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Long-Term Treatment with Egg Oral Immunotherapy Enhances Sustained Unresponsiveness That Persists After Cessation of Therapy

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Abstract

Background—We previously reported results of a randomized, placebo-controlled study of egg oral immunotherapy (eOIT), in which 27.5% of subjects achieved sustained unresponsiveness (SU) after 2 years. Here we report results of treatment through 4 years and long-term follow-up.

Objective—To evaluate the efficacy and safety of eOIT in participants treated up to 4 years.

Methods—Egg-allergic children (5–18 y/o) received eOIT (n=40) for up to 4 years or placebo (n=15) ≤1 year. The key outcome was the percentage of subjects achieving SU by Year 4. Safety and immunologic assessments were performed, and long-term follow-up questionnaires were administered after study conclusion (LFQ-1) and 1 year later (LFQ-2).

Results—Of 40 eOIT-treated subjects, 20/40 (50.0%) demonstrated SU by Year 4. For those subjects still dosing during Years 3–4, mild symptoms were present in 12/22 (54.5%) subjects. At the time of LFQ, more eOIT subjects [LFQ-1 23/34 (68%); LFQ-2 21/33 (64%)] were consuming

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unbaked and baked egg vs. placebos [LFQ-1 2/11(18%), p=0.006; LFQ-2 3/12 (25%), p=0.04]. Of subjects achieving SU, 18/20 (90%) completed the LFQ with 18/18 (100%) reporting consumption of all forms of egg. When compared to subjects not achieving SU, subjects achieving SU had higher IgG4 values (p=0.001) and lower egg skin prick test scores (p=0.0002) over time and a lower median baseline ratio of egg-specific IgE to total IgE (1.1% vs. 2.7%, p=0.04).

Conclusions—SU following egg OIT is enhanced with longer duration of therapy, and increases the likelihood of tolerating unbaked egg in the diet.

Keywords

egg allergy; food allergy; oral immunotherapy; desensitization; sustained unresponsiveness; immune tolerance; immunoglobulin E; follow-up

INTRODUCTION

Egg allergy is common in childhood, with a prevalence ranging from 0.5–2.5%.(1–4) Egg and egg-derived products are ubiquitous ingredients; therefore, avoiding accidental exposures leading to reactions is difficult.(5) Although the long-term prognosis of egg allergy is generally favorable, recent studies have suggested that resolution may occur more slowly than was previously appreciated, and a subset of egg allergic patients have egg allergy which persists into adolescence.(6, 7) Until and if tolerance develops spontaneously, patients are at risk for allergic reactions.

Several approaches to mitigating this risk have been examined in clinical trials. The best-studied approach is OIT, a procedure that aims to decrease reactivity to allergen with gradual escalation of daily doses followed by a maintenance treatment period.(8) Our group previously reported that 10 months of treatment with egg OIT (eOIT) was superior to placebo when comparing the successfully consumed dose during ongoing therapy (clinical desensitization) and that this benefit was enhanced with an additional year of therapy.(9) Sustained unresponsiveness (defined as lack of dose-limiting symptoms during an OFC to and subsequent open feeding of egg 4–6 weeks after stopping OIT) was achieved in 28% of the subjects on eOIT by month 24 with all reporting consumption of egg one year later. The results from this trial suggested that OIT may have a long-term disease-modifying effect as noted for outcomes assessed during two years of therapy.

Evidence of such an outcome would be a major breakthrough in the development of a food allergy treatment. Currently, the stability of treatment effects after such trials are not well understood. Two recent studies have provided long-term follow-up data after milk and peanut OIT, and both demonstrated that regular oral intake of the allergenic food appears to be required in order to maintain the protective effect following OIT but continued intake was difficult for some patients to continue.(10, 11) Another study comparing peanut OIT and SLIT demonstrated suppression of basophil effector cell function and dendritic cell-driven Th2 cytokine responses following peanut OIT, with some reversibility of those responses noted when antigen was discontinued in those achieving SU.(12, 13) These studies indicate that if allergen is avoided, clinical relapse can ensue, even among individuals previously considered to be treatment successes. This situation poses potential safety concerns if such

individuals incorrectly believe themselves to be protected.(14) Additional long-term studies after OIT treatment are necessary in order to further examine feasibility, safety, and durability of the treatment effect.

To investigate the effects of long-term OIT in patients with egg allergy, we continued the previously reported trial of eOIT for up to 4 years of treatment.(9) The proportion of subjects achieving sustained unresponsiveness in those 4 years was calculated. After the treatment phase of the study ended, an annual long-term follow-up questionnaire to assess egg consumption patterns was administered.

Materials and Methods

Study design and endpoints assessed

The current study is an extension of a previously published multi-center, randomized, double-blind, placebo-controlled study of eOIT.(9) As previously reported, subjects were enrolled and treated with placebo or eOIT for 10 months. Placebo-treated subjects were discontinued from dosing after 10 months and were followed as treatment controls through Year 2 and then surveyed for long-term follow-up. Subjects receiving eOIT continued dosing to Year 4 and discontinued dosing after passing an OFC off therapy (i.e., those achieving SU) following any yearly challenge point (Years 2, 3 or 4). The key outcome of this study was the percentage of subjects with SU to egg after up to 4 years of eOIT. SU was defined as lack of dose-limiting symptoms during a 10 gm egg white powder (~8 grams egg white protein) OFC and open feeding to a meal-size portion of whole cooked egg 4–6 weeks after stopping eOIT while maintaining an egg restricted diet. Secondary outcomes included safety during the additional years of treatment using methods previously reported(9), and immunologic assessments. Egg consumption was evaluated after the last subject completed the treatment phase by having all available subjects complete a long-term follow-up questionnaire, which was repeated approximately 1 year later. Tolerance to baked egg consumption was not assessed during the study entry or at any point in the study.

Study Population

Subjects were ages 5–18 years, from 5 US sites with inclusion/exclusion criteria previously reported.(9) The study was approved by each site's institutional review board, and written consent/assent was obtained. The study was conducted under an FDA investigational new drug application and monitored by an independent data and safety monitoring board from the National Institute of Allergy and Infectious Diseases.

Egg OIT dosing and participant follow-up

Dried Standard Egg White powder (raw, uncooked egg) was purchased from a commercial manufacturer (Deb-El Food Products) and was manufactured for individual doses for eOIT dosing. The daily OIT dose was mixed in a vehicle, such as pudding or applesauce, for dosing. Limiting physical activity was recommended for all participants for the first 2 hours after OIT dosing. Subjects who attained SU at any challenge point were instructed to incorporate egg into their diets ad libitum; however, there were no specific recommendation made on frequency, amount, or type of egg product.(9) Subjects who did not develop SU at

Year 2 or Year 3 were instructed to continue egg avoidance and to continue open-label dosing, per protocol, with 2,000 mg/day of eOIT for up to four years of treatment. Subjects who failed the SU OFC resumed eOIT maintenance dosing through dose escalations every 1–2 weeks beginning with 25% of their maintenance dose or their highest tolerated cumulative dose during OFC, whichever was lowest. Subjects who withdrew from dosing for any reason other than achieving SU or were originally in the placebo treatment arm were instructed to continue dietary avoidance of egg. An exception included one site's IRB mandate to crossover placebo subjects to eOIT treatment after Year 2 as part of a separate treatment protocol. Subjects who did not achieve SU after the 4-year study period were discontinued from dosing and instructed to continue dietary avoidance. For LFQ analysis, subjects were grouped into 4 categories based on their treatment and last known clinical outcome status: 1) Egg OIT: SU, 2) Egg OIT: desensitized, 3) Egg OIT: not-desensitized, and 4) Placebo.

Oral food challenge (OFC) assessment

At Years 2, 3 and 4, all subjects treated with eOIT underwent a 10 gm (cumulative dose) OFC to egg white powder to assess desensitization, i.e., the ability to consume egg while on OIT. Those who passed the desensitization OFC discontinued OIT dosing for 4–6 weeks and had a second (10 gm) OFC, followed by a 10 gram open egg feeding, to assess for SU, as previously reported.(9) The food challenge dose was mixed in a vehicle, typically pudding or applesauce, for dosing.

Long-term Follow-up Questionnaire

A scripted 25-item LFQ was administered twice (LFQ-1 after the last subject completed dosing and study OFCs; LFQ-2 approximately 1 year later). This survey (see On-line Repository) captured patterns of unbaked and baked egg consumption in egg eOIT participants and those previously treated with placebo OIT. In the LFQ, unbaked egg products referred to scrambled eggs, fried eggs, raw eggs, undercooked eggs, French toast or custard; baked egg products referred to cakes, muffins, and waffles. Subjects were asked to rate the frequency of symptoms to various forms of egg ingested to assess consumption patterns and adverse symptoms.

Assessment of Immunologic Markers/Mechanisms

Immunologic mechanisms were assessed during the study as previously described.(9) Skin prick testing (SPT), serum egg-specific IgE and IgG4 antibody levels, and basophil activation studies were performed at 6-month intervals through the end of the study (Year 4). Endpoint titration SPT was performed at Year 4 by measuring SPT wheal size using 5 serial weight/volume egg extract dilutions using commercially available egg white extract from Greer Laboratories (1:20, 1:200, 1:2,000, 1:20,000, 1:200,000).(15)

Statistical Methods

The study design was previously described(9) and, in brief, was adequately powered to compare the placebo arm to the eOIT arm for the primary endpoint (i.e., at 2 years) but was not specifically designed to ensure power for long-term comparisons of interest. The key

outcome of interest for the extension phase was the cumulative proportion of eOIT subjects achieving SU through 4 years. The proportion of eOIT subjects who did not achieve SU at 2 years but did achieve SU when treated after 2 years was also estimated. Comparisons of interest for immunologic assessments were between eOIT subjects achieving SU and those who did not. Differences between groups in continuous variables were assessed using exact Wilcoxon rank sum tests where computationally possible and the asymptotic Wilcoxon rank sum p-value in all other cases. Egg endpoint titration area under the curve was calculated as the sum of the wheal scores at each of the 5 serial weight/volume egg extract dilutions and assessed using the Wilcoxon rank sum test. Basophil activation, immunoglobulin levels, and SPT wheal scores were each evaluated in repeated-measurement models, with the baseline value, study visit, and SU status at Year 4 as covariates and heterogeneous compound symmetry within-person covariance. Hypothesis testing was performed using the log 10 transformation for IgE and IgG4 while summary statistics are reported on the observed scale. For the LFQ, analysis was primarily descriptive although differences between original treatment groups (placebo versus eOIT) in proportions of categorical variables were evaluated using Fisher's Exact test and in continuous variables using the Wilcoxon rank sum test. Logistic regression was used to identify baseline and Week 44 clinical and mechanistic factors (log transformed where noted) that might identify SU. All analyses were performed with the use of SAS software, version 9.2 (SAS Institute).

Results

Study Population

Fifty-five subjects enrolled initially (Figure 1); 6/40 (15%) eOIT and 2/15 (13.3%) placebo subjects withdrew from dosing before 24 months; one eOIT subject withdrew after 24 months, but prior to resuming dosing; 8 additional eOIT subjects withdrew from dosing after 24 months, before the end of study at Year 4 (see Table S1 in Online Repository). One of these subjects was withdrawn at month 30 for non-compliance due to regular whole egg consumption prior to passing any study OFC.

Sustained unresponsiveness is enhanced with duration of eOIT dosing

As previously reported, 0/15 (0%) placebo and 22/40 (55%) eOIT subjects were desensitized to 5 grams of egg white powder after 10 months of therapy, and 30/40 (75%) of eOIT subjects were desensitized to 10 grams of egg white powder after 22 months of therapy.(9) The number of eOIT subjects attaining desensitization reached 31/40 (77.5%) at Years 3 and 4 (Table 1). SU at Year 2 occurred in 11/40 (27.5%) of eOIT subjects(9) and increased to 18/40 (45.0%) in Year 3 and 20/40 (50.0%) in Year 4. Among the 22 eOIT subjects on treatment after Year 2, 41% achieved SU by Year 4. Logistic regression identified no baseline clinical features (e.g., age, atopic dermatitis, asthma, etc.) as significantly predictive of SU at Year 4.

Long-term OIT dosing induces mild symptoms in a majority of subjects

Symptoms reported during long-term egg OIT dosing—Of 22 subjects on eOIT in Years 3–4, 12 (54.5%) experienced symptoms with dosing, all categorized as mild (see Table S2 in Online Repository for symptoms scoring). Three subjects (13.6%) reported oral/

pharyngeal symptoms only; 9 (40.9%) reported skin, respiratory, or GI symptoms; 6 subjects (27.3%) were treated. Of 8,925 doses administered, 95% (8482 doses) were symptom-free (Table 2). Only 1.9% of doses resulted in symptoms lasting >30 minutes reported during home dosing; 1.6% of doses required treatment with antihistamines during either clinic (2 doses) or home dosing (144 doses). None required treatment with epinephrine for dosing-related symptoms during Years 3–4. Three subjects received 1 dose of epinephrine each during OFCs assessing SU. Dosing symptoms prior to and after Year 2 were summarized among the 22 subjects with eOIT dosing after Year 2. Prior to Year 2, the median percent of doses per subject with any symptoms was 8.0% for these 22 subjects. After Year 2 the median percent of doses per subject was 0.2% for these same 22 subjects, representing a reduction in dosing symptoms after Year 2 of the study.

Pre-Week 44 dosing reactions predictive of SU

We examined pre-Week 44 dosing reactions to determine if early dosing reactions were associated with long term clinical outcomes (see Table S3 in Online Repository). Reactions were compared between eOIT subjects achieving SU and all other eOIT subjects. The only statistically significantly difference was in the percentage of doses with moderate symptoms per subject (Wilcoxon p=0.03). Subjects with no moderate symptoms prior to Week 44 were significantly more likely to achieve SU by Year 4 compared to subjects who had any moderate symptoms in that time period (logistic regression p=0.047, OR=4.64, 95% CI: 1.02, 21.00) (see Table S4 in Online Repository).

Food allergy episodes (FAEs) and unsolicited adverse events (AEs) during Years 3–4—Thirty-five FAEs (food-induced reactions not related to study product) occurred in eOIT subjects in Years 3–4 with only 3 egg-related. Two occurred in eOIT desensitized subjects with symptoms of mouth/throat itching; one treated with antihistamines only. One reaction included abdominal pain and vomiting requiring epinephrine after the subject consumed lightly cooked egg, without any precipitating triggers (such as fever, viral infection, or exercise). This subject had achieved SU 19.4 months prior and subsequently reported on their LFQs unrestricted, asymptomatic egg consumption of baked and unbaked egg several times per week . A total of 141 AEs (events that were not dosing symptom/OFC related) were reported in 21/22 subjects during Years 3–4 of eOIT dosing, mostly MedDRA® categorized as System Organ Class Infections/infestations (40.4%). AEs were predominantly mild (95.7%) with 6 (4.3%) moderate.

Long-term follow-up questionnaire suggests persistence of SU after treatment cessation

Forty-five of 55 subjects (82%) completed the LFQ-1 and LFQ-2 (see Table S5 in Online Repository). The LFQ-1 was completed for eOIT and placebo subjects, respectively, a median of 61.7 (IQR: 57.0, 64.7) and 58.5 (IQR: 56.6, 65.6) months after study enrollment (p=0.74), 15.6 (IQR: 9.9, 26.1) and 37.1 (IQR: 34.4, 39.4) months after the last clinic visit (p<0.001), and at a median age of 12.3 (IQR: 11.2, 13.7) and 13.0 years (IQR: 10.7, 14.0) (p=0.93).

SU following OIT is associated with higher rates of successful egg consumption when compared to other treatment groups—During both LFQ

periods, a significantly higher proportion of eOIT subjects reported consumption of unbaked and baked egg compared to placebo subjects, potentially driven by those achieving SU (Tables 3 and 4). However, when considering dietary consumption of any form of egg (unbaked or baked), only during the LFQ-1 were eOIT subjects significantly different than placebo subjects (p=0.007) (Table 3).

Of eOIT subjects achieving SU, 18/18 (100%) surveyed reported dietary consumption of all forms of egg in LFQ-1 and LFQ-2, while only 4/9 (44%) in LFQ-1 and 2/8 (25%) in LFQ-2 of eOIT desensitized subjects and 1/7 (14%) in both LFQ-1 and LFQ-2 of eOIT not-desensitized subjects consumed unbaked egg (Table 4). Overall, eOIT subjects achieving SU reported increased frequency and amount of egg consumption in all forms when compared to other outcome groups (Table 5; Figure 2; Table S6 in Online Repository). Of subjects achieving SU, 3/18 (17%) had increased frequency of symptoms with egg consumption between LFQ-1 and LFQ-2, and 1/18 (6%) had symptom reduction. Three subjects at LFQ-1 and 4 subjects at LFQ-2 with SU reported symptoms after ingesting unbaked egg; 3 had increased symptoms to unbaked egg at LFQ-2 compared to LFQ-1, and one had decreased symptoms to unbaked egg. One subject with SU reported asymptomatic baked egg consumption in LFQ-1 but increased symptoms with baked egg consumption during LFQ-2. The longest period of time (median) that subjects achieving SU reported going without consuming unbaked egg was 14 days (IQR: 5, 21) in LFQ-1 and 14 days (IQR: 5, 14) in LFQ-2 and without consuming baked egg was 5.5 days (IQR: 2, 14) in LFQ-1 and 3.5 days (IQR: 2, 7) in LFQ-2. For eOIT subjects not achieving SU and those who were placebo-treated, dietary restriction of egg was variable, and successful dietary egg consumption in any form was reported at a lower frequency and lesser amount consumed than among subjects with SU (Tables 4 and 5; Figure 2).

Immunologic data over time

Mechanistic data were compared between subjects achieving SU and those who did not both at Year 4 specifically and over time. At Year 4, only egg SPT score (median: 3.0 vs. 7.0 mm, p=0.04) and endpoint titration SPT area under the curve (median: 3.5 vs. 11.0 mm, p=0.01) were significantly changed in subjects achieving SU at Year 4 (Figure 3; see Table S7–9 and Figure S1 in Online Repository). A repeated measures analysis was performed to examine whether there was a difference in immunologic values over time, after adjusting for baseline value and study visit, between subjects who reached SU and those who did not. Over time, egg IgG4 values were significantly greater in those achieving SU (p=0.001). Similarly, egg SPT scores were significantly lower in those achieving SU (p=0.0002). For egg IgE and basophil activation, there was a decrease over time in those achieving SU; however, the differences were not significant and therefore did not discriminate between those achieving SU and those who did not.

Immunologic predictors of SU

The baseline median egg-specific IgE level was lower, but statistically insignificant, in subjects achieving SU compared with those who did not (9.4 kU_A/L vs. 18.5 kU_A/L, p=0.07). Median percent egg IgE at baseline (i.e., the proportion of egg specific IgE to total IgE multiplied by 100) was significantly lower in subjects with SU (1.1% vs. 2.7%, p=0.04);

however, logistic regression analysis was not statistically significant [OR=0.89, 95% CI (0.78, 1.01), p=0.072]. Logistic regression of mechanistic outcomes identified baseline log10 egg IgE [OR=0.21, 95% CI (0.04, 0.99), p=0.049], Week 44 log10 egg IgG4 [OR=5.48, 95% CI (1.23, 24.38), p=0.026], and Week 44 log10 egg IgG4/IgE [OR=4.62, 95% CI (1.34, 15.84), p=0.015] as statistically significantly predictive of SU at Year 4; however, the area under the curve (AUC) for each model was below 0.75 (log 10 baseline egg IgE, AUC=0.67 (95% CI: 0.50, 0.84),Week 44 log 10 IgG4, AUC=0.71 (95% CI: 0.53, 0.88), and Week 44 log10IgG4/IgE, AUC=0.74 (95% CI:0.59, 0.90)) indicating limited utility as a predictor of treatment response.

Discussion

Numerous single- and multi-center studies have shown a beneficial impact of OIT on allergic responses in food allergic children including short-term protection for allergen desensitization and longer term benefits, such as SU.(9–11, 16–25) However, studies have suggested that long-lasting effects of therapy are unlikely without ongoing antigen exposure and that the effects of therapy may even be transient, highlighting the potential risk for relapse if allergen exposure is not maintained.(10–14, 26)

We previously reported successful desensitization in egg allergic children that improved in subjects from Year 1 (55%) to Year 2 (75%) on eOIT.(9) A smaller subset of subjects (27.5%) developed SU after discontinuing daily OIT treatment.(9) In this treatment extension and long-term follow-up study, we show improvements in achieving SU with continued eOIT with 45% in Year 3 and 50% in Year 4. This outcome may be comparable or slightly higher to rates of natural egg allergy resolution reported as 12–37% in a similar age group.(6) Findings are similar to those published for an open-label peanut OIT study in which 50% of subjects attained SU after 5 years of therapy(11) and slightly higher than reported in a milk OIT trial in which 40% of subjects achieved SU after 15 months of OIT. (10) In a more recent, shorter duration study comparing peanut OIT and SLIT, 3/11 (27%) OIT subjects attained SU after 18 months of therapy.(13)

Wide-scale clinical application of OIT has been hindered by reports of undesirable allergic side effects in randomized trials. In this study, we report long-term safety in Years 3 and 4 of eOIT dosing. Mild symptoms were noted in 54.5% of subjects during dosing with none reporting moderate or severe symptoms. During follow-up, one eOIT subject reported anaphylaxis after consuming unbaked egg 19.4 months after achieving SU. Subsequently, the subject was able to resume ad libitum egg consumption of both unbaked and baked egg without limitations and without symptoms as reported on long-term follow-up questionnaires. Interestingly, during Years 1–2 of eOIT dosing, 15% of eOIT subjects withdrew mostly due to dose-related symptoms (predominantly gastrointestinal),(9) a finding that was not present during Years 3–4. Participants did require unscheduled visits and staff phone calls for advice during illness, further highlighting the importance of close monitoring and staff availability during OIT.

This is the first multi-center OIT study to comprehensively survey allergen consumption and symptoms for up to 4 years of treatment and following study completion via an annual

questionnaire. Overall, 100% of subjects achieving SU were able to consume unbaked egg in their diet. As noted in the results section, only one case of anaphylaxis (due to unbaked egg) was noted in a subject with SU; however, that subject continued to consume unbaked egg in their diet without issues afterward. This finding in the SU treatment group was in direct contrast to other eOIT and placebo treatment groups which demonstrated an overall inability to consistently consume unbaked egg. When comparing baked egg consumption among treatment groups, all eOIT and placebo groups were able to incorporate some amount of baked egg into their diets by the second questionnaire; however, the group achieving SU was able to consume baked egg without limitations more frequently and in higher amounts.

Despite instructions to remain on an egg-free diet, placebo subjects or eOIT subjects who did not attain SU reported unbaked and baked egg consumption with many also reporting symptoms with consumption. This difference in recommended diets and the lack of systemic evaluation of placebo subjects is unfortunately a clear source of bias, as it is possible that if systematically challenged, more placebo treated subjects would be able to tolerate baked or unbaked egg than we found here. Full resolution of egg allergy in 2/12 (17%) placebo subjects and partial improvement in dietary egg consumption in 7/12 (58%) placebo subjects was noted by the second follow-up questionnaire (though one of these were on open-label egg OIT in another study protocol), suggesting the potential for underestimation of the impact of natural history.(6) In fact, at the time of the second questionnaire, there was no longer a significant difference in the inclusion of any form of egg in the diet between the active and placebo treated participants; however, significant differences were noted when considering dietary consumption of all forms of egg.

Our results support the hypothesis that administering OIT for 3 to 4 years results in enhanced rates of SU when compared to administration for only 2 years. We speculate that this effect is due to ongoing immunomodulation in specific T- and B-regulatory processes over time, but we cannot rule out the role of natural history in this uncontrolled study. Furthermore, ongoing, intermittent dietary allergen exposure may be required to maintain SU. Importantly, 17% of those achieving SU reported increased symptoms with variable egg consumption from daily to less than monthly, but all reported continued egg consumption, indicating that variable consumption may have led to symptoms in these subjects. In contrast, another study demonstrated that 100% of peanut OIT subjects treated over 5 years maintained symptom-free, ad libitum peanut ingestion while consuming an average of 555 (range 0–4000) mg/day at a frequency of 3 (range 0–7) times/week.(11) These results indicate that continued follow-up of patients with SU is critical to monitor for relapse.

Although multiple studies have indicated that significant immunomodulation is associated with positive response to OIT,(9, 11, 19, 24, 27, 28) none of the immune parameters tested have been consistently predictive of treatment outcomes. In this study, only egg SPT and endpoint titration SPT were different at Year 4 for subjects with SU, and only egg SPT and log10 egg IgG4 changed significantly over time. Although baseline log10 egg IgE and Week 44 log10 egg IgG4 were predictive of SU, the p-values were marginal, unadjusted for multiple comparisons, and the AUCs were low, thus we would not consider these immune parameters to be strong predictors. Egg-specific immunoglobulin components may provide additional predictors for success.(29) In the 5-year peanut OIT study, the baseline ratio of

peanut-specific IgE:total IgE and the baseline and end-of-study peanut-specific IgE levels were predictive of treatment success.(11) In a recent peanut OIT study, basophil activation and dendritic cell-driven Th2 cytokine responses to peanut were reduced in subjects achieving SU after only 18 months of treatment; however, these parameters reversed in the majority of subjects after OIT was discontinued and were not defined predictors of treatment success.(12) Our findings and interpretations are limited by the small size of the study. Further work is needed to identify biomarkers reliably predictive of a successful treatment response to OIT.

This study has several limitations. First, the initial placebo treatment in this study lasted only 10 months without subsequent food challenges; therefore, the long-term efficacy and safety parameters reported are not controlled. Second, as noted in our first report, the study population was enrolled based on egg allergy criteria that would predict persistence of allergy past 2 years of treatment even though the ability to predict is limited because the subjects did not have a baseline OFC. Thus, natural history may play a role in this long-term analysis. This study was not adequately controlled to assess spontaneous resolution of egg allergy; however, we feel that the majority of a group of children included with elevated egg-specific IgE and history of egg allergy at enrollment are unlikely to develop tolerance naturally. Participants were also not assessed for reactivity to baked egg at baseline, so some might have been baked egg tolerant at study entry. Third, due to the expanding numbers of OIT subjects achieving SU and subject drop out over the 4-year assessment period, the number of participants left to fully evaluate immunologic parameters was small. Thus, the end-of-study analysis is likely underpowered to detect differences that may exist. Finally, the long-term questionnaire data is based on recall reporting rather than prospective longitudinal data collection.

In summary, we demonstrate that the likelihood of SU to eOIT is enhanced with therapy exceeding 2 years with those achieving SU demonstrating an increased likelihood of tolerating unbaked egg in their diet compared to those who did not achieve SU. Half of eOIT-treated subjects achieved SU by Year 4, a treatment response that may require ongoing, intermittent dietary egg exposure to be maintained. For the majority of subjects, eOIT was administered with mild symptoms reported throughout the study. Extensive longitudinal immune evaluation did not identify biomarkers consistently predictive of treatment success. Future trials should focus on expansion of study population size and diversity of subjects with attention to immunologic assays that can help to personalize OIT to enhance predictable success.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DBPC	double-blind, placebo-controlled
kU$_A$/L	kilounits of antibody per liter
LFQ	Long-term follow-up questionnaire
OFC	double-blind, placebo-controlled oral food challenge
OIT	oral immunotherapy
eOIT	egg oral immunotherapy
SPT	skin prick test
SLIT	sublingual immunotherapy
SCIT	subcutaneous immunotherapy
SU	sustained unresponsiveness
MedDRA®	Medical Dictionary for Regulatory Activities

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Key Messages

- Longer duration of treatment with eOIT is associated with enhanced clinical response and increased likelihood of tolerating unbaked egg.
- Mild symptoms are commonly encountered throughout OIT treatment.
- Immune parameters studied do not consistently predict treatment response.

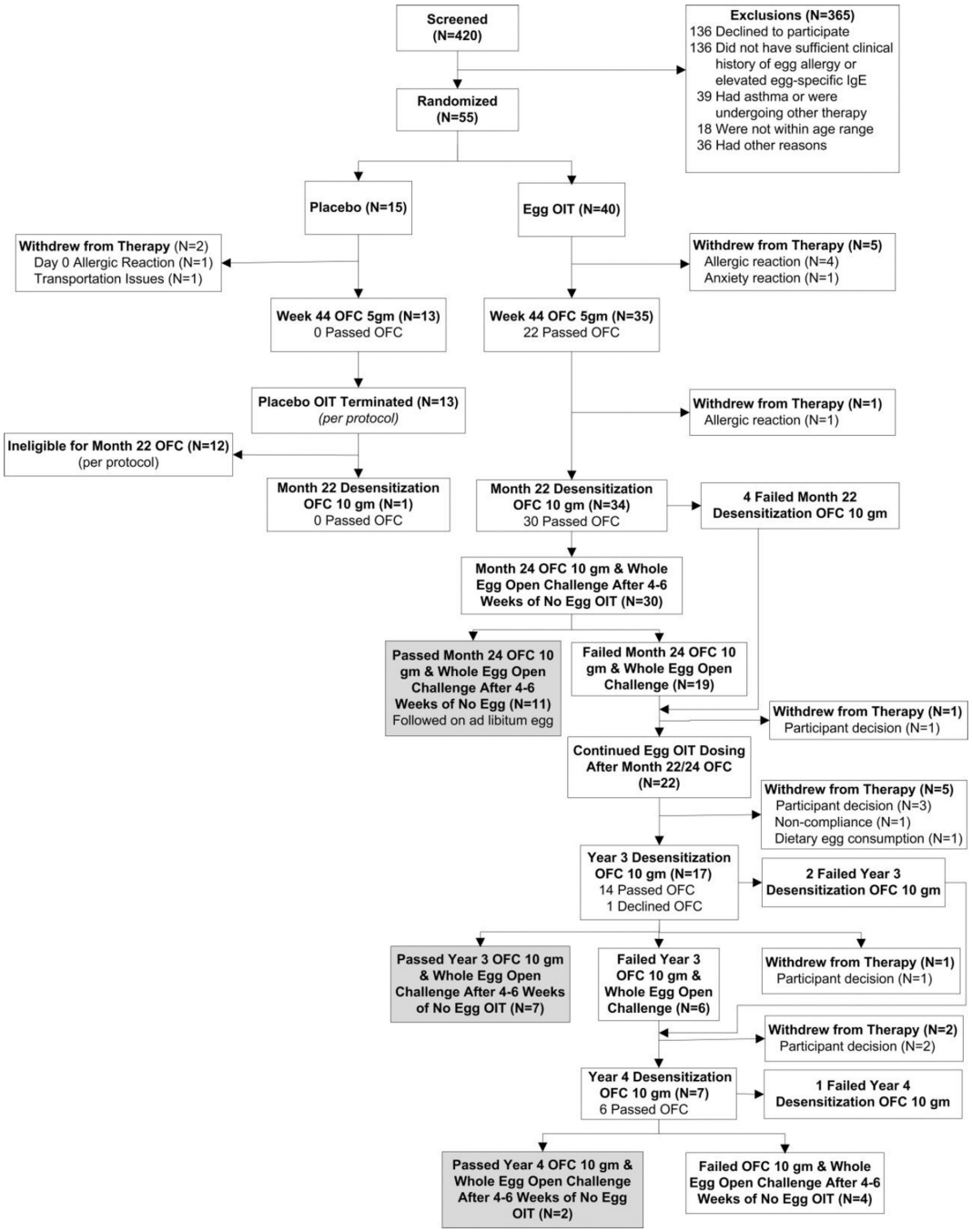
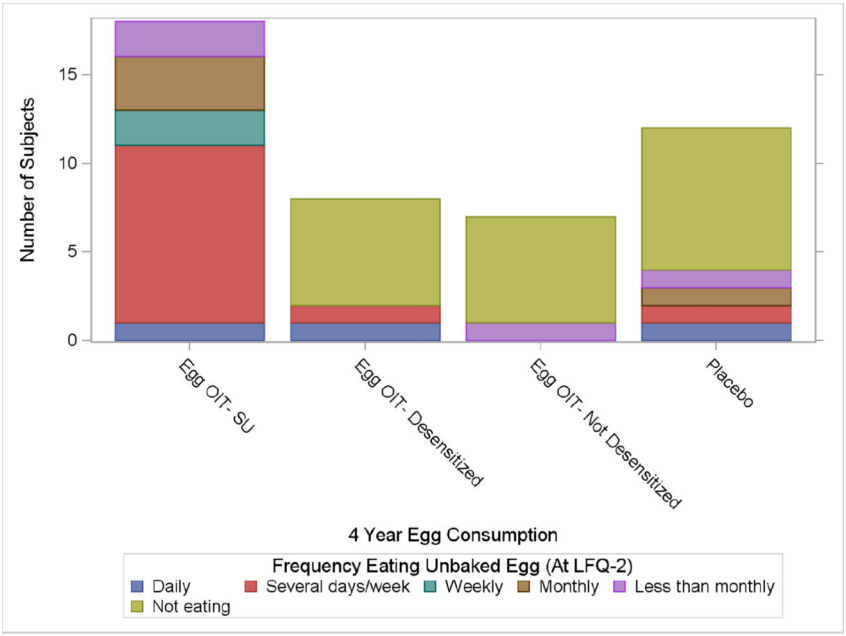
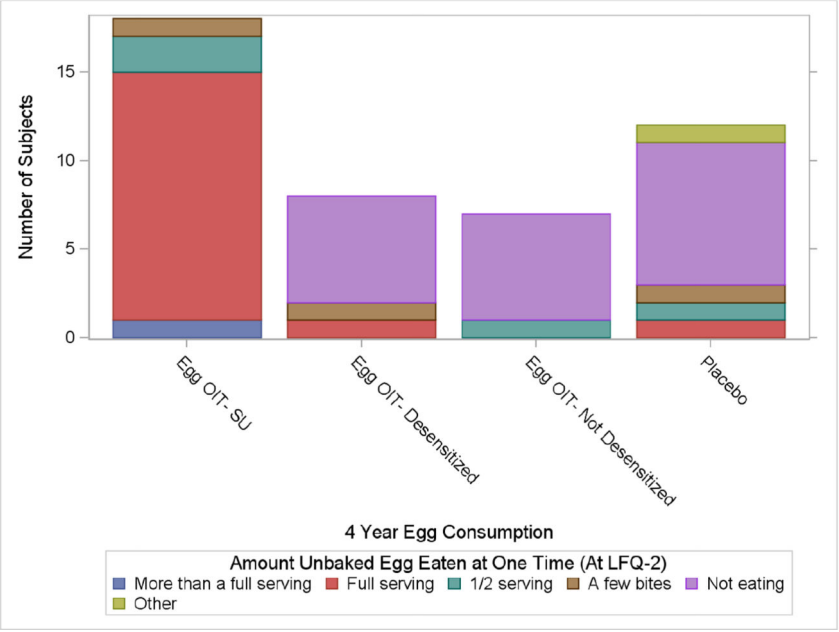


Figure 1. Study enrollment, randomization and treatment outcomes through Year 4
Greyed boxes represent participants achieving sustained unresponsiveness (SU).



Panel B



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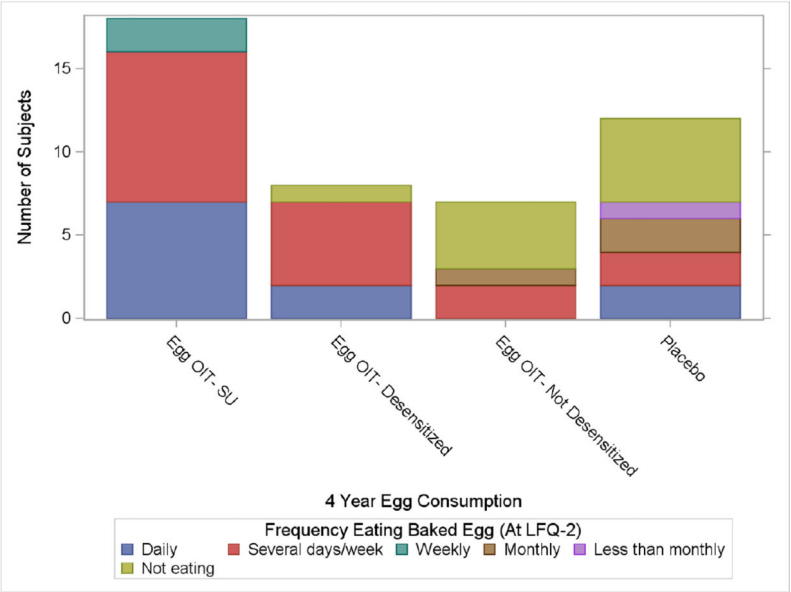


Panel D

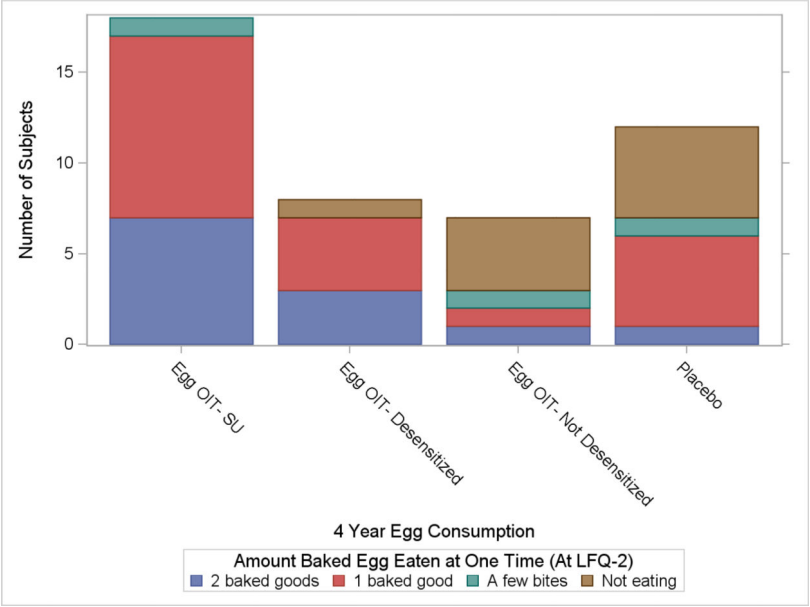


Figure 2. Long Term Follow-up Questionnaire (LFQ-2): Frequency and Amount of Egg Consumption by Treatment Outcome Status

Panel A. Frequency of eating unbaked egg. The percentage of subjects who were eating unbaked egg several days per week or more: Egg OIT–SU=61%, Egg OIT-D=25%, Egg OIT-Not-D=0%, Placebo=17%*. The percentage of subjects who were not eating unbaked egg: Egg OIT–SU=0%, Egg OIT-D=75%, Egg OIT-Not-D=86%, Placebo=67%.

Panel B. Amount of unbaked egg eaten at one time. The percentage of subjects who were eating a full serving or more of unbaked egg: Egg OIT–SU=83%, Egg OIT-D=13%, Egg OIT-Not-D=0%, Placebo=8%.

Panel C. Frequency of eating baked egg. The percentage of subjects who were eating baked egg several days per week or more: Egg OIT–SU=89%, Egg OIT-D=88%, Egg OIT-Not-D=29%, Placebo=33%**.

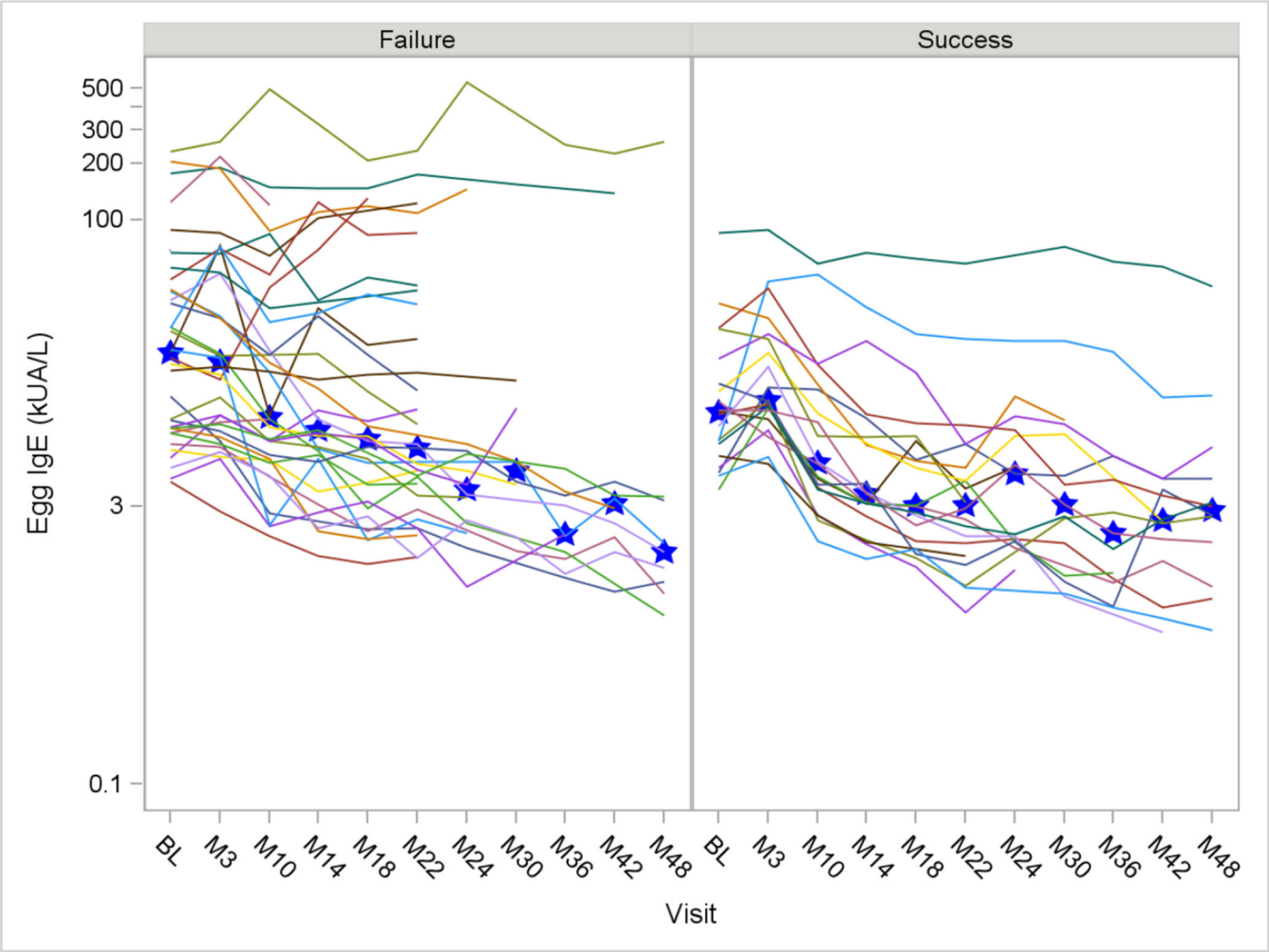
The percentage of subjects who were not eating baked egg: Egg OIT–SU=0%, Egg OIT-D=13%, Egg OIT-Not-D=57%, Placebo=42%*.

Panel D. Amount of baked egg eaten at one time. The percentage of subjects who were eating 1 or more of baked egg goods: Egg OIT–SU=94%, Egg OIT-D=88%, Egg OIT-Not-D=29%, Placebo=50%**.

**One Placebo subject enrolled in an egg OIT study after completing LFQ-1 and consumes egg white powder daily but does not consume baked egg.*

***One Placebo subject crossed over to egg OIT treatment per their site's IRB requirement after 2 years on study and consumes half a serving of concentrated egg less than monthly and 1 baked egg product daily.*

Panel A



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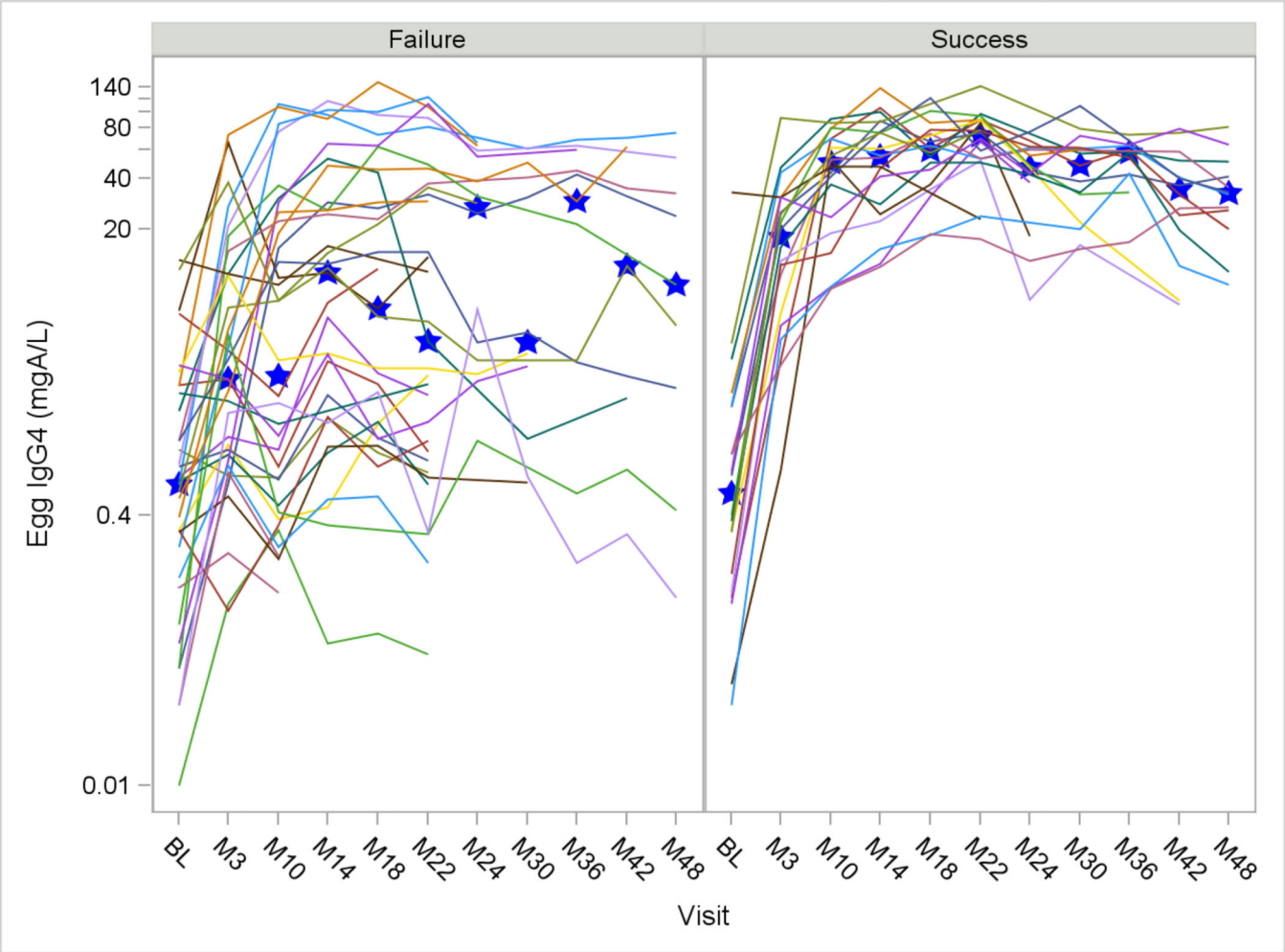

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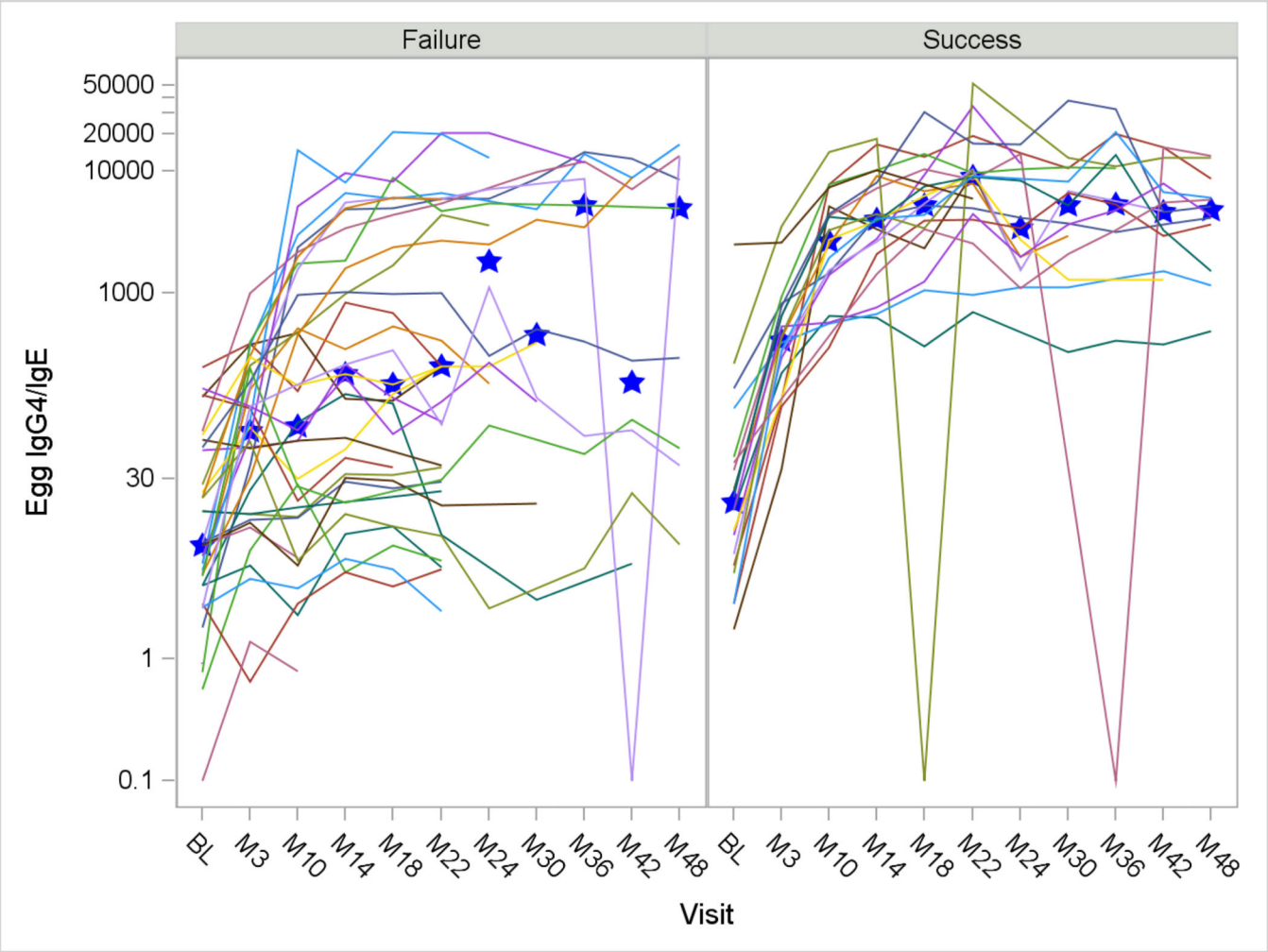
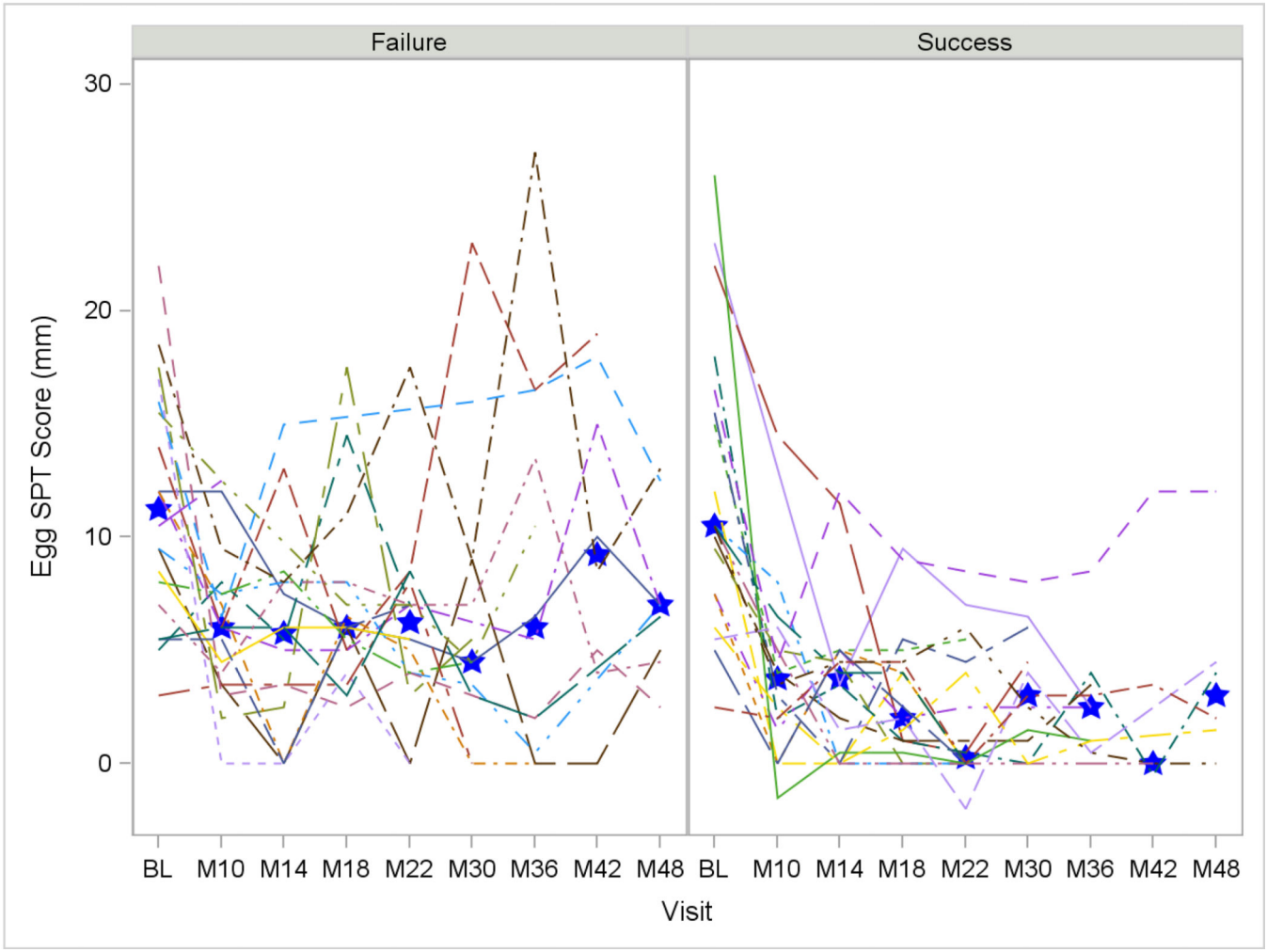


Panel D



Panel E

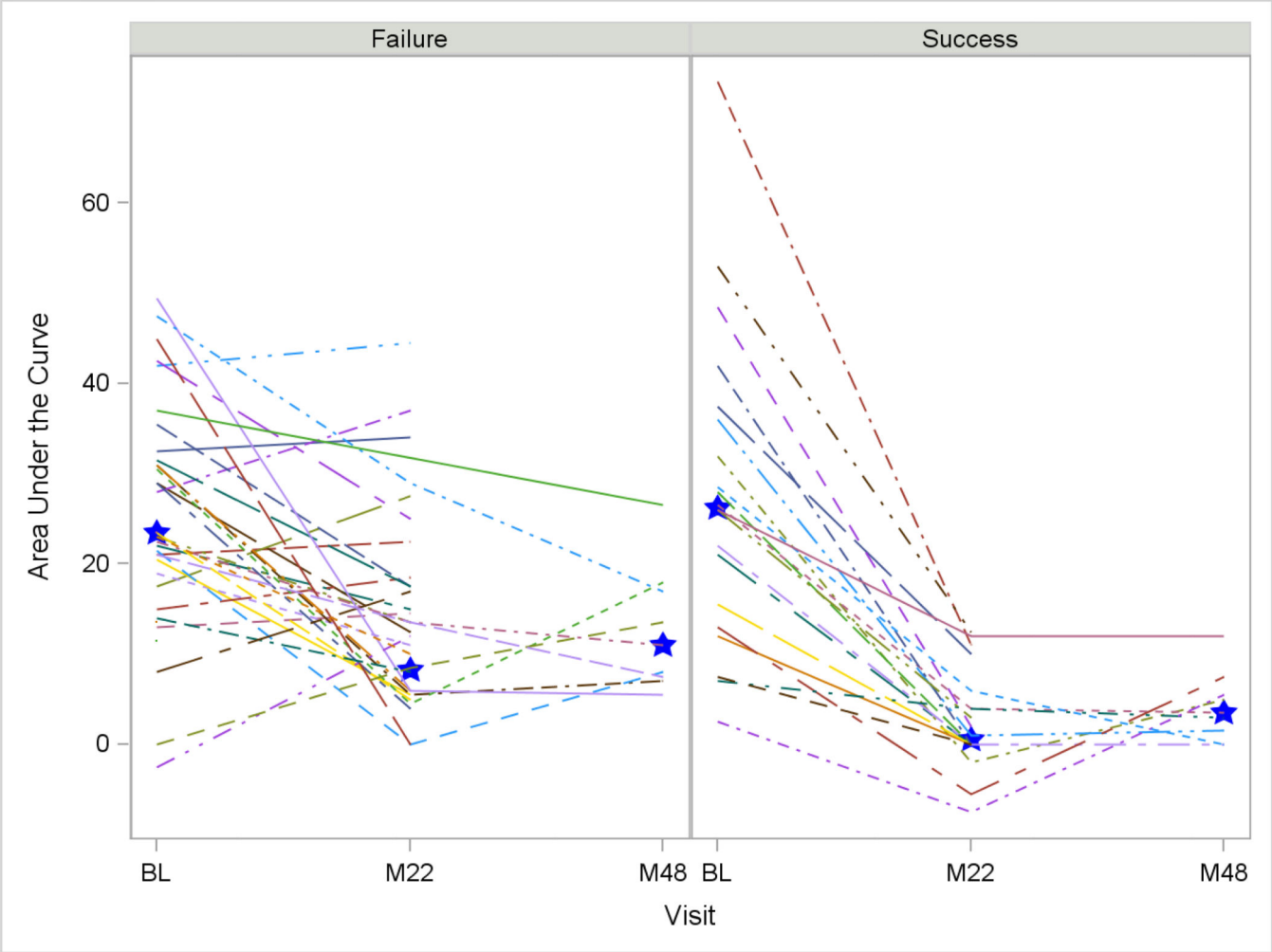


Figure 3. Immune Mechanistic Assessments by Year 4 Treatment Outcome Status for Egg OIT Subjects

Panel A. Change in Egg-Specific IgE Over Time; Panel B. Change in Egg-Specific IgG4 Over Time; Panel C. Change in Egg IgG4/IgE Over Time. Panel D. Change in Egg Skin Prick Test Over Time; Panel E. Change in Egg End Point Titration Area Under the Curve Over Time. Egg IgG4/IgE was calculated by converting IgG4 level from mgA/L to ng/ml and converting IgE level from kUA/L to ng/ml with the formula (IgG4 × 1000/IgE x 2.4). Egg End Point Titration Area Under the Curve was calculated by adding together the scores from all 5 dilutions. Graphs designate “Success” as those subjects treated with egg OIT who achieved sustained unresponsiveness versus “Failure” as those subjects treated with egg OIT who did not achieve sustained unresponsiveness. *Stars* represent median values.

Table 1

Food Challenge Defined Clinical Outcomes with Long-term Egg OIT

Time from Egg OIT Initiation	Desensitization	Sustained Unresponsiveness
Year 2 *	30/40 (75%)	11/40 (27.5%)
Year 3	31/40 (77.5%)	18/40 (45.0%)
Year 4	31/40 (77.5%)	20/40 (50.0%) **

* Previously reported: Burks, et al. NEJM 2012267:233–43.

** Among the 22 egg OIT subjects treated after 2 years, 41% (95% CI 21%-64%) achieved sustained unresponsiveness; one egg OIT subject who failed the 2 year tolerance OFC did not resume egg OIT dosing.

Table 2

OIT Doses (percent)[a] Associated with Symptoms During Years 3 and 4 of Study

			Symptom Type							Symptom Severity		
Visit Type	No. Doses	Any Symptom	Oral/Phary.	Skin	Resp.	GI	Other	Persist > 30 min	Treated	Mild	Moderate	Severe
Clinic	194	14.9	11.3	0.5	1.5	1.0	1.5	0.0	1.0	4.1	0.0	0.0
Home	8731	4.7	2.2	1.1	2.1	0.2	0.2	2.0	1.6	3.0	0.0	0.0
All	8925	5.0	2.4	1.1	2.1	0.3	0.2	1.9	1.6	3.0	0.0	0.0

[a]With the exception of number of doses, values are percent of doses.

GI=gastrointestinal; Phary.=pharyngeal; Resp.=respiratory.

Table 3

Long-term Follow-up Questionnaire: Egg Consumption by Treatment Group

	Egg OIT N (%)	Placebo* N (%)	P-value
Eating any egg in diet			
LFQ-1	28/34 (82)	4/11 (36)	0.007
LFQ-2	28/33 (85)	8/12 (67)	0.22
Eating unbaked and baked egg in diet			
LFQ-1	23/34 (68)	2/11 (18)	0.006
LFQ-2	21/33 (64)	3/12 (25)	0.04

* By the time of LFQ-2, two Placebo subjects were receiving egg OIT in other open-label study protocols; one had crossed-over to active OIT at Year 2 per their site IRB requirements and was eating unbaked and baked egg for LFQ-1 and LFQ-2; they are included in the group eating any egg in diet as well as the group eating unbaked and baked egg in diet for LFQ-1 and LFQ-2. The other subject entered a new study protocol after completion of LFQ-1 and was fully restricting egg except for study product eOIT dosing; for LFQ-2 they are included in the group eating any egg in diet.

Table 4

Long-term Follow-up Questionnaire: Egg Consumption by Treatment Outcome Status

		Unbaked Egg*		Baked Egg Only		No Egg in Diet		Total	
		LFQ-1	LFQ-2	LFQ- 1	LFQ-2	LFQ-1	LFQ- 2	LFQ- 1	LFQ-2
Egg OIT- SU	**Total**	18	18	0	0	0	0	18	18
	No Symptoms	15	14	0	0	0	0	15	14
	Symptoms	3	4	0	0	0	0	3	4
Egg OIT Desensitized	**Total**	4	2	4	5	1	1	9	8
	No Symptoms	4	2	3	4	0	0	7	6
	Symptoms	0	0	1	1	0	0	1	1
	Not in Diet	0	0	0	0	1	1	1	1
Egg OIT- Not Desensitized	**Total**	1	1	1	2	5	4	7	7
	No Symptoms	1	0	0	1	0	0	1	1
	Symptoms	0	1	1	1	0	0	1	2
	Not in Diet	0	0	0	0	5	4	5	4
Placebo	**Total**	2	4	2	4	7	4	11	12
	No Symptoms	1	3	1	1	0	0	2	4
	Symptoms	1	1	1	3	0	0	2	4
	Not in Diet	0	0	0	0	7	4	7	4
Total		**25**	**25**	**7**	**11**	**13**	**9**	**45**	**45**

*The ‘Unbaked Egg’ group includes subjects who had unbaked egg in their diet even if they did not have baked egg in their diet (one Placebo subject enrolled in an egg OIT study after completing LFQ-1 and consumes egg white powder daily but does not consume baked egg). For subjects in the ‘Unbaked Egg’ group, the symptoms shown are to unbaked egg only, not to unbaked or baked egg. For subjects with baked egg only in their diet (‘Baked Egg Only’ group), the symptoms shown are to baked egg only, not to unbaked egg.

Table 5

Long-term Follow-up Questionnaire (LFQ-2): Frequency and Amount of Egg Consumption by Treatment Outcome Status

	Egg OIT- SU		Egg OIT- Desensitized		Egg O IT- Not Desensitized		Placebo*	
	N	%	N	%	N	%	N	%
Total Subjects	18	100.0	8	100.0	7	100.0	12	100.0
Frequency Eating Unbaked Egg								
Daily	1	5.6	1	12.5	0	0	1	8.3
Several days/week	10	55.6	1	12.5	0	0	1	8.3
Weekly	2	11.1	0	0	0	0	0	0
Monthly	3	16.7	0	0	0	0	1	8.3
Less than monthly	2	11.1	0	0	1	14.3	1	8.3
Not eating	0	0	6	75.0	6	85.7	8	66.7
Amount Unbaked Egg Eaten at One Time								
More than a full serving	1	5.6	0	0	0	0	0	0
Full serving	14	77.8	1	12.5	0	0	1	8.3
1/2 serving	2	11.1	0	0	1	14.3	1	8.3
A few bites	1	5.6	1	12.5	0	0	1	8.3
Other	0	0	0	0	0	0	1	8.3
Not eating	0	0	6	75.0	6	85.7	8	66.7
Frequency Eating Baked Egg								
Daily	7	38.9	2	25.0	0	0	2	16.7
Several days/week	9	50.0	5	62.5	2	28.6	2	16.7
Weekly	2	11.1	0	0	0	0	0	0
Monthly	0	0	0	0	1	14.3	2	16.7
Less than monthly	0	0	0	0	0	0	1	8.3
Not eating	0	0	1	12.5	4	57.1	5	41.7
Amount Baked Egg Eaten at One Time								
2 baked goods	7	38.9	3	37.5	1	14.3	1	8.3
1 baked good	10	55.6	4	50.0	1	14.3	5	41.7

	Egg OIT- SU		Egg OIT-Desensitized		Egg O IT- Not Desensitized		Placebo*	
	N	%	N	%	N	%	N	%
A few bites	1	5.6	0	0	1	14.3	1	8.3
Not eating	0	0	1	12.5	4	57.1	5	41.7

* One Placebo subject enrolled in an egg OIT study after completing LFQ-1 and consumes egg white powder daily but does not consume baked egg.