Online Repository Materials

Gastrointestinal eosinophil responses in a longitudinal randomized controlled trial of peanut oral immunotherapy

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Table S1. Proportion of participants crossing eosinophilic gastrointestinal disease (EGID) thresholds at multiple gastrointestinal sites.

	Study Week		
Number of GI biopsy sites with eosinophil counts greater than EGID thresholds*	**0 (n = 20)**	**52 (n = 10)**	**104 (n = 11)**
0	16 (80%)	5 (50%)	7 (64%)
1	3 (15%)	2 (20%)	2 (18%)
2	1 (5%)	2 (20%)	2 (18%)
3	0 (0%)	1 (10%)	0 (0%)

*EGID thresholds: esophagus peak counts of ≥ 15 eos/hpf, average count of ≥ 30 eos/hpf for stomach (in at least 5hpf) and duodenum (in at least 2hpf).

Table S2a. Eosinophilic Histologic Scoring System (EoEHSS) grade scores

	Week 0		***Week 52***		***Week 104***	
	Placebo (n=5)	**PN-OIT (n=15)**	**Placebo (n=3)**	**PN-OIT (n=7)**	**Placebo (n=4)**	**PN-OIT (n=7)**
PE						
EI	0.2 (0-1)	0.14 (0-1)	0 (0-0)	0.71 (0-2)	0 (0-0)	0 (0-0)
BZH	0 (0-0)	0.21 (0-1)	0 (0-0)	0.67 (0-2)	0 (0-0)	0 (0-0)
DIS	0.4 (0-1)	1 (0-2)	1 (1-1)	1 (0-2)	1 (1-1)	0.86 (0-2)
LPF	NA	0 (0-0)	0 (0-0)	0.67 (0-1)	NA	NA
EA	0 (0-0)	0 (0-0)	0 (0-0)	0.29 (0-2)	0 (0-0)	0 (0-0)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0 (0-0)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Total score	0.09 (0-1)	0.19 (0-2)	0.14 (0-1)	0.41 (0-2)	0.14 (0-1)	0.12 (0-2)
ME						
EI	0.5 (0-1)	0.13 (0-1)	0 (0-0)	0.86 (0-2)	0 (0-0)	0.43 (0-1)
BZH	0.25 (0-1)	0 (0-0)	0 (0-0)	0.86 (0-2)	0 (0-0)	0 (0-0)
DIS	1 (1-1)	1 (0-2)	1 (1-1)	1.29 (1-2)	1 (1-1)	1 (0-2)
LPF	NA	NA	1 (1-1)	0.5 (0-1)	0 (0-0)	NA
EA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Total score	0.25 (0-1)	0.16 (0-2)	0.18 (0-1)	0.43 (0-2)	0.14 (0-1)	0.2 (0-2)
DE						
EI	0.2 (0-1)	0.47 (0-2)	0 (0-0)	1.29 (0-2)	0 (0-0)	0.86 (0-3)
BZH	0.2 (0-1)	0.4 (0-2)	0 (0-0)	1.14 (0-2)	0 (0-0)	0.57 (0-3)
DIS	1 (1-1)	1.07 (0-2)	1 (1-1)	1.29 (0-2)	0.75 (0-1)	0.86 (0-2)
LPF	NA	0.33 (0-1)	NA	0 (0-0)	0 (0-0)	0 (0-0)
EA	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.14 (0-1)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.14 (0-1)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0.29 (0-1)	0 (0-0)	0.29 (0-2)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.29 (0-2)
Total score	0.2 (0-1)	0.28 (0-2)	0.14 (0-1)	0.62 (0-2)	0.1 (0-1)	0.44 (0-3)

Table S2b. Eosinophilic Histologic Scoring System (EoEHSS) stage scores

	Week 0		***Week 52***		***Week 104***	
	Placebo (n=5)	**PN-OIT (n=15)**	**Placebo (n=3)**	**PN-OIT (n=7)**	**Placebo (n=4)**	**PN-OIT (n=7)**
PE						
EI	0 (0-0)	0 (0-0)	0 (0-0)	0.29 (0-2)	0 (0-0)	0 (0-0)
BZH	0 (0-0)	0.21 (0-1)	0 (0-0)	0.5 (0-1)	0 (0-0)	0 (0-0)
DIS	0.4 (0-1)	1 (0-2)	1 (1-1)	1.14 (0-2)	1 (1-1)	0.71 (0-1)
LPF	NA	0 (0-0)	0 (0-0)	0.67 (0-1)	NA	NA
EA	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0 (0-0)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0 (0-0)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Total score	0.06 (0-1)	0.17 (0-2)	0.14 (0-1)	0.33 (0-2)	0.14 (0-1)	0.1 (0-1)
ME						
EI	0 (0-0)	0 (0-0)	0 (0-0)	0.29 (0-2)	0 (0-0)	0 (0-0)
BZH	0.25 (0-1)	0 (0-0)	0 (0-0)	0.71 (0-2)	0 (0-0)	0 (0-0)
DIS	1 (1-1)	0.93 (0-2)	1 (1-1)	1.43 (1-2)	1 (1-1)	0.86 (0-1)
LPF	NA	NA	1 (1-1)	0.5 (0-1)	0 (0-0)	NA
EA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Total score	0.18 (0-1)	0.13 (0-2)	0.18 (0-1)	0.35 (0-2)	0.14 (0-1)	0.12 (0-1)
DE						
EI	0 (0-0)	0.27 (0-2)	0 (0-0)	0.86 (0-3)	0 (0-0)	0.43 (0-3)
BZH	0.6 (0-3)	0.47 (0-3)	0 (0-0)	1.14 (0-3)	0 (0-0)	0.57 (0-3)
DIS	1 (1-1)	1.07 (0-2)	1 (1-1)	1.57 (0-3)	0.75 (0-1)	1 (0-3)
LPF	NA	0 (0-0)	NA	0 (0-0)	0 (0-0)	0 (0-0)
EA	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.14 (0-1)
SL	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.14 (0-1)
SEA	0 (0-0)	0 (0-0)	0 (0-0)	0.29 (0-1)	0 (0-0)	0.14 (0-1)
DEC	0 (0-0)	0 (0-0)	0 (0-0)	0.14 (0-1)	0 (0-0)	0.14 (0-1)
Total score	0.23 (0-3)	0.25 (0-3)	0.14 (0-1)	0.6 (0-3)	0.1 (0-1)	0.36 (0-3)

Mean (Range). NA signifies that there was no sufficient material to assess the feature.

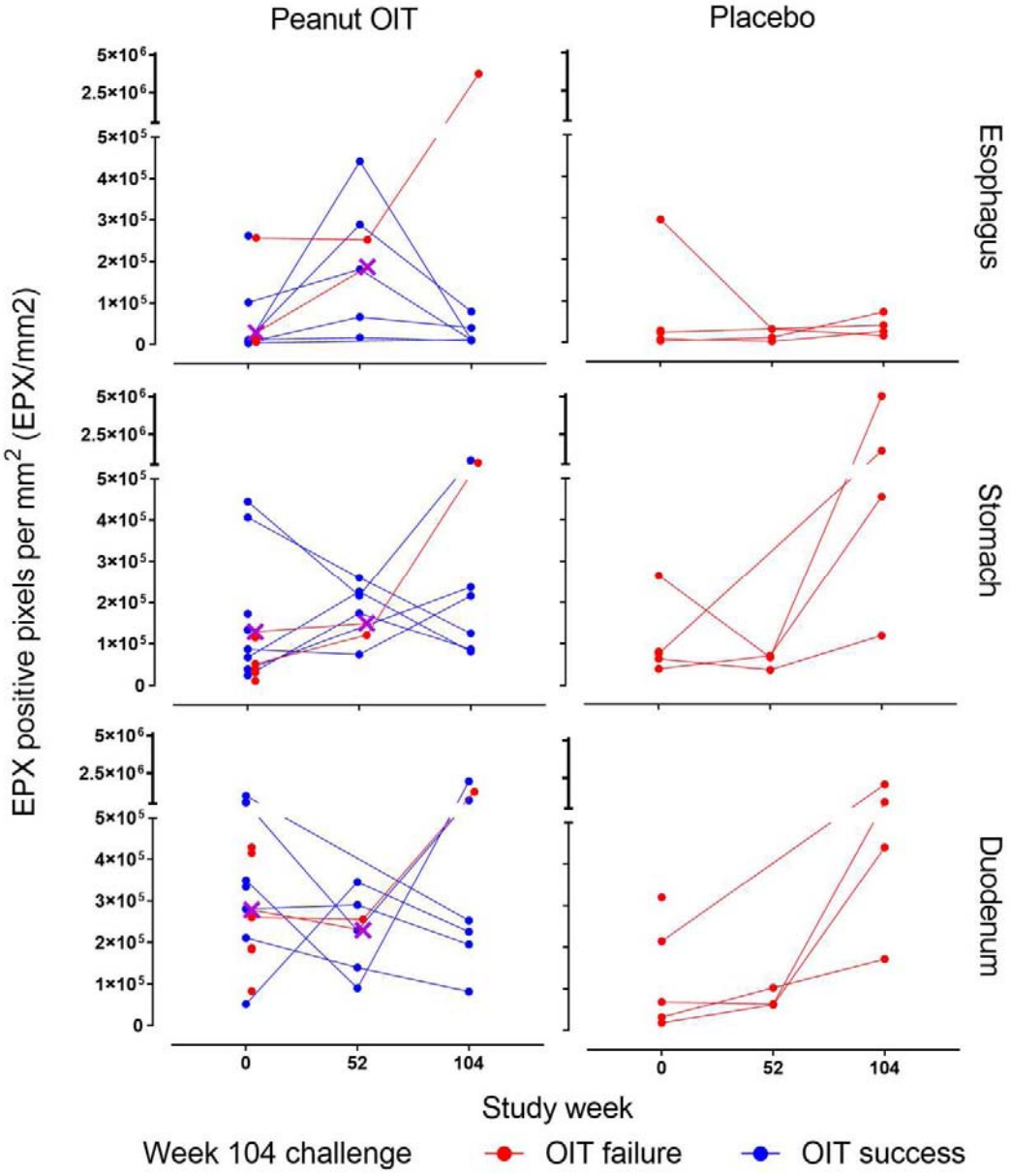
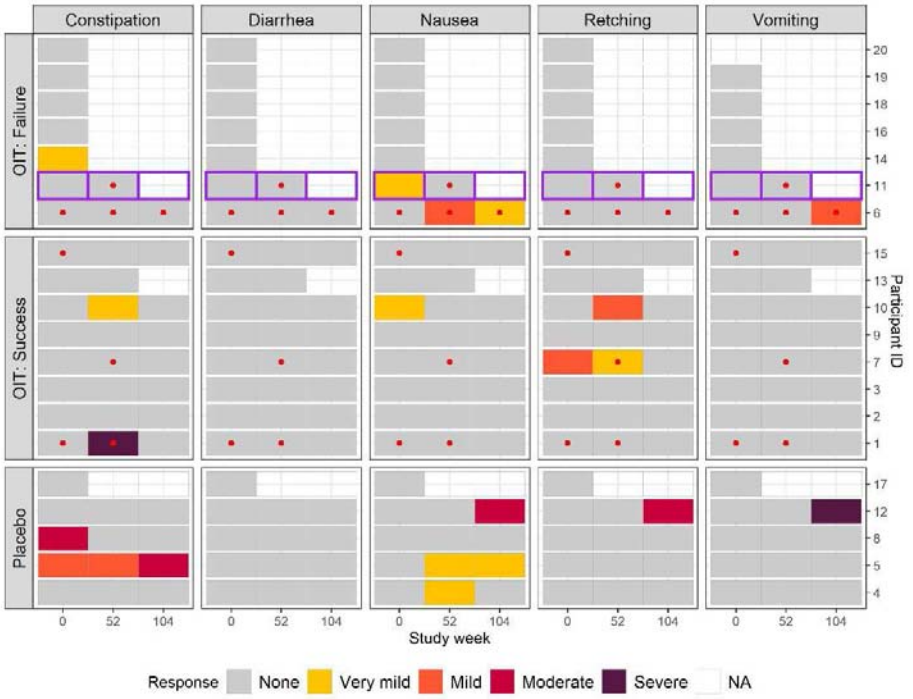
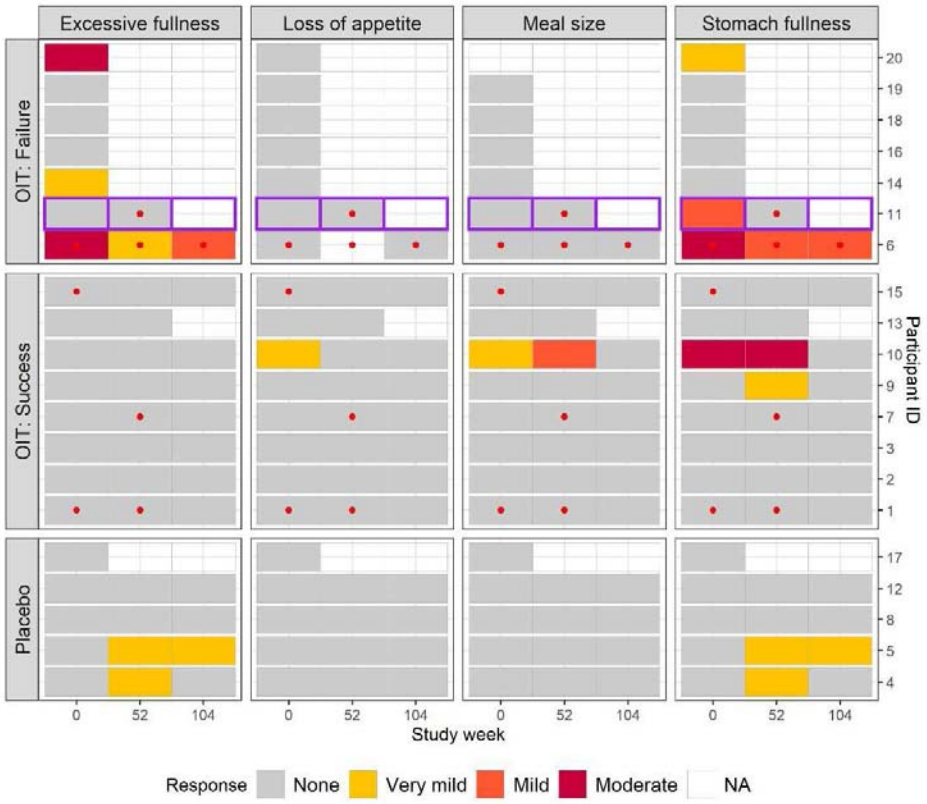


Figure S1. EPX/mm^2 over time by treatment arm, biopsy site, and week 104 outcome. Participant-level spaghetti plots of EPX/mm^2 over time by treatment, site, and week 104 peanut challenge outcome (success or failure). Data points are shown for Week 0 (peanut OIT n = 15, placebo n = 5), Week 52 (peanut OIT n = 7, placebo n = 3), and Week 104 (peanut OIT = 7, placebo = 4). Participant #11, who developed EoE during the study, is indicated by the purple X. EPX staining was quantified by automated image analysis.

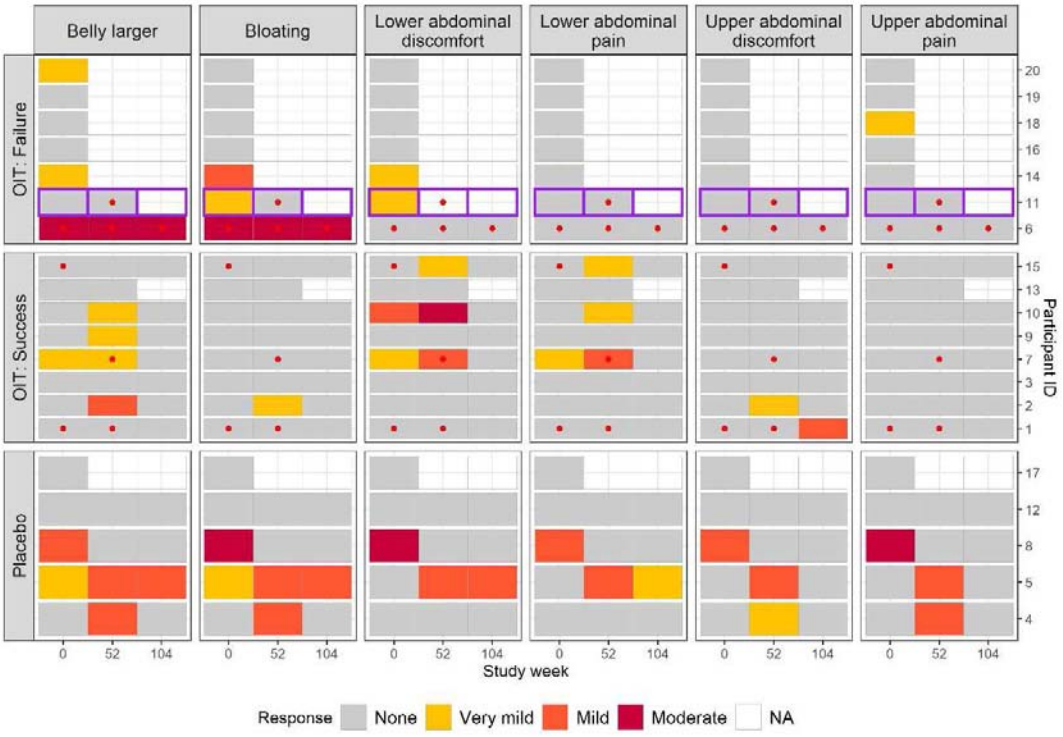
a) Nausea / diarrhea



b) Appetite



c) Bloating and abdominal pain



d) Heartburn and acid reflux

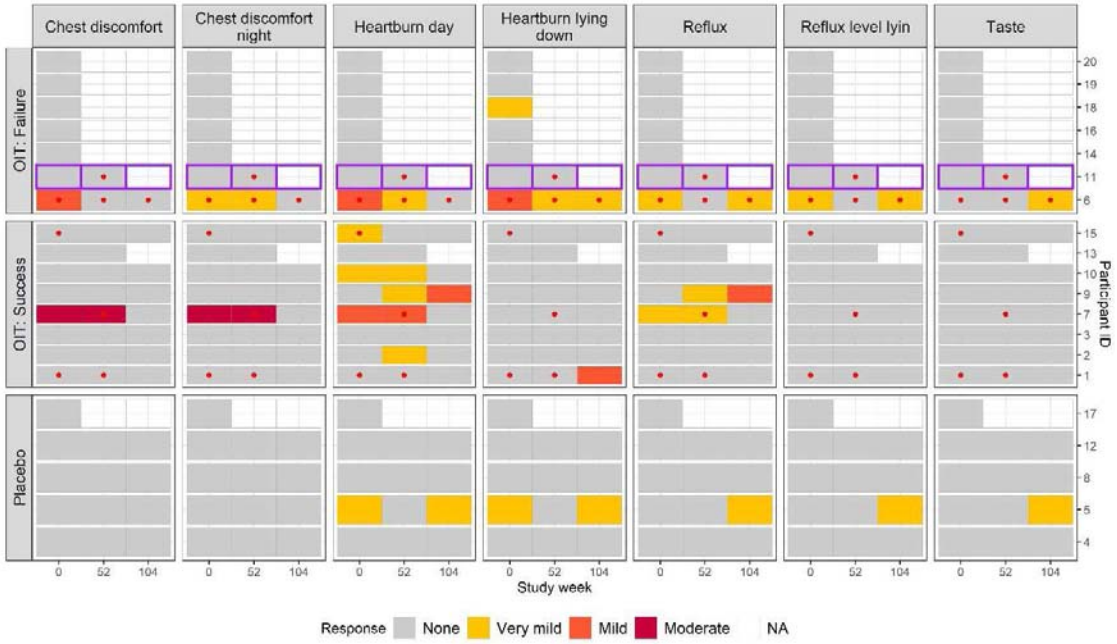
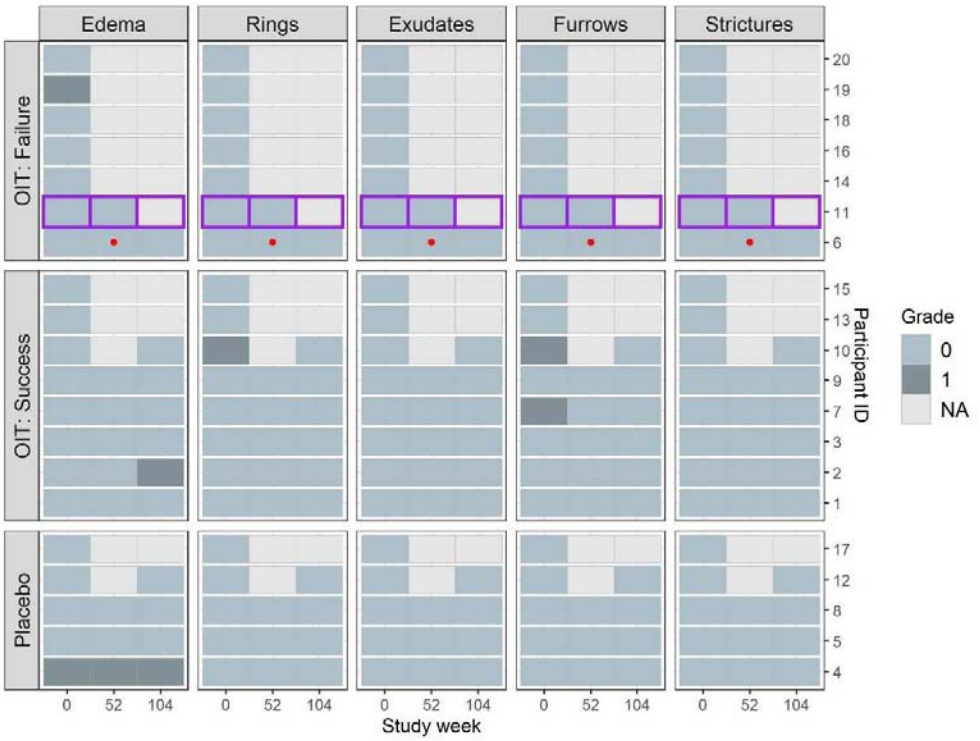
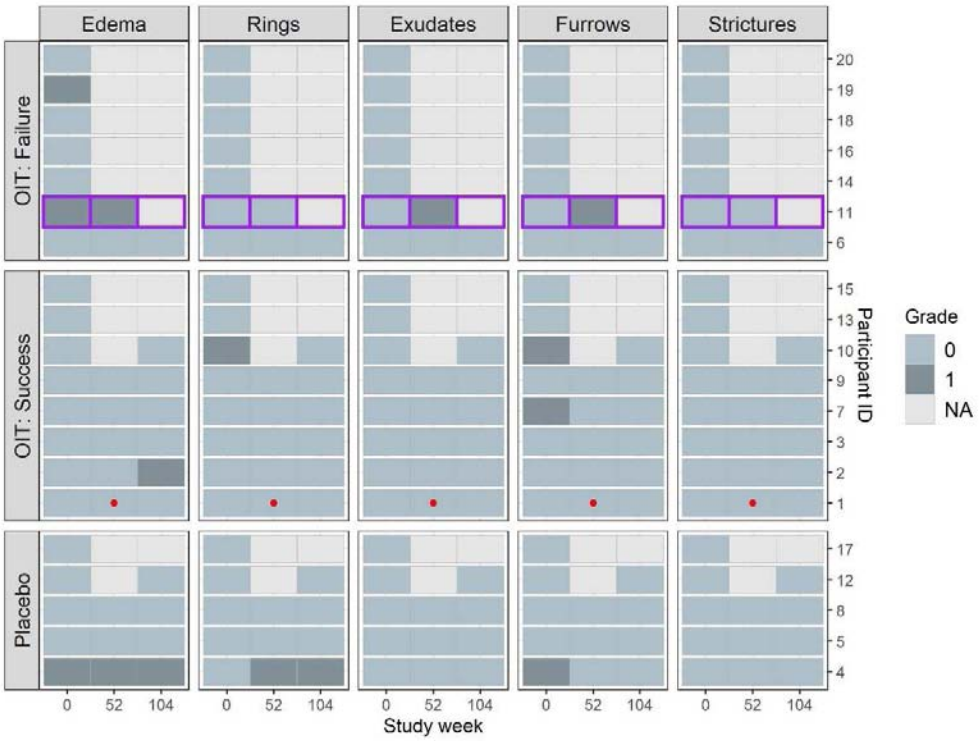


Figure S2a-d. Gastrointestinal symptom severity questionnaire results. Gastrointestinal symptom severity questionnaire results over time by treatment arm. Red dots denote that the participant had a peak GI eosinophil count ≥ 15 (cells/hpf) in at least one of the three esophageal sites in that study week. Participant #11 developed EoE and is outlined in purple. NA signifies that no response was provided for the questionnaire.

a) Proximal esophagus



b) Middle esophagus



c) Distal esophagus

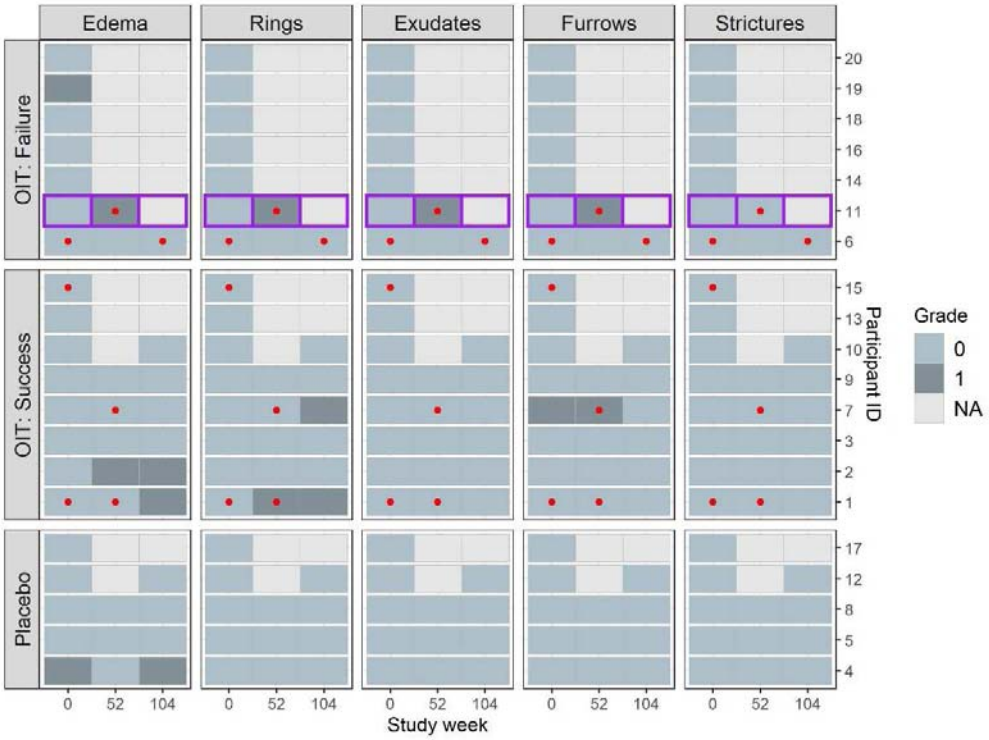


Figure S3a-c. EREFS over time by participant, treatment, grade, and site. EREFS within the a) proximal esophagus, b) middle esophagus, and c) distal esophagus over time by treatment arm and week 104 challenge outcome. Each row corresponds to a participant. Red dots denote that the participant had a peak GI eosinophil count ≥ 15 (cells/hpf) in the esophageal site in that study week. The participant who developed EoE during OIT is outlined in purple. NA signifies that an endoscopy was not performed.

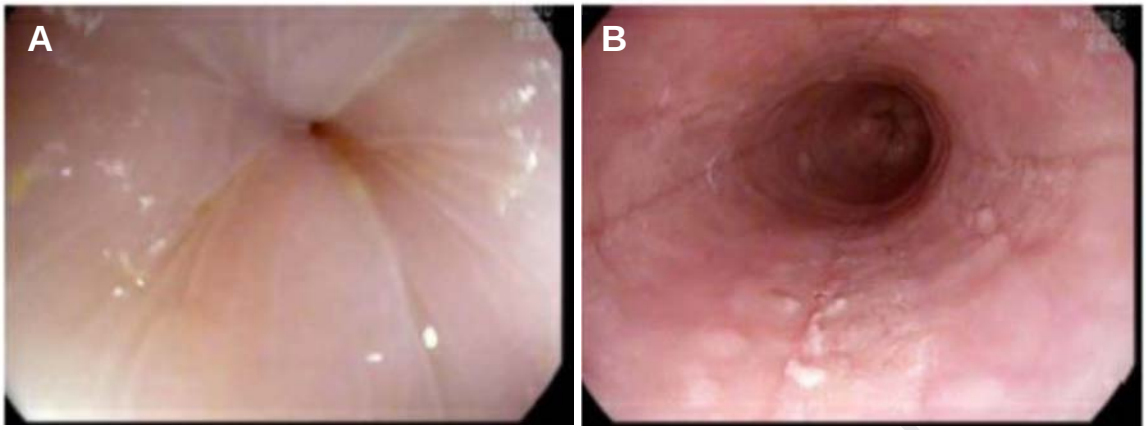
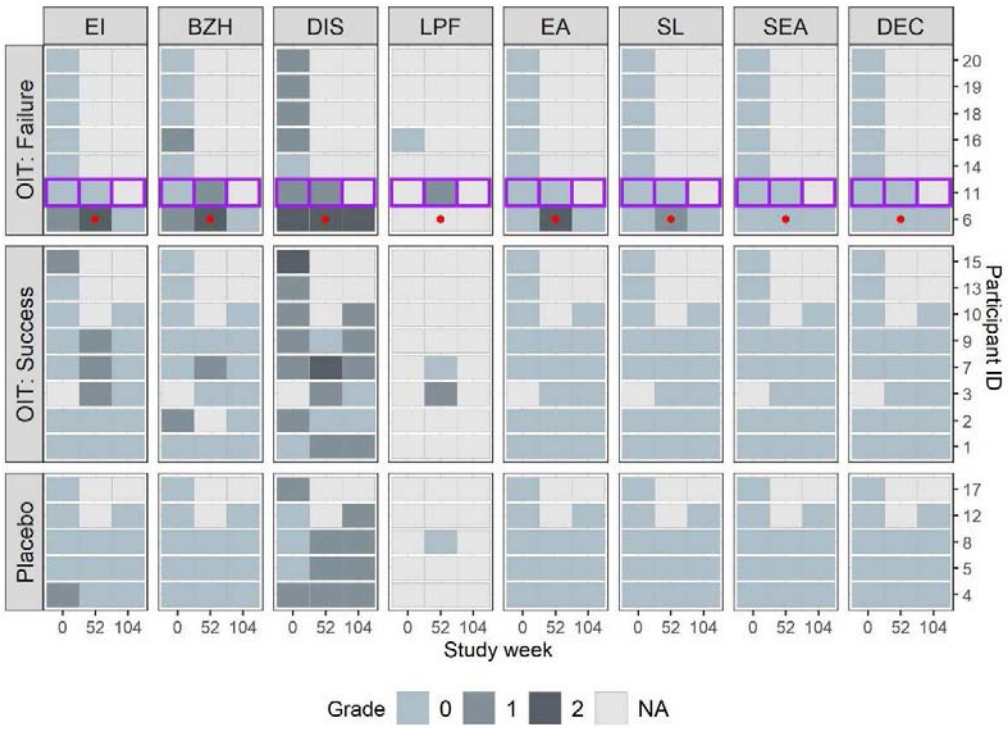
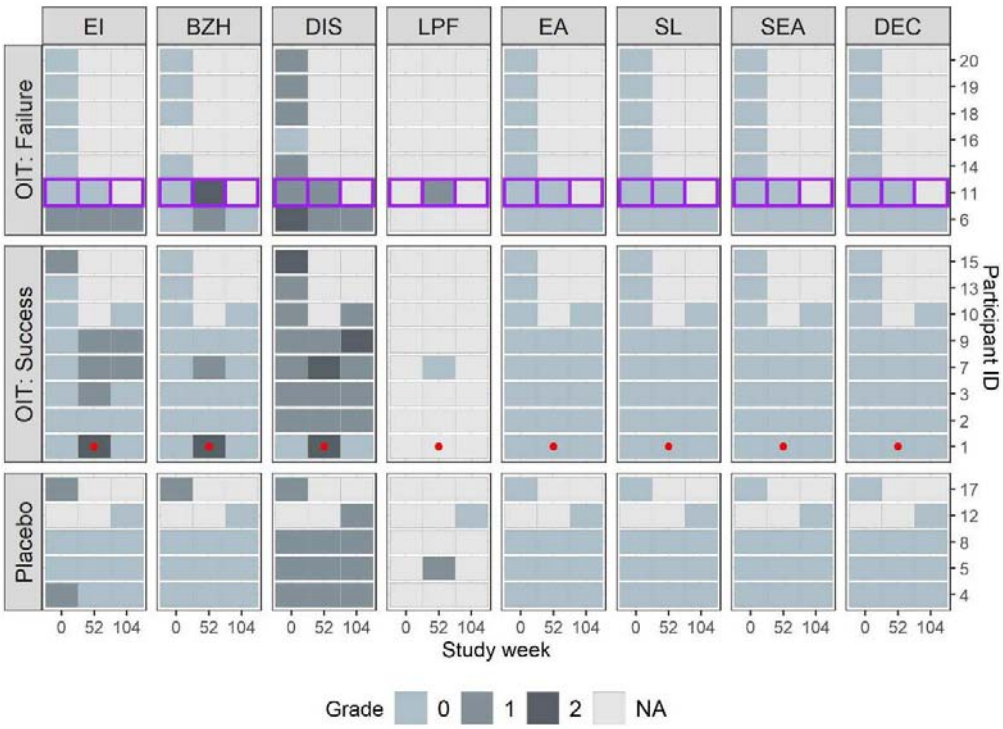


Figure S4a,b. Endoscopic gross images of the distal esophagus of the patient that developed EoE. EGD of participant #11 at baseline (**A**) was normal, whereas endoscopy at 52 weeks (**B**) revealed edema, rings, exudates and furrows.

a) Proximal esophagus



b) Middle esophagus



c) Distal esophagus

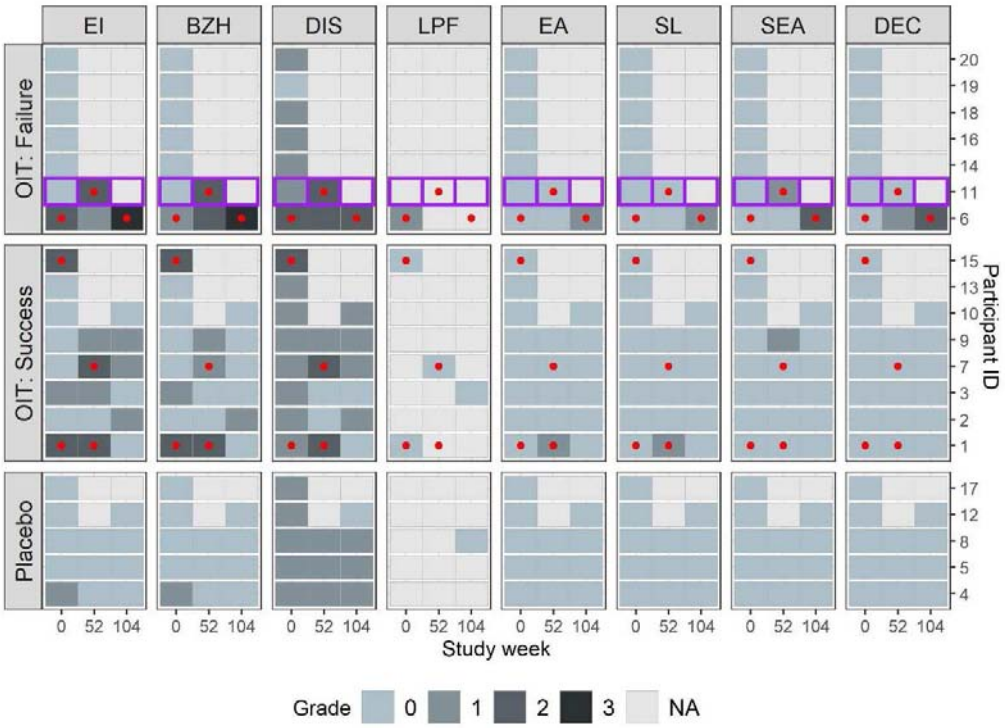
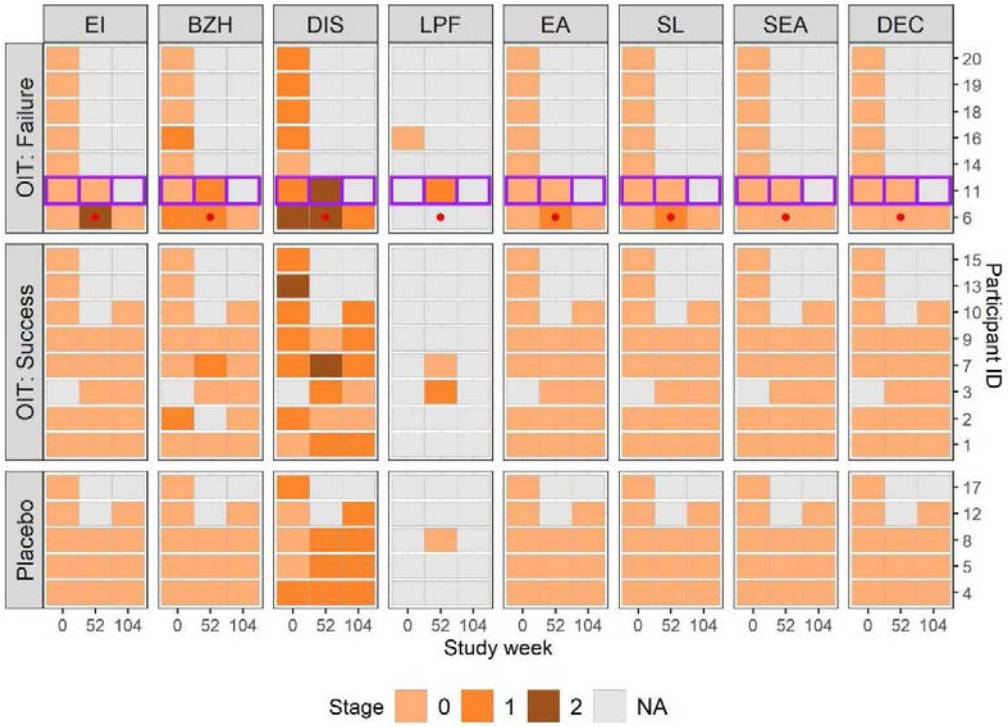
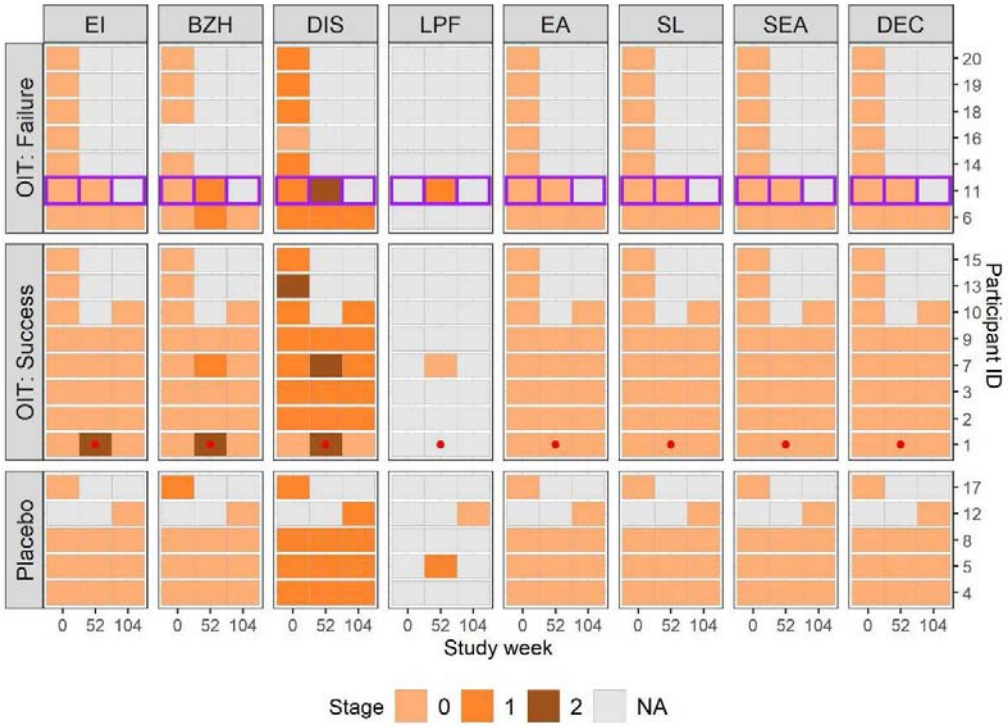


Figure S5a-c. Eosinophilic esophagitis histologic scoring system (EoEHSS) scores (grade) over time by participant, treatment arm, and biopsy site. EoEHSS scores (grade) over time within the a) proximal esophagus, b) middle esophagus, and c) distal esophagus by treatment arm and week 104 challenge outcome. Each row corresponds to a participant. Red dots denote that the participant had a peak GI eosinophil count ≥ 15 (cells/hpf) in the esophageal site in that study week. The participant who had EoE is outlined in purple. NA signifies that no biopsy was performed or insufficient material to assess. EI – eosinophil infiltration, BZH – basal zone hyperplasia, DIS – dilated intercellular spaces, LPF – lamina propria fibrosis, EA – eosinophilic abscess, SL – eosinophil surface layering, SEA – surface epithelial alteration, DEC – dyskeratotic epithelial cells. NA signifies that an endoscopic biopsy was not performed.

a) Proximal esophagus



b) Middle esophagus



c) Distal esophagus

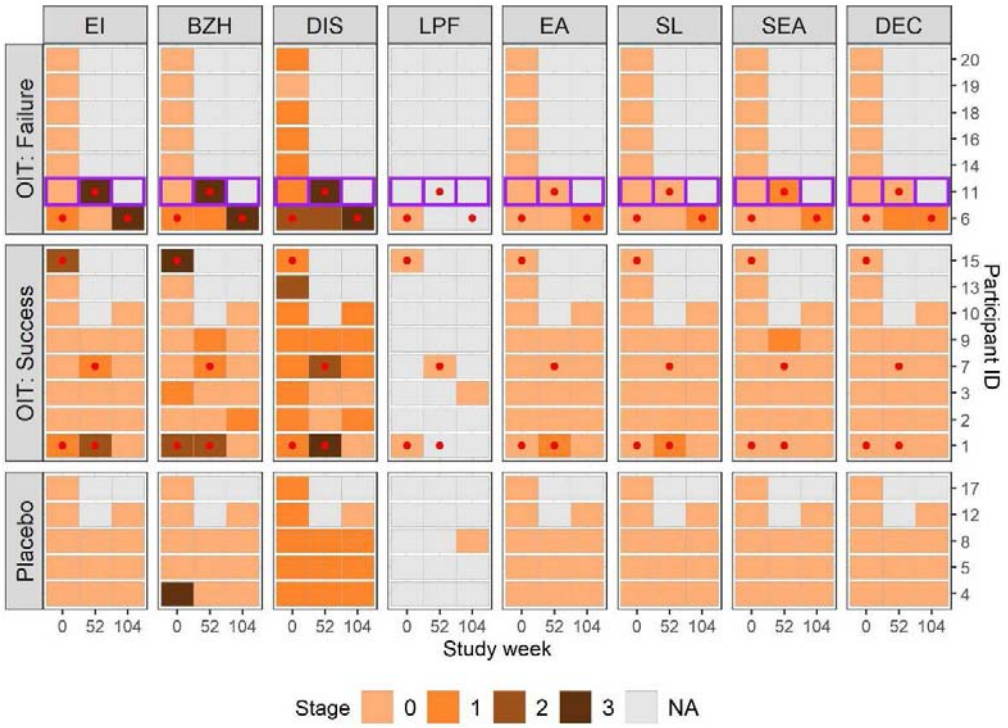


Figure S6a-c. EoEHSS scores (stage) over time by participant, treatment arm, and biopsy site. EoEHSS scores (stage) over time within the a) proximal esophagus, b) middle esophagus, and c) distal esophagus by treatment arm and week 104 challenge outcome. Each row corresponds to a participant. Red dots denote that the participant had a peak GI eosinophil count ≥15 (eos/hpf) in the esophageal site in that study week. Participant #11, who developed EoE, is outlined in purple. NA signifies that no biopsy was performed or insufficient material to assess. EI – eosinophil infiltration, BZH – basal zone hyperplasia, DIS – dilated intercellular spaces, LPF – lamina propria fibrosis, EA – eosinophilic abscess, SL – eosinophil surface layering, SEA – surface epithelial alteration, DEC – dyskeratotic epithelial cells. NA signifies that an endoscopic biopsy was not performed.

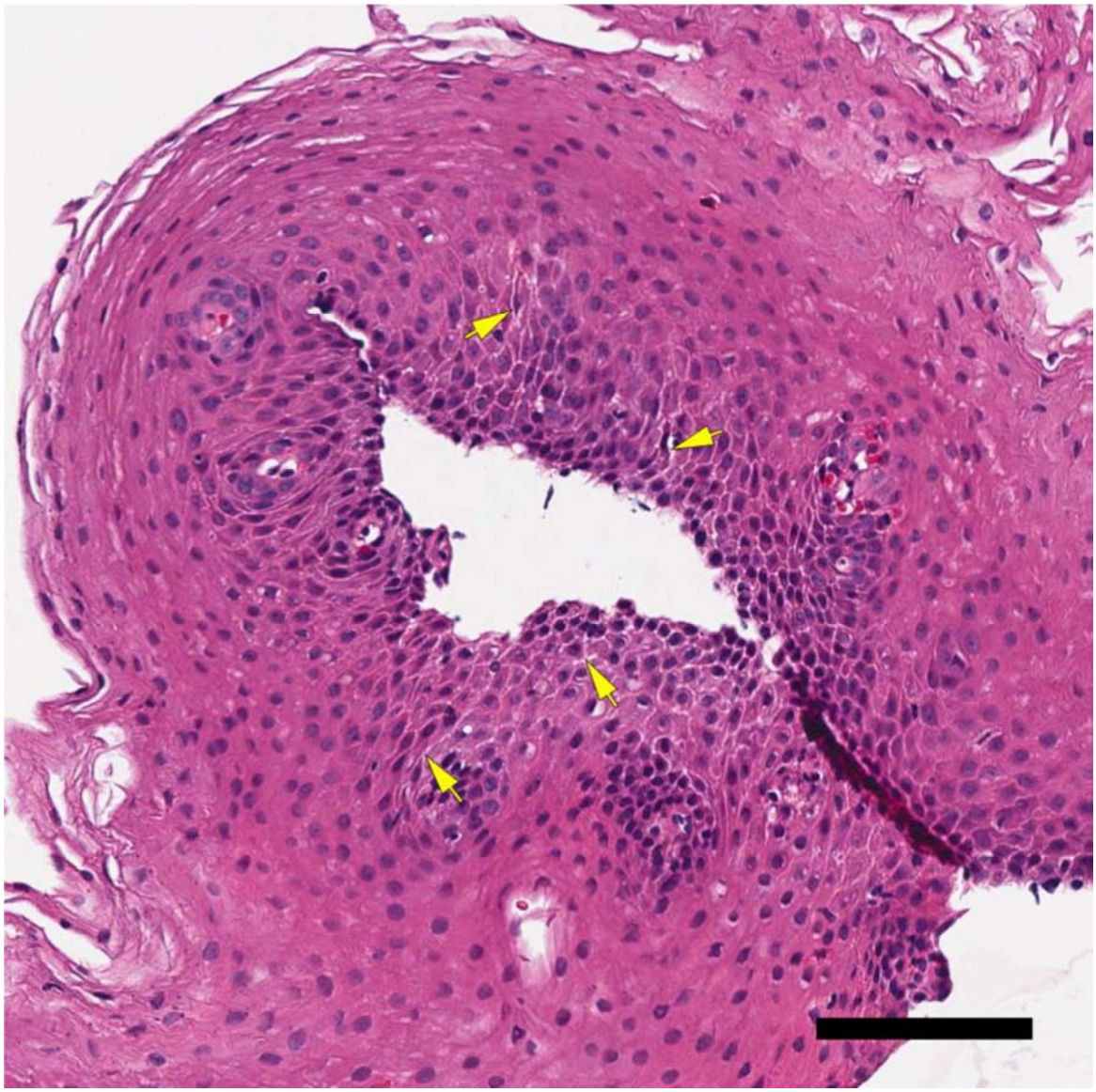


Figure S7. Histology showing dilated intercellular spaces (DIS) in a peanut allergic subject at baseline. Hematoxylin and eosin (H&E) stain from a tissue section of the proximal esophagus (participant #6) demonstrates DIS prior to initiation of peanut oral immunotherapy (OIT). Scale bar = 100 microns.

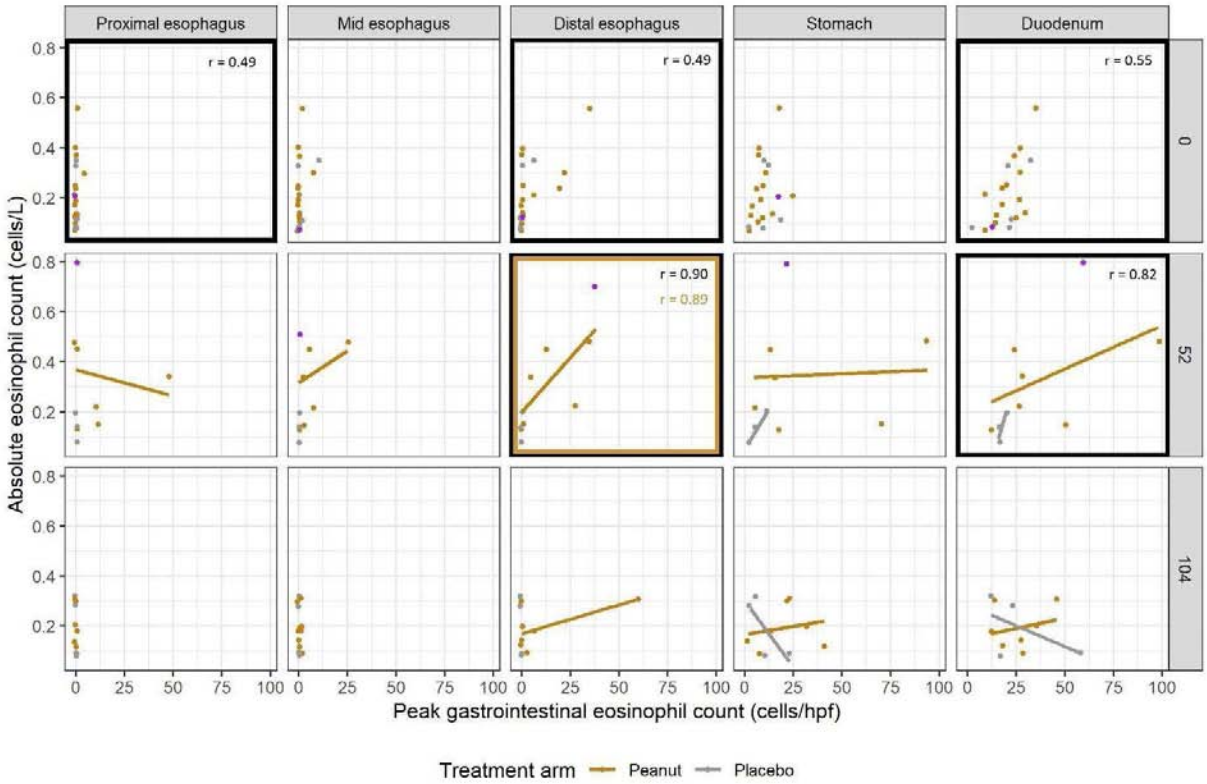


Figure S8. Correlation of peak gastrointestinal (GI) and absolute eosinophil counts by study week and treatment arm. The Spearman rank correlation comparing peak GI and peripheral blood eosinophils within each treatment arm, study week, and GI site was used and outlined in gold where significant. Correlations that were significant regardless of treatment arm are outlined in black ($p < 0.05$). Participant #11, who developed EoE, is identified by the purple points.

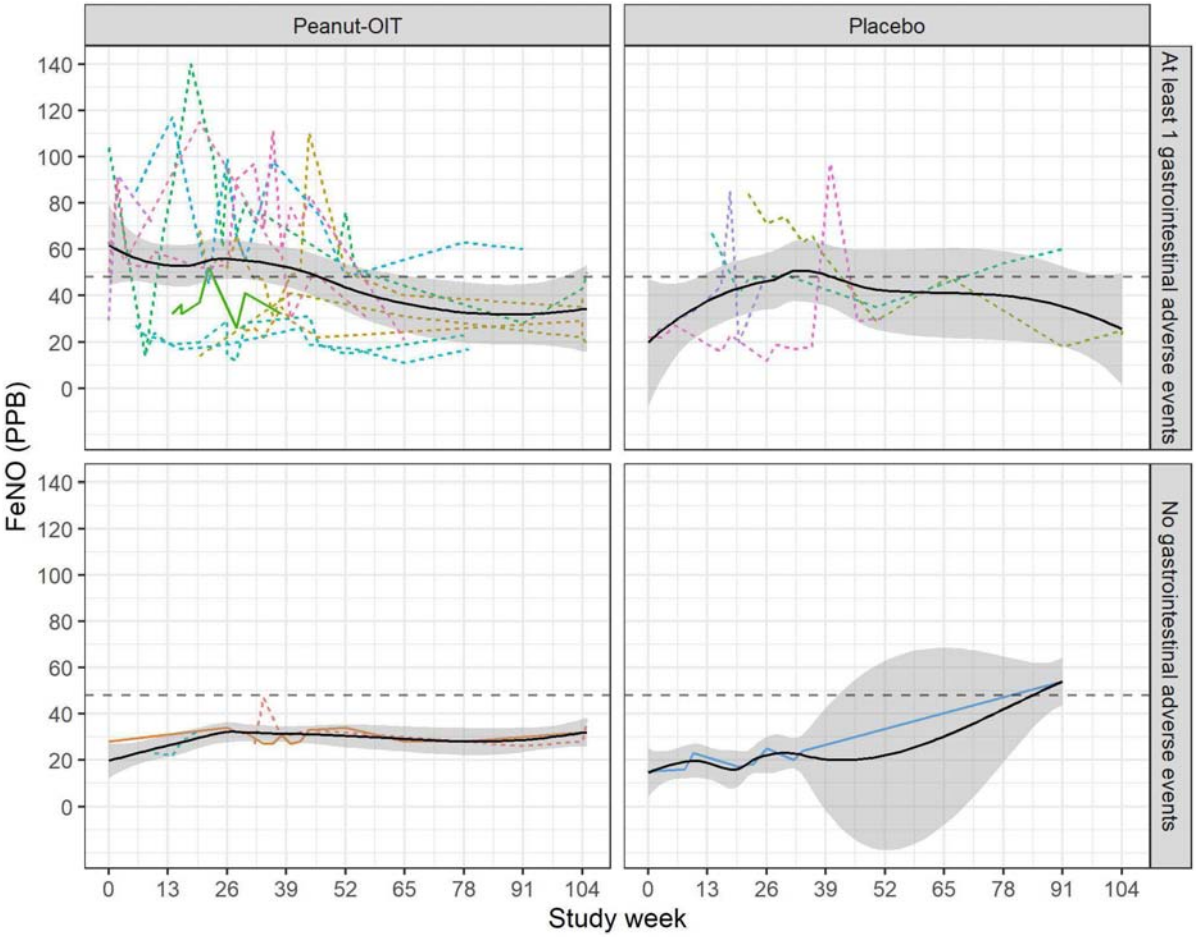


Figure S9. Fractional exhaled nitric oxide (FeNO) shown over time by participant, treatment arm, and whether or not GI adverse events (AEs) were experienced during the study. The solid black line within each plot represents the average value by LOESS smoothing with 95% confidence bands. The dashed gray line at 48 parts per billion represents a previously published threshold of discriminating between those who experience GI AEs and those who do not.[24] Participant line types are styled by history of asthma (dashed) and no history of asthma (solid).

Supplemental Methods:

Oral immunotherapy (OIT)

Details about peanut OIT dosing during the POISED trial have been previously published.[1] In brief, participants underwent an initial dose escalation day and continued to up-dose to 4,000 mg of peanut protein or placebo every two weeks over the course of one year. During the second year, participants maintained 4,000 mg of peanut or placebo daily. Daily diaries were reviewed for allergic reactions due to dosing with OIT.

Questionnaires

Questionnaires to document concerning gastrointestinal symptoms that could be consistent with EoE were provided to coincide with esophagogastroduodenoscopy (EGD), at baseline and weeks 52 and 104. Participants were given the questionnaires at the clinic visit closest to the scheduled EGD and asked to recall symptoms in the 4 weeks preceding the respective EGD.

Evaluation of gastrointestinal pathology

Sections from each segment of the gastrointestinal tract were stained with hematoxylin and eosin. A gastrointestinal pathologist (N.K.) blinded to clinical characteristics and demographic data of the individual participants quantified the peak eosinophil count (PEC) in a single high-power field (hpf) and performed standardized assessment of the severity and extent of histologic alterations using the EoE Histologic Scoring System (EoEHSS).[2] This scoring system assesses eight pathologic features characteristic of EoE: eosinophil infiltration (EI), basal zone hyperplasia (BZH), dilated intercellular

spaces (DIS), lamina propria fibrosis (LPF), eosinophilic abscess (EA), eosinophil surface layering (SL), surface epithelial alteration (SEA), and dyskeratotic epithelial cells (DEC).

Subjects with ≥5 eos/hpf were considered to have esophageal eosinophilia (EE) and those with ≥ 15 eos/hpf met histologic criteria for EoE. Subjects were also evaluated for gastric (>12 eos/hpf) or duodenal (>26 eos/hpf) eosinophilia. These values were based on normal reference ranges derived from healthy subjects.[3-5] Currently, there are no consensus guidelines for eosinophil thresholds in eosinophilic gastritis and eosinophilic duodenitis. We used 30 eos/hpf in the stomach (in at least 5 hpf) and duodenum (in at least 3 hpf) as histologic cutoffs for eosinophilic gastritis and duodenitis, which are values used in other studies.[5, 6]

Immunohistochemical (IHC) staining for eosinophil peroxidase (EPX) and analysis

In EoE, a majority of tissue eosinophils undergo cytolytic degranulation;[7] therefore, manual eosinophil counts by conventional histology may underestimate the extent of EI.[8, 9] EPX is an eosinophil-specific secondary granule protein that correlates with clinical symptoms in EoE.[10] EPX staining and analysis were performed as previously described.[11] Briefly, tissue sectioning and IHC staining was completed at the Pathology Research Core (Mayo Clinic, Rochester, MN) using the Leica Bond RX stainer (Leica). Tissue sections were digitized (Aperio AT Turbo, Leica Biosystems, Buffalo Grove, IL) and PEC were evaluated using an area equivalent to 1 hpf (0.24 mm^2). EPX

tissue deposition was quantified by an automated pixel algorithm with Aperio ImageScope software (version 11.2.0.780, Aperio Technologies, Vista, CA).

Statistical methods

Descriptive statistics were used to summarize baseline characteristics of the study cohort overall and by treatment assignment (combined peanut or placebo arm). The comparisons between peanut and placebo arms were performed using the Mann-Whitney U test for continuous variables and the Fisher's exact test for categorical variables. PEC, gastrointestinal questionnaire data, EREFS, EoEHSS scores, peripheral blood absolute eosinophil counts (AEC), fractional exhaled nitric oxide (FeNO), and EPX were plotted over time for each participant by treatment arm. The subject who developed EoE during the study (participant #11) is indicated in each figure.

The Wilcoxon rank-sum test was used to compare the changes in tissue eosinophil counts from week 0 to week 52 within each treatment arm and site. The Kruskal-Wallis rank sum test was used to determine whether differences in EoEHSS score existed across study time points within each treatment arm, grade/stage, and esophageal site. The Spearman rank correlation test was used to assess the correlation between peak gastrointestinal and peripheral blood AEC within each treatment arm, study week, and gastrointestinal site. All analyses were conducted using R v3.5.2.[12] A p-value < 0.05 was considered statistically significant.

Supplemental Results

EPX levels

Some of the observations seen with manual tissue eosinophil counts alone were more pronounced with EPX staining. For example, the one subject who failed desensitization in the active treatment group (participant #6) had marked EPX deposition in the distal esophagus (DE) at week 104 (88 eos/hpf, and EPX/mm^2 = 3,815,430.95). The baseline tissue eosinophilia in the placebo subject with 11 eos/hpf in the middle esophagus (ME) was also more distinct compared to placebo (EPX/mm^2 = 296,438 vs. median EPX/mm^2 of all other subjects = 4,573). In some participants, we noted discrepancies in the trends in eosinophil counts and EPX/mm^2 quantified from different sections of the same tissue biopsy. Manual counts of eosinophils in these subjects generally revealed higher eosinophil counts by EPX staining and/or more pronounced EPX deposition. EPX/mm^2 in the stomach and duodenum was more variable than levels measured in the esophagus likely due to the fact that eosinophils are resident in the stomach and duodenum of healthy individuals.[20-]

Biomarkers of gastrointestinal eosinophilia (GE)

We attempted to identify markers associated with GE during OIT. The peripheral blood AEC correlated strongly with EE of the DE and duodenal eosinophilia in all subjects at 52 weeks (r=0.90 and 0.82, respectively) (**Figure S8**). We also examined whether FeNO would identify subjects with gastrointestinal adverse effects (AE's) during OIT (**Figure S9**). We found that subjects with a FeNO > 48 ppb (n = 12)[23] were more likely

to experience gastrointestinal AE's, however this association was not statistically significant with the Fisher's exact test (92% vs 50%, p = 0.11). Additionally, subjects who experienced gastrointestinal AE's were more likely to have comorbid asthma compared to those with no gastrointestinal AE's (93% vs 40%, p = 0.032).

Endoscopic findings in the stomach and duodenum

Two subjects had gastric ulcers in the stomach and another had a nodule diagnosed microscopically as a tubular adenoma at baseline. At week 52, 1 subject on active treatment had erosion in the stomach and duodenum. At week 104, 1 subject on active treatment had erosion in the stomach.

Supplemental References

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