediatric allergy group at Johns Hopkins (C.K., R.A.W.), whose practices consist of peanut-allergic patients of similar age and severity as that of the lead site where the trial occurred. In addition, the clinic cohort at Johns Hopkins has formed the basis of several seminal and highly cited studies of the natural history of food allergies in US children[4-8], and importantly was not recruiting young children in peanut OIT at the time our study was enrolling. In retrospective fashion, we recruited a standard-care control cohort that was matched for age between 9-36 months at enrollment,

clinical history (e.g., both sensitized-not-ingested and clinically allergic), peanut allergy severity, absence of severe atopic dermatitis; severe/uncontrolled asthma; oat allergy; and eosinophilic disorders. Additional criteria for inclusion in the control cohort included having at least two visits separated by at least six months, as well as absence of recent immunomodulatory drugs or participation in a clinical trial. Though the standard of care for peanut allergy has not changed for young children since the initiation of this project, we did perform contemporaneous and consecutive enrollment into the control cohort; e.g., every qualifying clinical patient who was seen at Johns Hopkins during the period of trial enrollment 2009-2011 was included. We aimed to enroll at least 120 subjects in order to give at least 3 controls for each “case” receiving OIT in the trial.

## REFERENCES


1. Varshney P, Steele PH, Vickery BP, et al. Adverse reactions during peanut oral immunotherapy home dosing. J Allergy Clin Immunol. United States2009:1351-2.
2. Thyagarajan A, Jones SM, Calatroni A, et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. Clin Exp Allergy 2012;42:1197-205.
3. Vickery BP, Scurlock AM, Kulis M, et al. Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. J Allergy Clin Immunol 2014;133:468-75.
4. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol. United States2007:1172-7.
5. Savage JH, Matsui EC, Skripak JM, Wood RA. The natural history of egg allergy. J Allergy Clin Immunol. United States2007:1413-7.
6. Savage JH, Limb SL, Brereton NH, Wood RA. The natural history of peanut allergy: Extending our knowledge beyond childhood. J Allergy Clin Immunol 2007;120:717-9.
7. Savage JH, Kaeding AJ, Matsui EC, Wood RA. The natural history of soy allergy. J Allergy Clin Immunol 2010;125:683-6.
8. Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. J Allergy Clin Immunol 2001;107:367-74.

**Table E1 - Initial Day Escalation**

| Dose # | Dose | Interval (minutes) | % Increase |
|---|---|---|---|
| **1** | **0.1mg** | **30** | **n/a** |
| **2** | **0.2mg** | **30** | **100** |
| **3** | **0.4mg** | **30** | **100** |
| **4** | **0.8mg** | **30** | **100** |
| **5** | **1.5mg** | **30** | **87.5** |
| **6** | **3mg** | **30** | **100** |
| **7** | **6mg** | **30** | **100** |

123 **Table E2 - Build-up Phase**

| Dose # | Dose | Interval (weeks) | % Increase |
|---|---|---|---|
| **7** | **6mg** | | **as above** |
| **8** | **12mg** | **2** | **100%** |
| **9** | **25mg** | **2** | **108%** |
| **10** | **50mg** | **2** | **100%** |
| **11** | **75mg** | **2** | **50%** |
| **12** | **100mg** | **2** | **33%** |
| **13** | **125mg** | **2** | **25%** |
| **14** | **156mg** | **2** | **25%** |
| **15** | **195mg** | **2** | **25%** |
| **16** | **245mg** | **2** | **25%** |
| **17** | **306mg** | **2** | **25%** |
| **18** | **383mg** | **2** | **25%** |
| **19** | **479mg** | **2** | **25%** |
| **20** | **599mg** | **2** | **25%** |
| **21** | **749mg** | **2** | **25%** |
| **22** | **936mg** | **2** | **25%** |

| | | | |
|---|---|---|---|
| **23** | **1170mg** | **2** | **25%** |
| **24** | **1463mg** | **2** | **25%** |
| **25** | **1829mg** | **2** | **25%** |
| **26** | **2286mg** | **2** | **25%** |
| **27** | **3000mg** | **2** | **31%** |

124

125

**Table E3. Safety Data by Treatment Arm**

| | Overall | Buildup | Maintenance |
|---|---|---|---|
| Subjects Affected by AEs | | | |
| All subjects | 95% (35/37) | 92% (34/37) | 27% (9/33) |
| High Dose | 100% (17/17) | 100% (17/17) | 43% (6/14) |
| Low Dose | 90% (18/20) | 85% (17/20) | 16% (3/19) |
| Average Rate of AEs per person per dose (95% CI) | | | |
| All subjects* | 0.8% (0.3%, 1.4%) | 1.5% (0.9%, 2.2%) | 0.06% (0%, 0.1%) |
| High Dose* | 1.1% (0%, 2.3%) | 1.9% (0.6%, 3.2%) | 0.06% (0.01%, 0.1%) |
| Low Dose | 0.6% (0.3%, 0.9%) | 1.2% (0.5%, 2.0%) | 0.05% (0%, 0.2%) |
| Total Number of AEs | | | |
| All subjects | 211 | 195 | 16 |
| High Dose | 133 | 126 | 7 |
| Low Dose | 78 | 69 | 9 |
| Proportion of moderate severity AEs[+] | | | |
| All subjects | 17% (36/211) | 17% (33/195) | 19% (3/16) |
| High Dose | 13% (17/133) | 12% (15/126) | 29% (2/7) |
| Low Dose | 24% (19/78)[†] | 26% (18/69)[††] | 11% (1/9) |
| Study Withdrawals | | | |
| All subjects | 14% (5/37) | 11% (4/37) | 3% (1/33) |
| High Dose | 24% (4/17) | 18% (3/17) | 7% (1/14) |
| Low Dose | 5% (1/20) | 5% (1/20) | 0% (0/19) |

* Rate of AE = number of AEs/days on therapy. Because subject 36 was never able to start therapy due to inability to complete the modified rush, this subject was excluded from these calculations.

+All AEs were either mild or moderate (no severe AEs reported).

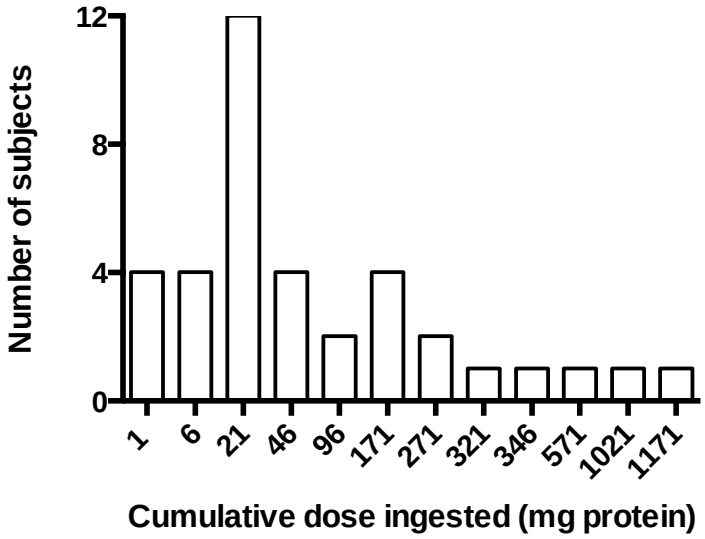
† p=0.04, compared to High Dose group

†† p=0.02, compared to High Dose group

**Table E4. Treatment of Likely-Related Adverse Events**

| Treatment | Events requiring treatment | | | Subjects requiring treatment | | |
|---|---|---|---|---|---|---|
| | Overall | Buildup | Maintenance | Overall | Buildup | Maintenance |
| Treated (all subjects) | 54 (26%) | 47 (24%) | 7 (44%) | 20 (54%) | 16 (43%) | 5 (15%) |
| Antihistamines | 52 (25%) | 46 (24%) | 6 (38%) | 20 (54%) | 16 (43%) | 5 (15%) |
| Albuterol | 3 (1%) | 1 (0.5%) | 2 (13%) | 3 (8%) | 1 (3%) | 2 (6%) |
| Epinephrine | 1 (0.5%) | 1 (0.5%) | 0 (0%) | 1 (3%) | 1 (3%) | 0 (0%) |
| Treated (High Dose) | 39 (29%) | 36 (29%) | 3 (43%) | 9 (53%) | 7 (41%) | 3 (21%) |
| Antihistamines | 38 (29%) | 35 (28%) | 3 (43%) | 9 (53%) | 7 (41%) | 3 (21%) |
| Albuterol | 1 (0.8%) | 0 (0%) | 1 (14%) | 1 (6%) | 0 (0%) | 1 (7%) |
| Epinephrine | 1 (0.8%) | 1 (0.8%) | 0 (0%) | 1 (6%) | 1 (6%) | 0 (0%) |
| Treated (Low Dose) | 15 (19%) | 11 (16%) | 4 (44%) | 11 (55%) | 9 (45%) | 2 (11%) |
| Antihistamines | 14 (18%) | 11 (16%) | 3 (33%) | 11 (55%) | 9 (45%) | 2 (11%) |
| Albuterol | 2 (3%) | 1 (1%) | 1 (11%) | 2 (10%) | 1 (5%) | 1 (5%) |
| Epinephrine | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |

# Supplementary Figure E1



# Supplementary Figure E2