Specific Peanut Epitopes as a Biomarker for Desensitization During Epicutaneous Immunotherapy

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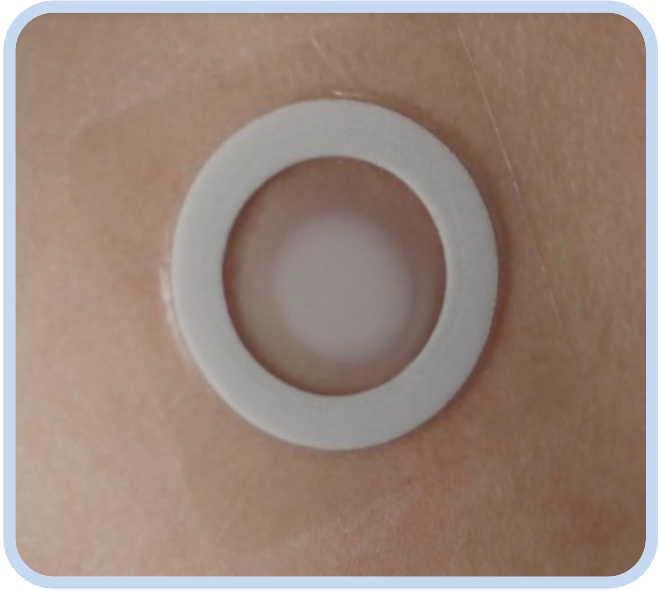
Background

- Epicutaneous immunotherapy (EPIT) is currently under investigation for the treatment of peanut allergy
  - **DBV712 250 µg** is a single, daily-dose patch applied to the back and is dosed at 250 µg (**~1/1000 of a peanut**)[1-3]
- Existing biomarkers such as total peanut-IgE and SPT used in the diagnosis of peanut allergy are not adequate for quantifying desensitization during immunotherapy
- Total peanut-IgG4 during EPIT is not highly correlated with treatment response during immunotherapy
- Measuring IgE and IgG4 reactivity to **epitopes** (smaller fragments of allergen to which an allergic individual can produce an antibody) from peanut protein allergens may help to quantify desensitization during EPIT



SPT=skin prick test.

1. Sampson HA, et al. *JAMA*. 2017;318:1798-1809. **2.** Tilles SA, et al. *Ann Allergy Asthma Immunol*. 2018;121:145-149. **3.** Parrish CP. *Am J Manag Care*. 2018;24:S419-S427.

Aims and Objectives

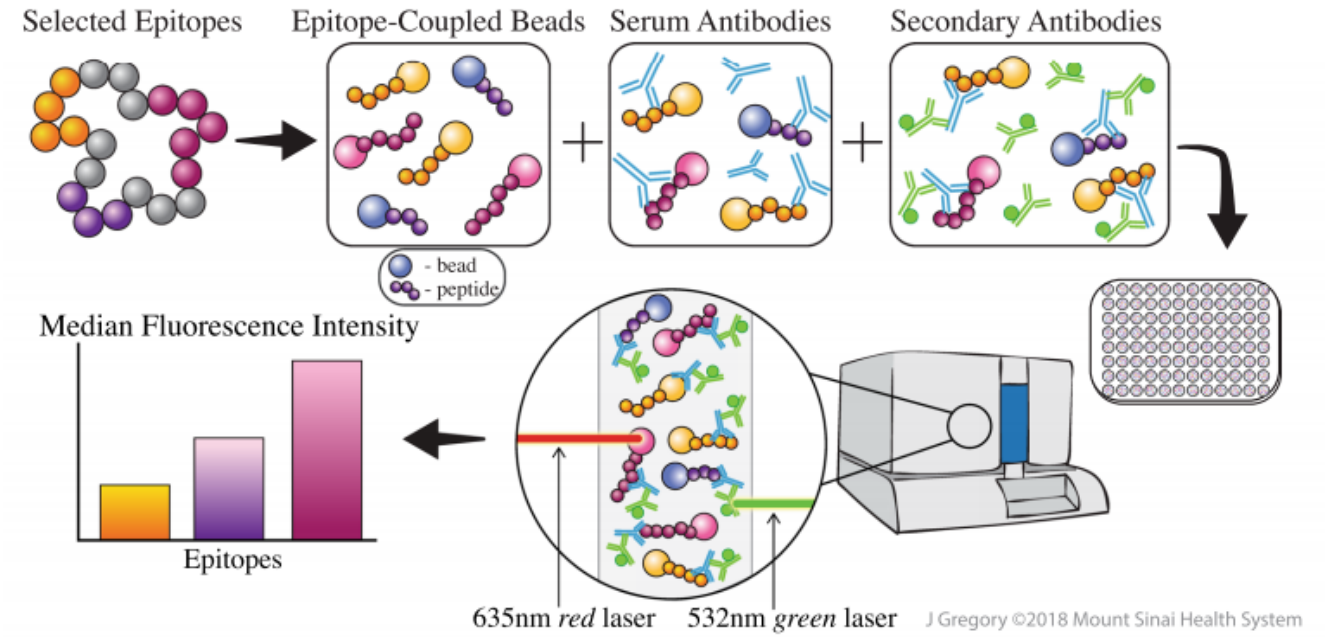
- Assess performance of the **Bead-Based Epitope Assay** (BBEA), which enables simultaneous quantification of antibodies recognizing sequential linear protein epitopes, in predicting desensitization during EPIT

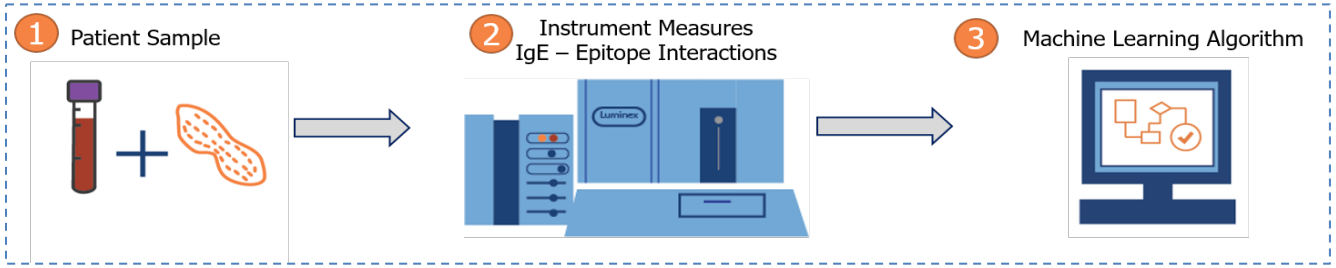
- The specific **objectives** of the study were to:
  - Analyze the samples from subjects who participated in PEPITES (Phase 3 DBRCT of DBV712 250 µg in peanut allergic children) for IgE and IgG4 reactivity to Ara h 1, Ara h 2, and Ara h 3 peanut allergen epitopes using the BBEA
  - Derive predictors of desensitization progress over time as well as predictors of desensitization above a peanut protein eliciting dose threshold (>300 mg) following 12 months of treatment with DBV712 250 µg

DBRCT=double-blind randomized controlled trial.

Methods: BBEA Technology[1]

- A **Bead-Based Epitope Assay** (BBEA) platform was used to monitor the reactivity of **IgE and IgG4** in subjects' serum to 64 linear **epitopes** from Ara h 1, Ara h 2, and Ara h 3





- The BBEA methodology enables simultaneous quantification of antibodies binding to sequential epitopes
- Epitopes are covalently coupled to unique fluorescent microspheres (Luminex)
- Epitope-labeled beads are mixed to form a master library
- Patient plasma and a secondary fluorophore-labeled antibody are then incubated with the beads
- The Luminex instrument uses dual-lasers for quantification (red for beads, green for secondary antibodies)
- For each epitope, the signal is quantified as a median fluorescence intensity (MFI)

Figure adapted from Suprun et al.[1] (http://creativecommons.org/licenses/by/4.0/)

1. Suprun M, et al. *Sci Rep.* 2019;9:18425. doi.org/10.1038/s41598-019-54868-7.

Methods: Subjects and Samples

Subjects

- **89 peanut-allergic subjects** who participated in the 12-month, randomized, double-blind, placebo-controlled **PEPITES** study[1]
  - Subjects received active treatment with DBV712 250 µg (n=61) or placebo (n=28)

Samples

- Serum samples were analyzed using the BBEA at 0, 3, 6, and 12 months for IgE and IgG4 reactivity to 64 linear epitopes from Ara h 1, Ara h 2, and Ara h 3 (esIgE and esIgG4)
  - The BBEA method was applied under SOPs to all subjects in triplicate and randomized across plates
  - Raw data was processed: noise removal, log normalized, triplicates merged
  - Analysis was performed using linear regression models
  - Accuracy, sensitivity, specificity, and AUC were generated
- Serum samples were analyzed for total peanut-specific IgE and IgG4

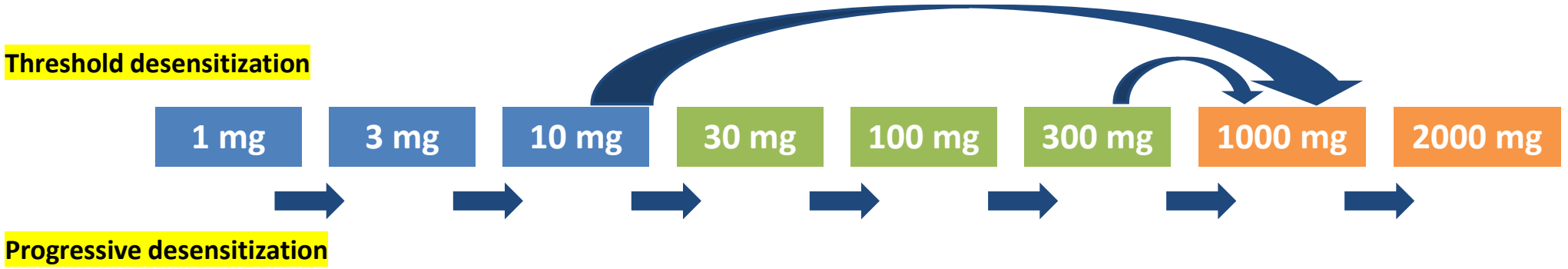
AUC=area under the curve; BBEA=Bead-Based Epitope Assay; esIgE=epitope-specific IgE; esIgG4=epitope-specific IgG4; SOP=standard operating procedure.

1. Fleischer DM, et al. *JAMA*. 2019;321(10):946-955.

Methods: Endpoints

Endpoints

- **Threshold desensitization** was defined as an eliciting dose (ED) >300 mg peanut protein at Month 12 (ie, ≥1000 mg) irrespective of baseline ED (all ≤300 mg)
- **Progressive desensitization** was defined as ED threshold increased by at least 1 dose from baseline to Month 12



Results: Epitope Mapping – Progressive and Threshold Desensitization Predictors

Threshold Desensitization (ED ≥1000 mg)

	12 Months
Best epitopes (IgG4)	*h2.010* *h3.102* *h1.029* *h1.090*
AUC	**0.97**
% accuracy (95% CI)	93% (90, 98)

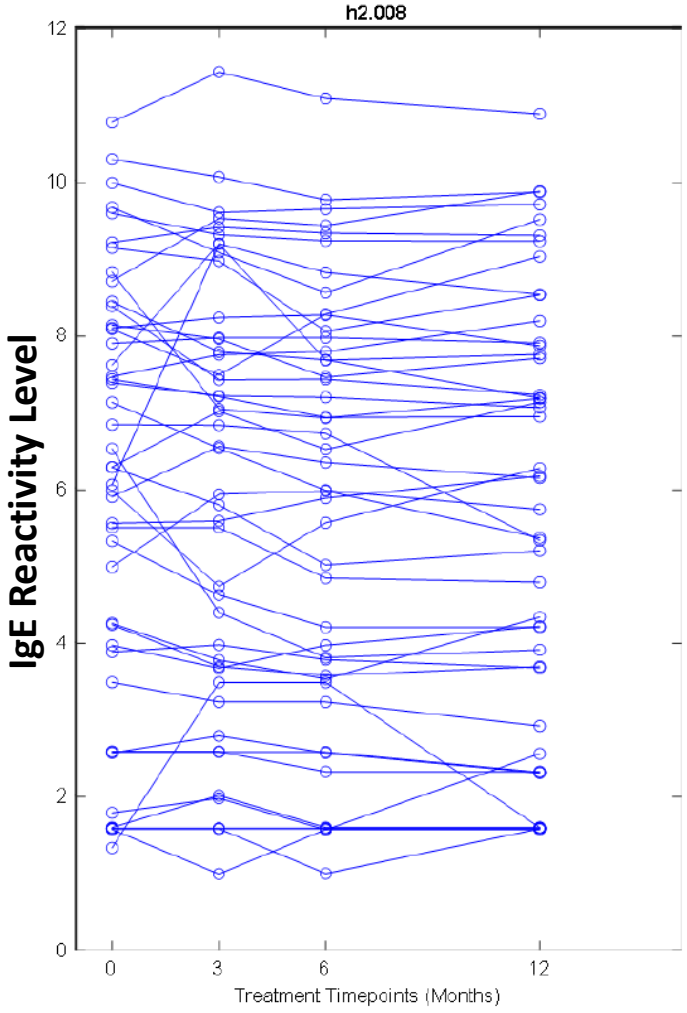
Progressive Desensitization (Improvement in ED)

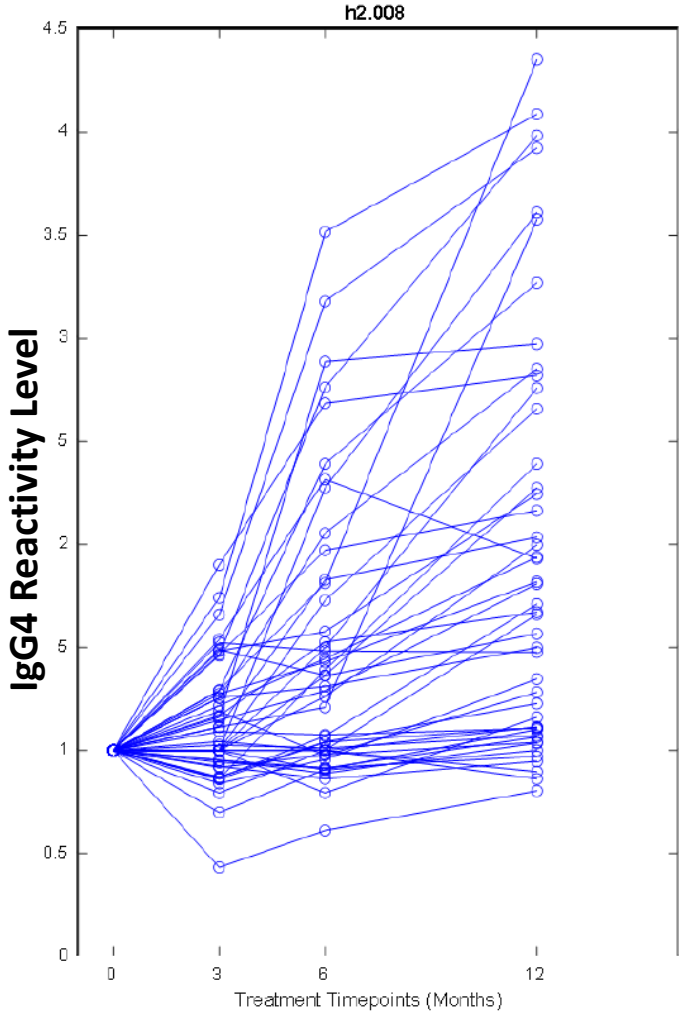
	12 Months
Best epitopes (IgE)	h1.041 h1.022 h2.008
AUC	**0.92**
% accuracy (95% CI)	91% (83, 93)

- Addition of baseline subject characteristics or AE rate during treatment did not improve performance in either analysis

AE=adverse event; AUC=area under the curve; CI=confidence interval; ED=eliciting dose.

Results: esIgE and esIgG4 to h2.008 Reactivity for All Subjects





- For epitopes such as h2.008, treatment increased IgG4 reactivity to epitopes over time
- Similar behavior was seen for h1.029

esIgE=epitope-specific IgE; esIgG4=epitope-specific IgG4.

Results: Threshold Desensitization

Threshold Desensitization at Month 12 – 4 Epitope Linear Regression Model (IgG4)

	Coefficient	SE	tStats	*P* value
(Intercept)	-0.535	1.593	-0.336	0.737
h2.010	6.357	3.312	1.919	0.055
h3.102	-5.910	2.970	-2.033	0.042
h1.029	-7.037	2.667	-2.639	0.008
h1.090	6.339	2.579	2.458	0.014
Chi^2-statistic vs constant model: 41.9, *P* value =1.77e-08, **AUC=0.97**				

AUC=area under the curve; SE=standard error.

Results: Progressive Desensitization at Month 12

Progressive Desensitization at Month 12 – 3 Epitope Linear Regression Model (IgE)

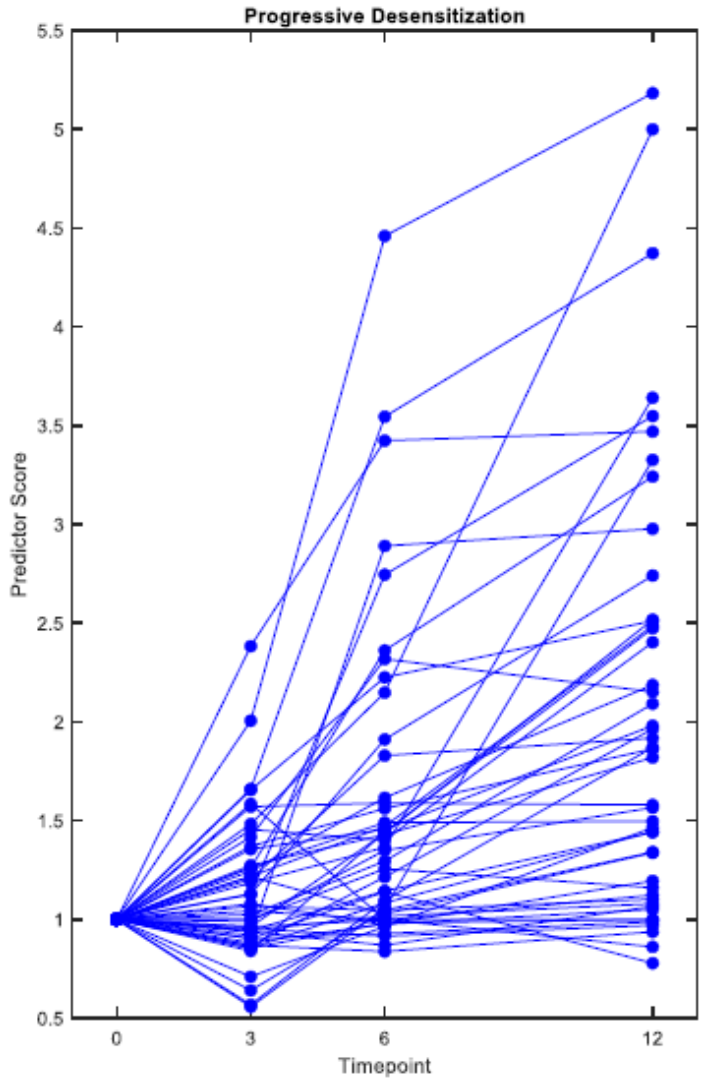
	Coefficient	SE	tStats	*P* value
(Intercept)	6.932	2.361	2.936	0.0033
h1.041	-0.634	0.279	-2.271	0.0232
h1.022	0.585	0.237	2.463	0.0138
h2.008	-0.423	0.226	-1.872	0.0612
Chi^2-statistic vs constant model: 23.2, *P*=3.71e-05, **AUC=0.92**				

AUC=area under the curve; SE=standard error.

Results: Progressive Desensitization Over Time

Progressive Desensitization

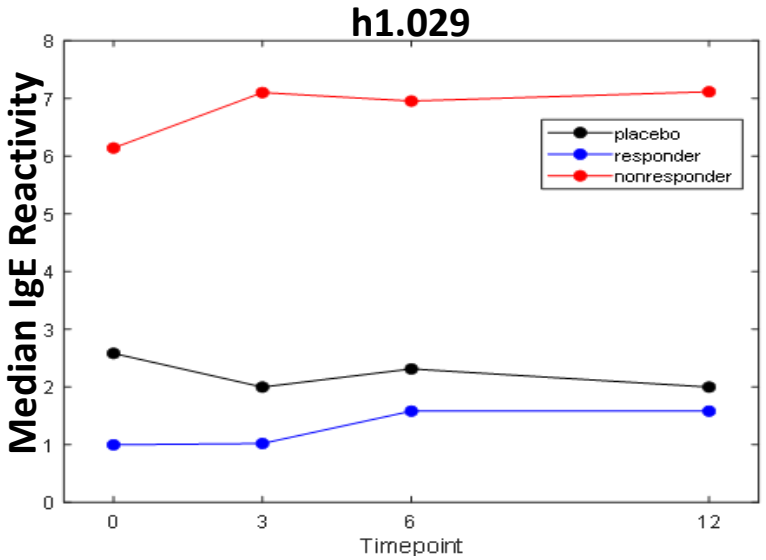
	3 Months	6 Months	12 Months
Best epitopes (IgE)	h1.041 h1.022 h2.008	h1.041 h1.022 h2.008	h1.041 h1.022 h2.008
AUC	0.90	0.91	0.92
% accuracy	89%	89%	91%

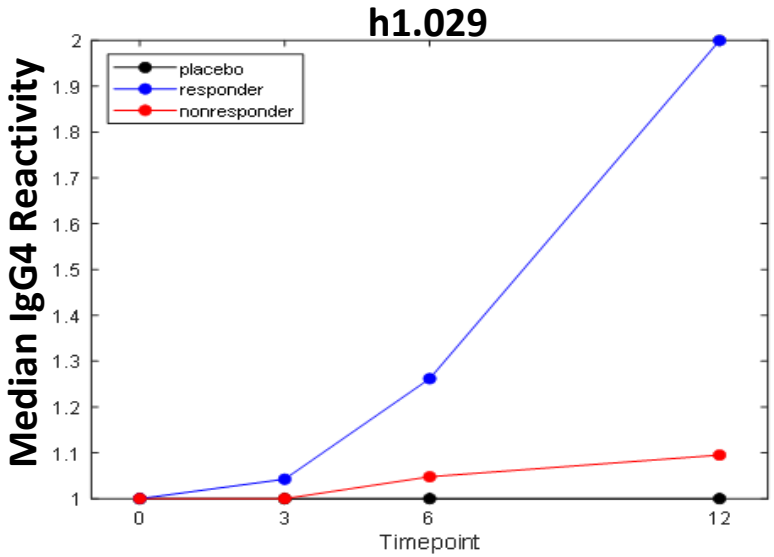


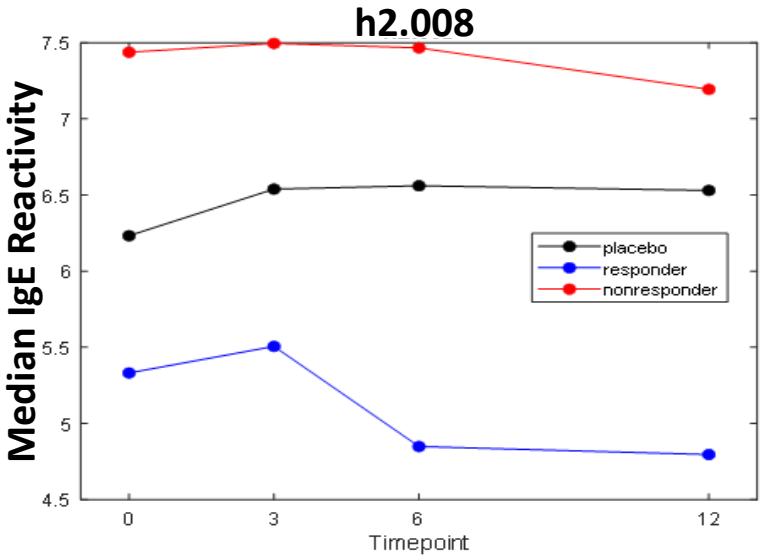
Scores above 1 indicate progressive desensitization over time.

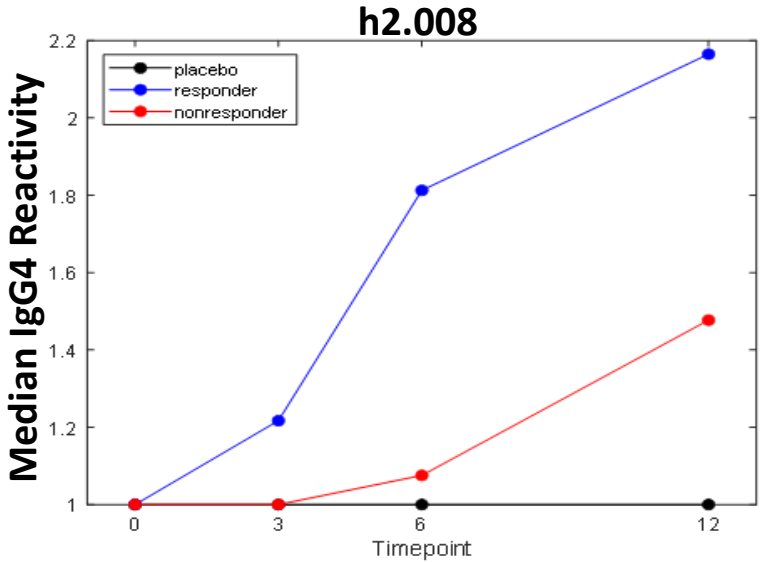
AUC=area under the curve.

Results: Responders vs Non-Responders vs Placebo – Epitopes h1.029 and h2.008









- IgG4 reactivity to each epitope differentiated between placebo, responders, and non-responders with a markedly different trajectory over the 12 months by group
- Faster increases in IgG4 reactivity to h1.029 and h2.008 was associated with treatment response
- IgE reactivity to h1.029 and h2.008 remained stable or slightly declined over 12 months of treatment

Results: Comparison of esIgE/IgG4 to Total Peanut-sIgE/sIgG4

Total peanut-sIgE and sIgG4/sIgE were demonstrated to be poorer predictors of progressive desensitization, with AUCs of 68% and 64%, respectively, at 12 months

sIgE Predictor of Progression Desensitization

	Coefficient	SE	tStats	*P* value
(Intercept)	1.4447	0.45808	3.1538	0.0016113
x1 [h1.041]	-0.0012408	0.0010524	-1.1789	0.23842
Chi^2-statistic vs constant model: 1.36, *P*=0.244, **AUC=0.68382**				

sIgE and sIgG4 Predictor of Progression Desensitization

	Coefficient	SE	tStats	*P* value
(Intercept)	1.2014	0.53651	2.2392	0.025142
x1 [h1.041]	-0.0016422	0.0011868	-1.3837	0.16644
x2 [h1.022]	0.061838	0.076652	0.80674	0.41981
Chi^2-statistic vs constant model: 2.14, *P*=0.344, **AUC=0.64216**				

AUC=area under the curve; esIgE=epitope-specific IgE; esIgG4=epitope-specific IgG4; SE=standard error; sIgE=peanut-specific IgE; sIgG4=peanut-specific IgG4.

Summary and Future Directions

BBEA may be a highly accurate tool for monitoring desensitization during EPIT

- Highly accurate models for predicting both threshold and progressive desensitization were able to be built using 3, 4, and 5 epitope (esIgE and esIgG4) algorithms
- Overall peanut-esIgE reactivity did not change significantly with treatment over time, while peanut-esIgG4 reactivity increased
- Faster increases in esIgG4 reactivity to h1.029 and h2.008 were associated with treatment response
- Lower esIgE reactivity to h1.029 and h2.008 was associated with response to treatment
- Total peanut-sIgE and/or sIgG4 were shown to be relatively poor predictors of progressive desensitization in this population
- Analyses are ongoing to validate these findings in a larger sample and to examine predictors for desensitization beyond 12 months

BBEA=Bead-Based Epitope Assay; EPIT=epicutaneous immunotherapy; esIgE=epitope-specific IgE; esIgG4=epitope-specific IgG4; sIgE=peanut-specific IgE; sIgG4=peanut-specific IgG4.

Acknowledgements

Dianne E Campbell[1,2], Paul Kearney[3], Bob Getts[3], Todd D Green[2,4], David M Fleischer[5], Hugh A Sampson[2,6]

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